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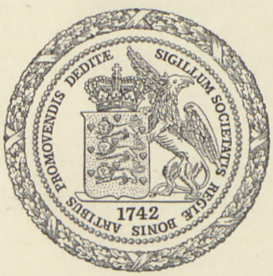
BIND XIX



KØBENHAVN
I KOMMISSION HOS EJNAR MUNKSGAARD
1943—46

INDHOLD

DET KGL. DANSKE VIDENSKABERNES SELSKAB
BIOLOGISKE MEDDELELSER, BIND XIX, NR. 1

SOME MARINE ALGAE FROM MAURITIUS

III. RHODOPHYCEAE

PART 2

GELIDIALES, CRYPTONEMIALES, GIGARTINALES

BY

F. BØRGESEN

KØBENHAVN
I KOMMISSION HOS EJNAR MUNKSGAARD
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As was the case with the previous parts of this publication the present part is based upon the collections of Dr. TH. MORTENSEN and Dr. R. E. VAUGHAN to which, as has been mentioned in the former part, has been added the rather copious collection of Dr. JADIN sent to me from the Muséum National d'Histoire Naturelle, Paris.

The present part contains the following three orders of the *Rhodophyceae*: *Gelidiales, Cryptonemiales* and *Gigartinales*. The systematic arrangement of the families of these orders is in accordance with KYLIN's classification in "Anatomie der Rhodophyceen", 1937.

Dr. G. HAMEL, Laboratoire de Cryptogamie, Paris, has been so kind as to send me, on my request, some material of the typical specimen of *Phyllophora Maillardi* Mont. et Millard. so that I might be able to compare this species with some other related forms.

I am much indebted to Professor HARALD KYLIN, Botaniska Laboratoriet, Lund who has most kindly given me some valuable information concerning type-specimens of algae kept in J. AGARDH's herbarium and likewise concerning the determination of some specimens.

Director, Professor, Dr. H. J. LAM and Dr. I. TH. KOSTER, Rijks Herbarium, Leiden have most courteously sent me a photo of the type-specimen of *Platoma Pikeana* Weber since, because of the war, the specimen itself could not be sent. I take the opportunity here of expressing my sincere thanks to Director LAM and Dr. KOSTER for their kindness.

Further I am much indebted to Dr. O. HAGERUP who with

the greatest readiness made a series of microtomic preparations of some algae for me.

Cand. mag. Louis Harmsen has produced the two fine microphotographs for me and Miss Ingeborg Frederiksen has kindly helped me with some of the drawings.

To the Trustees of the Carlsberg Foundation I am much indebted for a grant for continued algological researches.

II. Gelidiales.

Fam. 1. Gelidiaceae.

Gelidiella Feldm. et Hamel.

1. **Gelidiella acerosa** (Forssk.) Feldm. et Hamel.

Feldmann et Hamel, Observ. s. quelq. *Gélidiacées*, 1934, p. 533. — *Fucus acerosus* Forssk., Fl. Ægypt. — Arab., 1775, p. 190. *Echinocaulon acerosum* (Forssk.). Børgs., Revis. Forssk. Alg., p. 5.

Some few specimens "growing on coral debris" are found in the collection of Dr. Vaughan.

Jadin mentions it in his list. I have seen only a small specimen (no. 514) of his, which is very like the form which Kützing called *G. ramelliferum* (Tab. Phycol., vol. 18, pl. 39). On the other hand, a well-developed specimen from Réunion (no. 174) is present in his collection; in his list p. 163 he calls it *Gelidium rigidum* Vahl. About its habitat at the Mascarene Isls. he writes: "Cueilli dans les anfractuosités des rochers exposés aux lames fortes ou aux courants violents".

Mauritius: Savinia, R.E.V. no. 302, Aug. 1939. Mahébourg, Sept. 1890, Jadin no. 514.

Geogr. Distr.: Most warm seas.

Gelidium Lamour.

1. **Gelidium pusillum** (Stackh.) Le Jolis.

Le Jolis, Alg. Cherb., p. 139. Feldmann et Hamel, *Gelidiales*, 1936, p. 236, where the literature is mentioned.

Several small specimens are found in Dr. Jadin's collection (no. 203); they agree quite well with the figures of Kützing in

Tab. Phycol., vol. 18, pl. 37, figs. i, k. They are about 3—4 mm high, a specimen from Réunion is even smaller, the segments reaching a height of 1—2 mm only and in size it is thus like var. *minusculum* Weber-v. Bosse, Siboga Algues, 1921, p. 226. While these specimens had broad oblong erect segments, another specimen (no. 246) had nearly cylindrical erect segments, only a little compressed and broadened above.

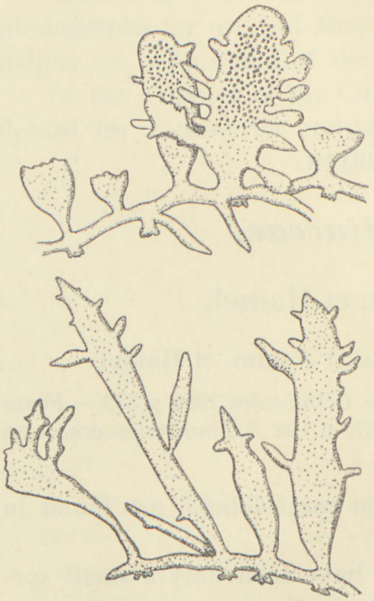

Fig. 1. *Gelidium pusillum* (Stackh.) Le Jolis var. *pulvinatum* (Ag.) Feldm. (× 7).

var. *pulvinatum* (Ag.) Feldm.

Feldmann, Algues de France, no. 36; Feldmann et Hamel, *Gelidiales*, p. 237, fig. 19 C. *Acrocarpus pulvinatus* Kütz., Spec. Alg., p. 762; Tab. Phycol., vol. 18, pl. 37, figs. a—h. *Gelidium pulvinatum* Thuret in Bornet, Algues de Schousboe, 1892, p. 268.

In the material of Dr. Vaughan (no. 309) a small *Gelidium* forms a low growth upon pieces of shells. This plant (Fig. 1) agrees very well with Kützing's above-quoted figures. The plant has sporangia in roundish or oval groups in the broad lobes of the thallus.

The *Phyllophora reptans* Suhr (Beiträge, 1839, p. 285, tab. III, fig. 10) seems to me, from the description and good figures of Suhr, to be this variety of *Gelidium pusillum* since it quite resembles Kützing's figures. Kylin (Verzeichnis, 1938, p. 6, fig. 2 A—C), on the other hand, who has been able to examine a specimen of *Phyllophora reptans* Suhr found in J. Agardh's herbarium, Lund, regards it as a separate species = *Gelidium reptans* (Suhr) Kylin, while Schmitz (1894, p. 194, Anm. 5) who has also been interested in the *Phyllophora reptans* Suhr expresses as his view, in accordance with Agardh (Spec. II, p. 480) and Grunow (1870, p. 82), that it is "vielleicht nur eine sehr winzige Form von *Suhria pristoides* (Turner) J. Ag.".

Mauritius: Mahébourg, July 1890, JADIN no. 246. var. *pulvinatum*. Savinia, R.E.V., no. 309, Aug. 1939, "in rock crevices and on barnacles in exposed situations".

Geogr. Distr.: Extensive in temperate and warm seas.

2. **Gelidium micropterum** Kütz.

KÜTZING, Tab. Phycol., vol. 18, p. 21, pl. 59 c–g. Comp. BØRGESEN, Contributions, III, 1938, p. 212.

To this species described upon material from the Cape I have referred some small specimens in Dr. JADIN's collection (no. 203 bis) which in his list, p. 163 are called *G. corneum*. The specimens are about 3 cm high with flat main segments from which in their upper parts some few branches issue which again along both sides are provided with short oblong roundish branchlets. In some of the specimens nearly cylindrical, quite thin filaments are given out. The specimens are sterile. A specimen from Réunion likewise in JADIN's collection (no. 37) agrees perfectly with those from Mauritius.

About its habitat JADIN writes: "Tapissant les rochers dans les parties ombragées, toujours couvert à marée basse".

Mauritius: Flacq, June 1890, JADIN no. 203 bis.

Geogr. Distr.: Cape, Mascarene Islands, India.

3. **Gelidium crinale** (Turn.) Lamour.

LAMOUROUX, J. in BORY, Diction. class. d'Hist. nat., vol. 7, 1825, p. 191. FELDMANN et HAMEL, *Gélidiales*, p. 240, fig. 22, where the literature is quoted. — *Fucus crinalis* Turner, Fuci, pl. 198.

A single quite small specimen in Dr. JADIN's collection is most probably this species. In his list, p. 163 JADIN mentions this species from Réunion only.

Mauritius: Without locality, JADIN 1890.

Geogr. Distr.: Atlantic Ocean, Mediterranean Sea, Red Sea, Indian Ocean etc.

4. **Gelidium cartilagineum** (L.) Gaill.

GAILLON, B., Resumé méthod. de classification des Thalassiophytes, 1828, p. 15. J. AGARDH, Spec. Alg., vol. II, p. 473; Epicr., p. 550. KÜTZING, Tab. Phycol., vol. 18, pl. 44. BØRGESEN, Mar. Alg. Can. Isl., 1927, p. 90, figs. 48, 49. KYLIN, Floridienstudien, 1928, p. 25. — *Fucus cartilagineus* L.,

Spec. pl., Edit. II, vol. II, p. 1630. TURNER, Fuci, pl. 124. *Gelidium rigidum*, Kütz. Tab. Phyc., vol. 18, pl. 44 (non *Fucus rigidus* Vahl). For more synonyms compare DE-TONI, Syll. Alg., vol. IV, p. 152.

Of this species I have only seen a single specimen belonging to the collection of the Riksmuseum, Stockholm. It has been collected by Colonel PIKE, has no. 45, and has been determined by DICKIE as *Gelidium regidum* Vahl. The specimen is a little smaller in all respects but otherwise agrees very well with specimens from the Cape.

Mauritius: Grand River, 1867, Colon. PIKE.

Geogr. Distr.: Mauritius, Madagascar, Cape, Canary Islands, Brazil, Philippine Islands etc.

5. **Gelidium biserratum** nov. spec.

Frons caespitosa, perennis, subplana, anceps, c. 22 cm alta et ultra? et $1^1/_2$—$2^1/_2$ mm lata, ad basem filamentis subteretibus, decumbentibus et repentibus instructa. Margines thalli serrati, dentibus acutis, subtriangularibus, aequidistanter praediti.

Superficies thalli glabra, nuda.

Rami hic illic sparsi aut subopposite, ex marginibus orti.

Substantia in sicco cartilaginea, color rubro-purpureus.

Organa fructificationis ignota.

Mauritius: Tombeau Bay, 10. Febr. 1939, R. E. VAUGHAN legit.

The growth of the plant (Fig. 2) is undoubtedly caespitose; it forms tufts about 22 cm and perhaps more. The base is composed of decumbent thin filaments, the tips of which are very likely able to form new erect shoots. In one of the specimens an ordinary branch has become decumbent and on coming in contact with the substratum has formed a broad flat hapter; above this hapter three young shoots and below it a single one have begun to grow out, thus forming the beginning of a new tuft.

The thallus is flat c. $1^1/_2$—$2^1/_2$ mm broad; a transverse section (Fig. 3 *a*) shows it to be lengthened lanceolate, about 300 μ in the middle, tapering towards both sides.

Along the edges of the thallus densely placed upward directed short teeth are found with a distance of about 1—2 mm between their apices. These toothlike prominences remain undeveloped

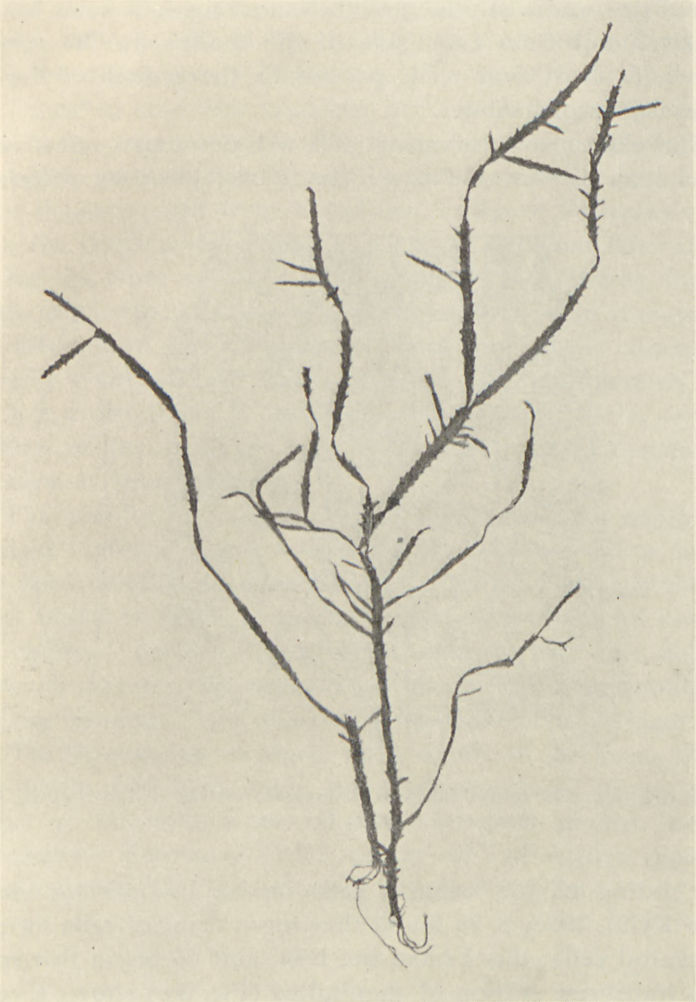

Fig. 2. *Gelidium biserratum* nov. spec. Habit of the plant. (× 3/4).

in most cases but now and then they grow out into branches. As a rule the branches grow out near the upper end of the segments in which the plant is divided, this segmentation no doubt originating from the periodic growth of the plant due to the more or less favourable seasons of the year. For I take it for granted that the plant is perennial; when the favourable season sets in the growth commences again, the thallus attains its

normal size, and when the growth is most vigorous some branches are given out from each side of the thallus. In the specimen figured, at least four such periods in the growth of the plant are easily recognizable.

The plant grows by means of a well-developed apical cell; so far as I have been able to see this is two-sided; by a horizontal

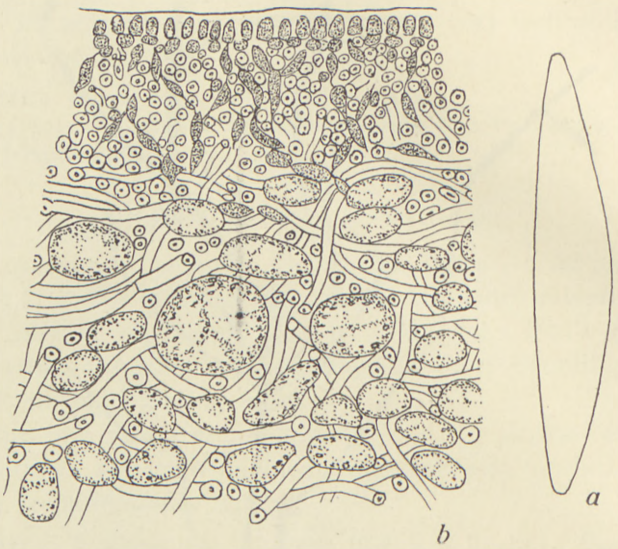

Fig. 3. *Gelidium biserratum* Børgs. *a*, transverse section of the thallus; *b*, fragment of the same. (*a*, × 40; *b*, × 350).

wall cutting off the segment cells below. In *Gelidium cartilagineum* Kylin, 1928, p. 25 found that these segment cells form four pericentral cells; this I have not been able to see in this species.

A transverse section of the thallus (Fig. 3 *b*) shows a cortical layer composed of a single or two rows of oblong cells and underneath this a tissue of uncoloured densely placed rhizoids intermixed with assimilating filaments formed by spindle-shaped or more irregularly formed cells. The central large part of the thallus is composed of cells which are roundish or oblong in transverse section, largest near the periphery, between which rhizoids are densely interwoven in all directions.

Apart from its smaller size this species is surely most nearly related to *Gelidium subcostatum* Okamura, Icones Jap. Alg., 1909,

p. 233, pl. XLVI; comp. also OKAMURA in SCHMITZ, Neue jap. Florid., 1894, p. 190, pl. X); a comparison of the habit figures of both the plants easily shows this. The plant from Mauritius is less ramified and the branches are not bi-tri-partite as is the case in the Japanese plant. The very vigorous midrib formed in the older parts of the Japanese plant is not developed in the more delicate plant from Mauritius; compare OKAMURA's Fig. 2 with the transverse section (Fig. 3 *a*) of *Gelidium biserratum*.

But in other respects both plants have certainly the same mode of growth; the Japanese plant is surely also perennial and has alternating periods of growth according to the climate. This is easily observable on studying OKAMURA's fine figure in Icones.

It is a pity that the plant from Mauritius is sterile; any comparison of the fructiferous organs with those of *Gelidium subcostatum* is thus excluded.

The plant is said to have been dredged at a depth of 80 fathoms (160 metres); if this be right it deserves notice, and if it had been possible to communicate with Dr. VAUGHAN the statement ought to have been confirmed; though, to be sure, it is not without parallel. In a small notice: "Sur une collection d'Algues marines recueillies à une profondeur remarquable près des Iles Canaries" I have given an account of some algae which Dr. TH. MORTENSEN dredged at a depth of between 100 and 200 metres and also made some references to former observations of the occurrence of algae at great depths. Compare FELDMANN's Summary (1937, pp. 71—72) of earlier reports on the occurrence of algae growing at great depths.

Suhria J. Ag.

1. Suhria vittata (L.) J. Ag.

AGARDH, J., Alg. medit., p. 68; Spec. Alg., II, p. 480; Epicr., p. 554. — *Fucus vittatus* L., Syst. Nat., II, p. 718. TURNER, Fuci, tab. 64. *Sphaerococcus vittatus* Ag., Spec. Alg., p. 233. For more synomyms comp. DE-TONI, Syll. Alg. IV, p. 164.

Of this species I have seen only a small specimen belonging to the Riksmuseum, Stockholm. It has been collected "prope insulam Mauritii" by Dr. GRÖNDAL and according to his state-

ment it seems to have been found floating and it is therefore a question if this species really belongs to the flora of Mauritius.

To be sure *Suhria vittata* is mentioned in the list of JADIN (p. 163) who writes about it: "Cette plante parait rare, je ne l'ai pas trouvée et n'ai reçu qu'un exemplaire recuelli sur la plage par DARUTY". This specimen I have been able to examine and thus to state that it is not *Suhria vittata* as it has quite another structure. The specimen of JADIN shows much likeness to *Meristotheca tasmanica* J. Ag., Epicr., p. 583 according to the photo of the original specimen in J. AGARDH's herbarium published by KYLIN in *Gigartinales*, 1932, p. 29, pl. 12, fig. 29. KYLIN points out that its anatomical structure answers to that of *Faucheopsis* Kylin. The specimen of J. AGARDH is sterile but that from Mauritius is tetrasporic; the tetrasporangia are cruciately divided and its anatomical structure might very well correspond to that of this genus or a related genus of the *Rhodymeniaceae*.

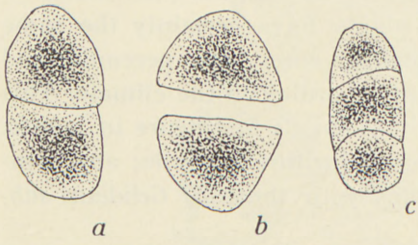

Fig. 4. *Suhria vittata* (L.) J. Ag. Sporangia, *a*, and *b*, divided by a transverse wall into two spores; *c*, a sporangium divided in 3 spores. (× 600).

The above-mentioned specimen of Dr. GRÖNDAL has sporangia. These are said to be cruciatety divided in *Suhria*; compare for instance SCHMITZ & HAUPTFLEISCH in ENGLER u. PRANTT, Nat. Pflanzenf., 1. Teil, Abt. 3, 1897, p. 348. But all the sporangia I met with in this specimen were divided by a single transverse wall into two spores only (Fig. 4) with the only exception of a single sporangium which was divided into 3 spores by transverse walls. Because of this observation I made an examination of some specimens of *Suhria* found in the Botanical Museum, Copenhagen, and was able to ascertain that in these specimens all the sporangia were divided into two spores, none being cruciately divided.

Mauritius: "Prope insulam Mauritii", Dr. GRØNDAL.
Geogr. Distr.: Moluccas, Cape, South America, Brazil.

III. Cryptonemiales.

Fam. 1. Rhizophyllidaceae.

Desmia Lyngb., J. Ag.

1. Desmia Hornemanni Lyngb.

Lyngbye, H. C., Tentamen Hydrophyt. Dan., 1819, p. 35, pl. 7, fig. c. J. Agardh, Spec. Alg., II, 2, 1852, p. 641; Epicr., p. 357. Papenfuss, G., Notes on South African Mar. Alg., I, 1940, p. 216, fig. 12. — *Chondrococcus Hornemanni* (Mert.) Schmitz, Mar. Florideen deutsch Ost-Africa. 1896, p. 170 (in part). Børgesen, Some Indian Rhodophyceen, 1933, p. 117. For more synonyms compare Papenfuss, l. c.

In the above-quoted paper Papenfuss urges the resumption of Lyngbyes generic name *Desmia*, re-established by J. Agardh in 1852. Schmitz, 1896, p. 169 rejected the name of Lyngbye, because it comprehends two Phaeophyceae also besides *Desmia Hornemanni* and substitutes the genus *Chondrococcus* Kütz. published 1847 in Bot. Zeit. p. 23, a genus not much better than that of Lyngbye, comprising as it does several genera of *Rhodophyceae*. Referring the reader for more detail to the paper of Papenfuss I join him in the above-mentioned procedure.

In a note Papenfuss furthermore points out that Mertens' name *Fucus Hornemanni* in Göttinger Gel. Anzeiger, no. 64 (1815), is a nomen nudum, the species being first described by Lyngbye (1819).

In Jadin's list, p. 170 this species is mentioned as *Desmia ambigua* Grev. About its habitat Jadin writes: "Les exemplaires cueillis par moi le furent sur les récifs, donc exposés aux lames violentes".

Mauritius: Ilôt Brocus, R. E. V., Aug. 1938, no. 194. Flacq, Jadin no. 465, Sept. 1890.

Geogr. Distrib.: Seems to be widely spread in the Indian Ocean and adjacent parts of the Pacific.

2. **Desmia tripinnata** (Her.) J. Ag.

AGARDH, J., Spec. Alg., vol. II, 1852, p. 640. PAPENFUSS, Notes etc. p. 218, fig. 13 .— *Rhodhymenia tripinnata* Hering in KRAUSS, Pflanzen des Cap.- u. Natal-Landes, 1846, p. 209.

In connection with what I have said about various forms of *D. Hornemanni* in Kew Bulletin 1933, p. 117 I am really most inclined to consider this species as nothing but a thin delicate form of *Desmia Hornemanni.* If nevertheless I mention it as a separate species here it is because Dr. PAPENFUSS during a visit he paid me in Copenhagen saw some few specimens of a delicate form from Mauritius which he considered to be *Desmia tripinnata.* In his paper quoted above, PAPENFUSS, who has collected the plant in South Africa, himself points out that the only differences separating it from *D. Hornemanni* are that it has a "smaller size and more delicate fronds". And further, what is perhaps the most essential fact, that it grows at a level different from that of *D. Hornemanni.*

PAPENFUSS quotes *Chondrococcus Hornemannii,* Kylin, 1938, p. 8 as a synonym of this species, but it seems to me that the figure of *Plocamium cincinnatum* Kütz., Tab. Phyc. vol. 16, pl. 47 cited by KYLIN as the type figure of this species shows a plant which is a good deal more robust than PAPENFUSS's fig. 13.

Mauritius: Cannoniers Point "in shallow water", Th. M., Oct. 18, 1929.

Geogr. Distr.: South Africa.

Fam. 2. Squamariaceae.

Peyssonnelia Decaisne.

1. **Peyssonnelia Gunniana** J. Ag.

Epicrisis, 1876, p. 387. WEBER-van BOSSE, Liste, p. 272, fig. 90.

Some specimens are found in JADIN's collection. They have been determined by FOSLIE.

The specimens are numbered nos. 538 and 543 respectively

but no locality is mentioned. It is not certain therefore whether the specimens are from Mauritius or from Réunion; in JADIN's list p. 170 this species is mentioned from Réunion only.

Geogr. Distr.: Tropical Australia, Tasmania, Malayan Archipelago.

Hildenbrandia Nardo.

1. Hildenbrandia prototypus Nardo.

NARDO, S., in OKEN's Isis, 1834, p. 675. HAUCK, F., Meeresalgen, p. 38. ROSENVINGE, L. KOLDERUP, Mar. Alg. Denm., p. 202.

Several specimens of this species are found in JADIN's collection. The filaments of the thallus have a breadth of 4—5 μ. As is usually the case the sporangia, divided by oblique walls, are of a very variable shape and size.

About its habitat JADIN says p. 172: "Formant des taches lie de vin sur les rochers, les rendant très glissant quand ils sont mouillés d'eau de mer. Cette Algue recouvre beaucoup de roches, grosses ou petits; quand ell croît sur les gros rochers plats, la marche sur ceux-ci devient très difficile". This description shows that it grows here under similar conditions as I found it in the environs of Bombay (Børgesen, 1935, p. 51) where it covered large nearly horizontal rocks and during low-tide was able to endure the burning tropical sun.

Mauritius: Flacq, June 1890, JADIN no. 548. Mahébourg, Aug. 1890, JADIN no. 546. Bay de la Grande Rivière, July 1890. JADIN no. 539.

Geogr. Distr.: Wide-spread in cold and warm seas.

Fam. 3. Corallinaceae.

Subfam. 1. Melobesieae.

The few species mentioned of this group are taken from JADIN's list. The determinations are in most cases, perhaps all, due to FOSLIE.

Lithothamnion Philippi.

1. **Lithothamnion Lenormandii** (Aresch.) Foslie.

Foslie, Norwegian forms of *Lithothamnion*, 1895, p. 178. — *Melobesia Lenormandii* Aresch., in J. Agardh, Spec. Alg., vol. II, V, p. 514.
Mauritius: Flacq, Jadin no. 562, Aug. 1892.
Geogr. Distr.: Extensive.

2. **Lithothamnion incrustans** (Phil.) Foslie.

Foslie, The Norwegian forms of *Lithothamnion*, p. 122. — *Lithophyllum incrustans* Phil. in Wiegm. Arch. 1837, vol. I, p. 388.
Mauritius: Flacq, Jadin, June 1890, "sur les récifs".
Geogr. Distr.: Atlantic Ocean, Mediterranean Sea, etc.

Lithophyllum Phil.

1. **Lithophyllum incrassatum** Foslie.

Foslie, Algologiske Notiser, VI, 1909, p. 18.
Mauritius: Mahébourg, Jadin, Sept. 1890. Port-Louis, Jadin, Aug. 1890.
Geogr. Distr.: South Africa, Madagascar, Mauritius.

Porolithon Foslie.

1. **Porolithon onkodes** (Heydr.) Foslie.

Foslie, Algol. Notiser, VI, 1909, p. 57. — *Lithophyllum onkodes* Heydr. in Ber. d. deutsch. Bot. Ges., 1897, p. 410. Weber and Foslie, The Corallinaceæ of the Siboga-Expedition, 1904, p. 57, pl. XI, figs. 5—10.
Mauritius: Flacq, Jadin, Oct. 1890, "Recueilli sur la plage".
Geogr.: Distr.: Indian and Pacific Oceans.

Melobesia Lamour.

1. **Melobesia farinosa** Lamour.

Lamouroux, J., Polyp. flexib., p. 315, tab. 12, fig. 3. Compare De-Toni, Syll. Alg., vol. IV, p. 1764, where the literature is mentioned.
Mauritius: Flacq, Jadin, July 1890.
Geogr. Distr.: Extensive.

2. **Melobesia mauritiana** (Foslie) Lemoine.

Lemoine, Sur quelques Mélob. des Comores, 1918, p. 89. — *Heteroderma mauritianum* Foslie, Algol. Notiser, VI, 1909, p. 56.

Mauritius: Flacq, Jadin 1890, "Sur des coquilles".

Geogr. Distr.: Mauritius, Comore Isls.

Subfam. 2. Corallineae.

Amphiroa Lamour.

1. **Amphiroa fragilissima** (L.) Lamour.

Lamouroux, Hist. Polyp. corallig. flex., 1816, p. 298.

Areschoug, Corallineae, 1852, p. 531. Weber-van Bosse and M. Foslie, The *Corallinaceae*, 1904, p. 89, pl. XVI, figs. 1, 2, 5.

In some specimens of this species found in Dr. Vaughan's collection the swollen padlike end of the joints characteristic of this species were not much developed or not at all, but since the anatomical structure of the thallus was in good accord with that of this species I do not hesitate to refer them to it.

Two forms were present, one with slender joints (no. 289), and one with somewhat more robust joints (no. 288), but the building up of the central strand was quite alike in both forms, having 3—4 to 7—8 rows of long cells interrupted by a row of short cells, thus in good accord with the statement of Mme Weber. In specimens from Madagascar Pilger, *Corallinaceæ*, 1908, p. 47 found 5—7 rows of long cells; compare his fig. 13, pl. 6.

Concerning the habitat etc. of the two forms Dr. Vaughan says: "No. 288 forms low cushions pink or grey in colour. Lagoon near shore" and about 289, "very fragile orange red thallus common in shallow water with sea grasses".

Mauritius: Black River Bay, July 1939, R. E. V. nos. 288 and 289.

Geogr. Distr.: Widely distributed in warm seas.

2. **Amphiroa Beauvoisii** Lamour.

Lamouroux, J., Hist. Polyp. corallig. flex., 1816, p. 299. Bornet, Algues de Schousboe, 1892, p. 349. — *Amphiroa pustulata* Mertens in Flora 1836,

p. 487, tab. 1. *Amphiroa exilis* Harv., Nereis Austr., p. 95. *Amphiroa polyzona* Mont., Fl. d'Algerie, 1847—49, p. 136. *Amphiroa algeriensis* Kütz., Tab. Phyc., vol. VIII, p. 21, fig. 44.

Dr. VAUGHAN's collection contains an *Amphiroa* broken to pieces, which seems to be referable to this species of which I have not had any original or well-determined specimen for comparison.

The plant has been described by LAMOUROUX upon a specimen from Portugal but his description is very short. In Algues de Schousboe BORNET points out that the above-mentioned synonyms belong to this species; and about the figure of KÜTZING he says that it gives a good representation of the type-specimen of the plant in LAMOUROUX's herbarium; the plant from Mauritius seems to agree quite well with this figure.

In Algues Siboga, pl. XVI figs. 18 and 19 Mme WEBER gives two figures of joints and nodes of this species, respectively from Naples and Durban; the plant from Mauritius is in good accordance with these figures. In the central strand I have found mostly three rows of long cells followed by one row, sometimes two rows of short cells. The long cells had a length of 70 to 90 μ, while the length of the short cells varied from 22—35 μ. Now and then also a row of quite short cells, about 15 μ long only, was present. As is pointed out by Mme WEBER the node consists of the entire central strand and almost entire cortical layer.

Referring to some figures of *Am. zonata* Yendo and *Am. echigoensis* Yendo (1902, pp. 10—11, pl. 1, figs. 11—14 and figs. 15—16) Mme WEBER (l. c., p. 101) remarks that these species seem to come near to *Am. Beauvoisii*; these figures show much likeness to the anatomical structure of the plant from Mauritius.

The thallus is terete below, more or less flattened above; its surface its rather uneven, sometimes with a tendency to be annulated, and the ramification is very irregular.

Another specimen, no. 351, was likewise broken to pieces. About its appearance etc. Dr. VAUGHAN writes: "Very fragile, pale pink calcareous segments".

Mauritius: Ilôt Brocus, 31. Dec. 1938, R. E. V. no. 219, "in rocky pools". Pte aux Sables, Aug. 1939, R. E. V. no. 351.

Geogr. Distr.: Mediterranean Sea, Portugal, Morocco, Cape, Brazil.

3. **Amphiroa crassa** Lamx. forma **minuta** Web.-v. Bosse.

Weber-van Bosse and Foslie, The *Corallinaceae* of the Siboga-Expediton, p. 98.

In Dr. Vaughan's collection an *Amphiroa* occurs which forms dense, firm cushions c. 2 cm high, upon rocks. This small plant seems to be the same as the dwarfish form of *Am. crassa* which Mme Weber, l. c., p. 98, has described as forma *minuta*; compare her figure 3, pl. XV.

As stated above, the plant forms dense low cushions formed by the stiff, much entangled, and very irregularly articulate and ramified filaments. Also the filaments are very irregularly shaped, in some cases terete below and flattened or lingulate above, in others flattened below and gradually subterete and slender upwards. The breadth of the filaments varies from $^1/_2$—$1^1/_2$ mm.

As regards the anatomical structure, the central strand consisted in one specimen of 3 rows of long cells interrupted by a row of short cells followed by 3 rows of long cells, and so on. The long cells were about 80 μ long and the short ones about 25 μ long. In another specimen the central strand contained two rows of long cells and one row of short cells, the long cells reaching a length of 80—110 μ and the short ones about 30—50 μ. The node consists of many rows of cells and is quite surrounded by the calcified cortical layer; when the cortical layer does not split, the nodes are not visible.

Mauritius: Souillac, "on reef", R. E. V. no. 340.
Geogr. Distr.: Indian and Pacific Oceans.

Cheilosporum (Decsne.) Aresch.

1. **Cheilosporum acutilobum** Decsne.

Decaisne, J., Sur les Corallines, 1842, p. 125. Montagne et Millardet, Algues, 1862, p. 0—16.

Some specimens in Dr. Vaughan's collection seem to be in good accordance with the detailed description of Montagne and Millardet; the species was originally described by Descaisne upon a specimen from Mauritius but I have not seen an authentic specimen.

The plant (Fig. 5) grows in dense tufts about 3—4 cm high. The basal decumbent and very intricate branches are subterete and composed of joints isodiametric or a little longer than broad, about $^1/_2$ mm broad; upwards the joints gradually become flattened out and broader, acquiring the characteristic cordate, bialate shape with falcate pointed ends. The breadth from point to point is about $1^1/_2$ mm. One specimen in Dr. JADIN's col-

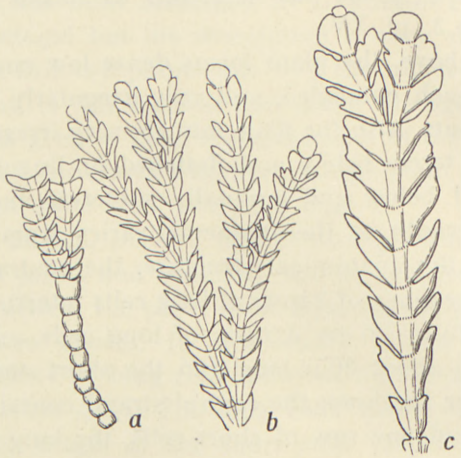

Fig. 5. *Cheilosporum acutilobum* Decsne. *a*, part of the thallus from near the base; *b*, upper part of thallus; *c*, a deviating piece of the thallus. (*a* and *b* × 3; *c* × 6).

lection was larger, nearly 6 cm high, and the breadth of the thallus about 2 mm, thus approaching the size of *Cheilosporum culthratum*; compare ARESCHOUG, 1852, p. 545.

The specimens were sterile.

It seems to me that several of the small forms of *Cheilosporum* are closely related and not easily distinguishable; an examination of their abilily of variation is certainly much needed. MONTAGNE et MILLARDET do not conceal that they consider *Amphiroa elegans* Harv., Nereis Australis, p. 101, pl. 38, described upon a specimen from Mauritius, to be very closely related to the species of DESCAISNE or perhaps the same species. Nearly allied to *Cheilosporum acutilobum* are surely also forms of the polymorphous species *Cheilosporum culthratum* (Harv.) Aresch., l. c., p. 545.

In the middle of one of the tufts from Mauritius some

branches were found in which the joints instead of the falcate pointed ends at both sides were abruptly cut off, and ended in a nearly entire, or more or less deaply sinuated edge (Fig. 5 *c*). These branches show much likeness to the plant which in Alg. Bombay, 1935, p. 52, fig. 23, I have referred to *Cheilosporum spectabile* Harv. and in some degree also to *Cheilosporum multifidum* (Kütz.) Yendo = *Amphiroa multifida* Kütz., Tab. Phyc., vol. 8, tab. 56, about which KÜTZING says in a note that it seems only to be one of the many forms of *Cheilosporum cultbratum*. In addition MONTAGNE and MILLARDET also mention *Cheilosporum multifidum* in their paper on the algae of Réunion, p. 0—15.

Mauritius: R. E. V. no. 32 (no locality), "in exposed situations constantly washed by waves; dull pink in colour". JADIN 1892, no locality. DICKIE, 1875, p. 193, mentions this species from Mauritius.

Geogr. Distr.: Mascarene Islands.

Corallina Lamour.

1. Corallina polydactyla Mont. et Mill.

MONTAGNE, C., and M. MILLARDET, Algues, 1862, p. 0—18, pl. XXV, fig. II.

While referring some specimens in Dr. JADIN's and Dr. VAUGHAN's collection to this species, I must point out that I have not seen any authentic specimen of the plant; but the specimens from Mauritius seem to be in good accordance with the description and figures quoted above.

The plant forms a dense roundish tuft about 3 cm high. Fig. 6 shows two small pieces of the thallus. The articulate main axis is composed of subtriangular joints. These are broadest above, and from their upper margins in the well developed thallus two pinnae are given off on both sides of the main axis (Fig. 6 *a*); in the less vigorous specimens only a single pinna becomes developed (Fig. 6 *b*). The more vigorous pinnules are furcated, the rest remain simple and taper gradually upwards to the tips, the joints of which they are composed also at the same time increasing more or less in length. As a characteristic of this species the authors point out that these elongated and often

somewhat curved branchlets may have the appearance of the skeleton of a hand.

The pedicellate conceptacles are obovate in shape, a little narrowed above and provided above with two apposite branchlets composed of a single or often two joints.

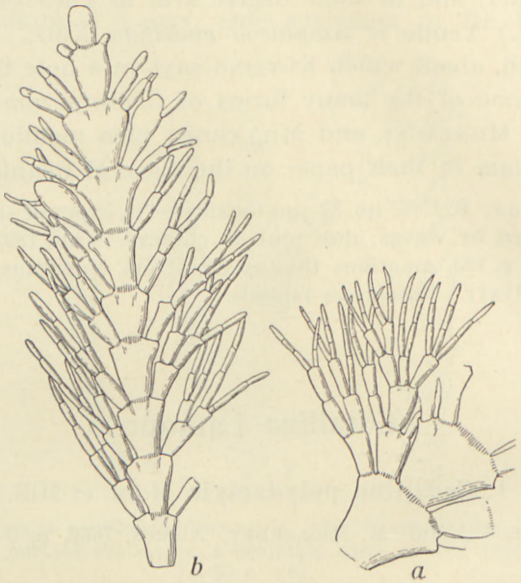

Fig. 6. *Corallina polydactyla* Mont. et Mill. Fragments of the thallus. (× c. 10).

About its habitat Dr. VAUGHAN writes: "growing in rock crevices usually in exposed situations constantly washed by waves". This species is also mentioned in JADIN's list, p. 170 but the specimens of his I have seen are the following species.

Mauritius: R. E. V. no. 31 (no locality).
Geogr. Distr.: Mascarene Islands.

2. **Corallina mauritiana** nov. spec.

Frondes caespitosae, c. 6 cm altae, articulatae, compressae, distichae, ad basem subsimplices, ex articulis subteretibus nudis compositae, ad apicem versus plus minus abunde et irregulariter ramosae, ex articulis compressis, triangularibus, pinnulatis, c. 600 μ altis et 600—900 μ latis compositae.

Rami bipinnati. Pinnae circumscriptione fere lineares, c. 3 mm

longae et 2—3 articulos oblongo-triangulares continentes, suberectae, angulis acutis ortae, pinnulatae.

Pinnulae erectae, fere cylindricae, simplices aut interdum furcatae, aequicrassae, apicibus late rotundatae, ex articulis 2—6 compositae.

Conceptacula ex transmutatione pinnularum orta, urceolata vel pyriformia, corniculata.

Cornua simplicia aut interdum furcata, 2—3 articulos continentia.

Mauritius: Without locality, JADIN nos. 210, 121, 453, 262. Flat Island, 17. Oct. 1929, TH. M.

The characteristic feature of *Corallina mauritiana* (Figs. 7 and 8) is its very regularly featherlike ramification. The thallus has a well marked articulate midrib, composed of nearly triangular joints. From the upper corners of these at both sides straight, obliquely upward directed pinnae are given out. The angle between the pinnae and the joints of the midrib is very narrow (Fig. 8).

The pinnae consist of 2, most often of 3, more rarely of more joints and from the uppermost of these, but sometimes

Fig. 7. *Corallina mauritiana* nov. spec. Habit of the plant. (× 2).

also from the lower ones, pinnules issue. The pinnules are articulate, nearly terete and upward directed. The pinnae and pinnules all lie on the same plane as the main axis.

The base of the plant forms a plexus of intermingled irregularly ramified and more or less decumbent filaments composed of nearly terete joints; from this base the erect part of the thallus arises.

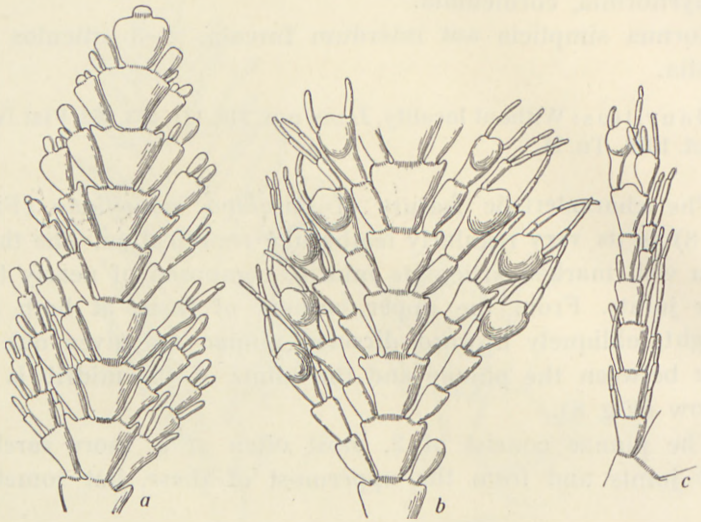

Fig. 8. *Corallina mauritiana* nov. spec. Pieces of the thallus, *b* and *c* with conceptacles. (× c. 8).

Corallina mauritiana may show some likeness to *Corallina rosea* Lamarck, compare HARVEY, Nereis austr., 1847, p. 105, tab. 40. I have seen no material of this species but a comparison with HARVEY's description and figure shows that rather essential differences are present. Thus the ramuli in *Corallina rosea* are broader, carrying pinnules on both sides of the axis, while in *Cor. mauritiana* these are less developed, often quite wanting on the lowermost joints; furthermore the pinnules are straight upward directed in the latter species, while those in *Cor. rosea* are spreading.

The herbarium of the Botanical Museum, Copenhagen, contains a small specimen from Port Natal determined as *Cor. rosea* but with no information about the collector or who has determined it. Most probably this plant is the same as the one ARESCHOUG,

Corallineæ, p. 524, mentions in a special note, pointing out that the specimen in question is perhaps *Jania Cuvierii natalensis* mentioned by HARVEY, l. c., p. 105, but at the same time declaring that he dare neither refer it to *Jania rosea* nor consider it as a separate species. On a superficial inspection, the plant from Natal may show some likeness to *C. mauritiana*, but its thallus is in all respects smaller and the ramuli are more developed and have more spreading pinnules.

From *Corallina polydactyla* *Corallina mauritiana* differs by less branching of the main filaments. In *Corallina mauritiana* the pinnae together with the pinnules have nearly the same length, are straight and upward directed, the result being that the thallus keeps nearly the same breadth and has nearly straight parallel flanks, while in *Corallina polydactyla* the pinnules are of unequal length, are not directed so straight upwards and the pinnules are more crooked; because of this the breadth of the thallus is variable. And in *Corallina polydactyla* the pinnules (horns) terminating the conceptacles are composed of a single or now and then two joints and their tips are more acute, while in *Corallina mauritiana* the horns consist of 2—3 joints and are obtuse above (Fig. 8 *b*).

Finally the colour of *Corallina mauritiana* in the dried condition is a dull greyish red, while in *Corallina polydactyla* the colour of the thallus is a fine rosy red with shining surface.

The specimens of *Corallina mauritiana* in JADIN'S collection which I have seen have numbers, and according to these JADIN in his list refers them to *Corallina polydactyla* Mont. JADIN also mentions *Corallina plumifera* Kütz., a species which is also included by DICKIE in his list of algae from Mauritius, p. 173; but no explanatory remarks as to why it is referred to KÜTZING'S species (Spec. Alg., p. 705 and Tab. Phycol., vol. VIII, pl. 71, II c, d) are given. It cannot be denied that *Corallina mauritiana* shows some likeness to KÜTZING'S figure II d, though with the exception that the conceptacles in KÜTZING'S figure are without pinnules, while such are present in the plant from Mauritius. But as to the other figure of KÜTZING, fig. II c, the likeness is not a good one; this figure suggests that *Corallina plumifera* Kütz. is most probably a form of the very polymorphous species *Corallina Cuvierii*, a possibility already alluded to by DE-TONI in Sylloge Alg., vol. IV, p. 1854.

Jania Lamour.

1. Jania rubens (L.) Lamour.

Lamouroux, Hist. Corall. flexib., 1816, p. 272. Areschoug, *Corallineae*, 1852, 557. — *Corallina rubens* L., Systema Nat., ed. 12, vol. 1, p. 1304.

Two small specimens are found in Dr. Vaughan's collection; one of these being an epiphyte upon *Digenea*.

I have also seen some specimens from Dr. Jadin's collection; it is mentioned in his list as a very common species.

Suneson (1937, p. 38) classes *Jania* to *Corallina*, referring to the view of Rosenvinge (1927, p. 275) who considers *Jania* as a subgenus of *Corallina*, because, according to him, *Jania* differs only from *Corallina* by its dichotomous ramification. Meanwhile, as Mme Weber (1904, p. 85) points out, an anatomical character by which *Jania* differs from *Corallina* is also present, seeing that the cells in the central strand of *Jania* are proportionally longer as compared with the shorter ones in *Corallina*, and further the cells in the nodes of *Jania* are shorter when compared with the long cells of the node in *Corallina*. Mme Weber refers to Kützing's figures in Phycologia generalis, pl. 79, figs. I and II of *Corallina officinalis* and *Jania rubens* respectively, which show the differences pointed out by her very clearly. I therefore prefer to keep up the genus *Jania*, and in this I am, indeed, in agreement with by far the greater number of algologists.

Mauritius: Pte aux Roches, 7. Febr. 1939, R.E.V. no. 263. Flacq and Mahébourg, July—Sept. 1890, Jadin nos. 431, 449.

Geogr. Distr.: Widely spread in temperate and warm seas.

2. Jania tenella Kütz.

Kützing, Tab. Phycol., vol. VIII, p. 41, pl. 85, fig. II.

On a piece of *Galaxaura* a quite small *Jania* was found, the thallus of which had only a breadth of about 60 μ.

Mauritius: Pte aux Sables, R. E. V. no. 354, Aug. 1939.

Geogr. Distr.: Mexico, Mediterranean Sea, Malayan Archipelago etc.

Fam. 4. Grateloupiaceae.

Grateloupia C. Ag.

1. Grateloupia filicina (Wulf.) Ag.

AGARDH, C., Spec. Alg., p. 223. J. AGARDH, Spec. Alg., vol. II, p. 180. — *Fucus filicinus* Wulf. in JACQUIN, Collectanea, vol. III, 1789, p. 157, tab. 5, fig. 2 (not seen).

Of this species I have seen some few specimens in Dr. JADIN's collection; some of these are undetermined, some are determined as *Hypnea nigrescens*. JADIN mentions *Grateloupia filicina* in his list p. 170. About its habitat he writes: "Sur des débris de coraux, à une faible profondeur".

Mauritius: Port Louis, Fort-Georges, Aug. 1890, JADIN.
Geogr. Distr.: Most warm seas.

Halymenia J. Ag.

1. Halymenia maculata J. Ag.

AGARDH, J., Till Algernes Systematik, VII, *Florideæ*, 1884, p. 12.

This species has been described by J. AGARDH upon a specimen from Mauritius gathered by MELVILL. I have not seen any specimen of it. It is not mentioned in JADIN's list.

Geogr. Distr.: Endemic.

Carpopeltis Schmitz.

1. Carpopeltis rigida (Harv.) Schmitz.

SCHMITZ, FR., Florideen von Deutsch-Ostafrika, 1896, p. 167. OKAMURA, Icones Jap. Alg., vol. II, 1912, p. 63, pl. LXVI. — *Cryptonemia rigida* Harv., Alg. Ceylon Exsicc. no. 51 (nomen nudum). J. AGARDH, Epicrisis, 1876, p. 163. *Phyllophora Maillardi* Mont. et Millard., Algues Réunion, 1862, p. 8, pl. 24. *Suhria(?) Zollingeri* (Sonder) Grun., Alg. Novara, 1870, p. 82, tab. X, fig. 3.

As will appear from the above-mentioned synonyms, this plant has been described as a distinct species several times.

Harvey was the first to name the plant, in Alg. Ceylon Exsiccatae no. 51, but as a nomen nudum only; afterwards J. Agardh published a description of it, keeping Harvey's name.

Then Montagne et Millardet in their paper on the algae of Réunion described the plant as a new species: *Phyllophora Maillardi*; their description is accompanied by some good figures. And finally Grunow in 1870 mentions the plant as *Suhria*(?) *Zollingeri*, basing his description upon material from the Nicobar Islands.

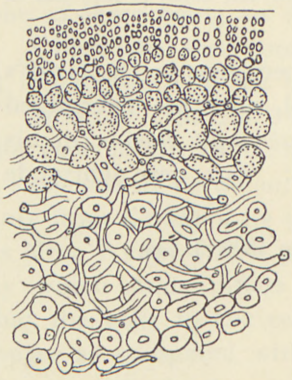

Fig. 9. *Carpopeltis rigida* (Harv.) Schmitz. Fragment of a transverse section of the thallus. (× c. 400).

When mentioning the synonymy of this species Schmitz in the paper quoted above (1896, p. 167) arrives at the result: "dass *Phyllophora Maillardi* mit *Suhria Zollingeri* entschieden zusammengehört und wie diese vermutlich mit *Cryptonemia rigida* zu vereinigen ist".

So as to arrive at a definite result concerning this question which was thus not quite settled, I asked Dr. Hamel, Muséum National d'Histoire Naturelle, Paris to let me see, if possible, a small piece of *Phyllophora Maillardi* M. et M. Upon my request Dr. Hamel most kindly sent me a small piece of the original material by means of which I have been able to establish the identity of the plant from Réunion with those which I have for determination from Mauritius. A comparison of the plant from Réunion with Harvey's plant and some specimens I have collected at Galle showed further evidence that the plant from Ceylon was the same as that from Réunion.

If finally we turn to *Suhria Zollingeri* Grunow, the determination of which Grunow based upon material from the Nicobar Islands, we have here in the Botanical Museum, Copenhagen, some excellent material collected during the Galathea-Expedition in the years 1845—47.

These specimens not only agree perfectly with Grunow's figures but also with Harvey's and other specimens from Ceylon, and with those from the Mascarene Islands. Hence there can be no possible doubt that they are all one and the

same species, and referable to the genus *Carpopeltis* established by SCHMITZ in Flora, 1889, p. 453.

Of this species several specimens are found in the collections. As the anatomy of this plant seems to be known only from the short description of SCHMITZ in ENGLER und PRANTL, Natürl. Pflanzenfam., I Teil, 2. Abt., 1897, p. 514, I give here a part of a transverse section of the thallus (Fig. 9). The cortical layer consists of short densely placed anticlinal more or less furcated filaments, composed of oblong cells, smallest near the periphery, becoming gradually larger inwards, forming an even transition to the somewhat larger, at first roundish, soon more irregularly shaped cells of the peripheric medulla; the innermost part of this tissue is composed of thick-walled, densely interwoven filaments, among which rhizoids are intermingled. The thallus is very tough and cartilaginous, which is in good accord with the very exposed habitats of this plant.

Concerning the habitat of this species JADIN, p. 170 writes: "Croissant sur des rochers exposés aux lames violentes".

Mauritius: Flacq, June 1890, JADIN no. 217. Pointe aux Roches, R. E. V. no. 237.

Geogr. Distrib.: Seems to be wide-spread in the Indian and adjacent parts of the Pacific Ocean.

Fam. 5. Kallymeniaceae.

Kallymenia J. Ag.

1. Kallymenia perforata J. Ag.

AGARDH, J., Bidrag till Florideernes Systematik, p. 9; Epicr., p. 219. BØRGESEN, Mar. Alg. D. W. I., p. 358, fig. 353.

Two small specimens are found in Dr. MORTENSEN's collection. I have compared them with some preparations of a plant from Ceylon (FERGUSON, Alg. Ceylon exsicc., no. 16) and spe-

cimens from the West Indies, with which the plant from Mauritius seems to agree perfectly.

The specimens were dredged at a depth of about 25 fathoms.

Mauritius: Between Gunners Quoin and Flat Island, TH. M., 15. Oct. 1929.

Geogr. Distr.: Ceylon, West Indies, Malayan Archipelago.

IV. Gigartinales.

Fam. 1. Nemastomaceae.

Nemastoma J. Ag.

1. Nemastoma coliformis J. Ag.

AGARDH, J., Till Algernes Systematik, IV., 1884, p. 11, KYLIN, H., Gigartinales, p. 7, tab. 1, fig. 2.

This species is described by J. AGARDH upon material from Mauritius. KYLIN's above-quoted figure shows the original specimens.

I have not seen any specimen of it.

Geogr. Distr.: Endemic.

Titanophora nov. gen.

Thallus 10—20 cm altus et ultra(?), disco ad substratum adfixus, calce incrustatus, exsiccatione fragilis, subcompressus, irregulariter lobatus, interdum ex marginibus proliferus.

Lobi in parte basali latiores, sinu rotundato sejuncti, ad apicem versus tenuiores, iterum furcati, apicibus obtusis vel subacutis.

Superficies frondis inaequale-subverrucosa, colore in sicco albido-rubescente.

Stratum corticale ex filamentis brevibus subdichotomis, cellulas breves continentibus et glandulis sparsis intermixtis formatum.

Medulla ex filamentis crassioribus, irregulariter inter se confluentibus, sparsim furcatis composita.

Gonimoblasti per totam superficiem frondis sparsi, in superiore parte medullae immersi.

Carposporae, ex cellulis omnibus filorum gonimoblastorum ortae, per porum rotundatum superne in strato corticali formatum, liberatae.

Species typica: *Titanophora Pikeana* (Dickie) Børgs.

1. **Titanophora Pikeana** (Dickie) Børgs., nov. comb.

Galaxaura Pikeana Dickie, Alg. Mauritius, 1873, no. 195. *Halymenia Pikeana* J. Agardh, Till Alg. System. VII, Florideae, 1884, p. 15. *Platoma Pikeana* Weber, Alg. Siboga, p. 253, pro parte.

Some undetermined material collected by Dr. Jadin contained an alga (Fig. 10) incrusted with chalk which upon examination turned out to be identical with *Galaxaura Pikeana* Dickie (1873, p. 195), an alga which, when superficially observed, may bear some likeness to *Galaxaura*.

This alga J. Agardh (1884, p. 15) refers to the genus *Halymenia*, placing it with a plant from the West Indies (*Halymenia incrustans*) in a separate section named *Titanophora*, because of the chalk-incrusted thallus of the plants in question.

Later the late, much regretted Mme Weber (1921, p. 253) collected an alga on a reef at New Guinea which she presumed to be the same species as that from Mauritius. She placed it in the genus *Platoma* as it is also rather closely related to this genus, but nevertheless it comes nearer to another genus of the *Nemastomaceae*, namely *Schizymenia*, as will be demonstrated in more detail later. Meanwhile it seems better to place these plants in a separate genus for which I propose the name *Titanophora*, giving the section of J. Agardh generic rank.

Yet I should like also to point out here that I do not think that the plant of Mme Weber is the same as that of Dickie, but another species of *Titanophora*.

In the sequel I shall first give a description of the plant from Mauritius. This can be based only upon dried material, just as I have had no occasion to compare with authentic material, since the collection of *Rhodophyceae* from Mauritius of the Kew

Herbarium in which DICKIE's specimens are incorporated could not be sent because of the war.

It cannot be denied that the description of DICKIE is rather poor; nevertheless some of the few characters mentioned in his diagnosis give some idea of the plant. Meanwhile so as to make

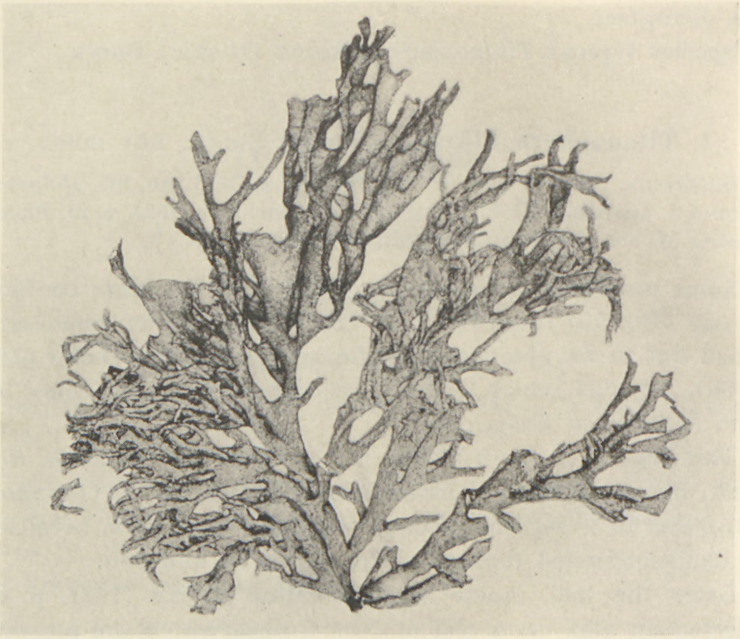

Fig. 10. *Titanophora Pikeana* (Dickie) Børgs. Habit of the plant. (× 4/5).

sure as far as possible of the identity of JADIN's plant with that of DICKIE, Professor KYLIN, Lund, has most kindly on my request made a comparison of JADIN's specimen with those found in J. AGARDH's herbarium and informed me that they agree perfectly. Two specimens are found in AGARDH's herbarium; one of these, which was most probably collected by Colonel PIKE himself, FARLOW sent to AGARDH, the other one was collected by MELVILL. Professor KYLIN has also most kindly sent me some fragments of both specimens by which I have been able to establish the identity of their anatomical structure with that of JADIN's plant.

The plant is about 10 cm high. The thallus (see Fig. 10) is

subterete to complanate, repeatedly irregular, subdichotomously divided into longer or shorter lobes of variable breadth; below the divisions the segments are broadened out subcuneately, reaching a breadth of up to 1 cm, the upper lobes having only a breadth of 1—2 mm. The angles between the lobes are more or less broadly rounded. The whole thallus is incrusted with chalk and rather brittle; the surface is unevenly warted. A transverse section of a decalcified piece of a lobe shows it to be oval, and nearly terete in the uppermost tips. The apices of the segments are obtuse, which is best observable when the thallus is decalcified. The colour of the specimen upon which the examination was based is reddish, the same as that of the specimens in J. AGARDH's herbarium; another specimen of Dr. JADIN's collection, most probably cast ashore, has a greenish colour and a more smooth surface.

The incrustation of chalk consists of a porous rather soft mass filling up the whole interior of the thallus; in this mass the filaments of the medullary tissue are imbedded. However, the cavities of the cystocarps are free from incrustation; this has also been observed by Mme WEBER (p. 254).

Concerning the anatomical structure of the plant I must point out that I have only had the opportunity of examining a full-grown dried specimen in which young apices were wanting, for which reason I have not been able to observe the development of the thallus.

Before entering upon a description of the anatomical structure of the plant I should like to express my acknowledgements to Dr. O. HAGERUP who most kindly made a series of microtomic preparations of the plant for me.

A transverse section of the thallus shows that the cortical tissue forming the assimilating part of the frond consists of oblong or more irregularly shaped cells, largest innermost, smaller towards the periphery (Fig. 11 *a*). From the uppermost of these cells papilliform subcylindrical cells, broadly rounded above, protrude freely. For lack of young well-preserved material I have not been able to follow the development of the cortical layer, but I suppose it to be homologous to that of the following species in which according to Mme WEBER's description

and figures the cortical layer consists of filaments several times furcated. The freely projecting papilliform cells in *Titanophora Pikeana* (Fig. 11 *a*, Pl. I, fig. 1) would thus be the apices of the short filaments.

The cells from which the papilliform cells issue, forming the closely connected peripheric layer, are short and broad in trans-

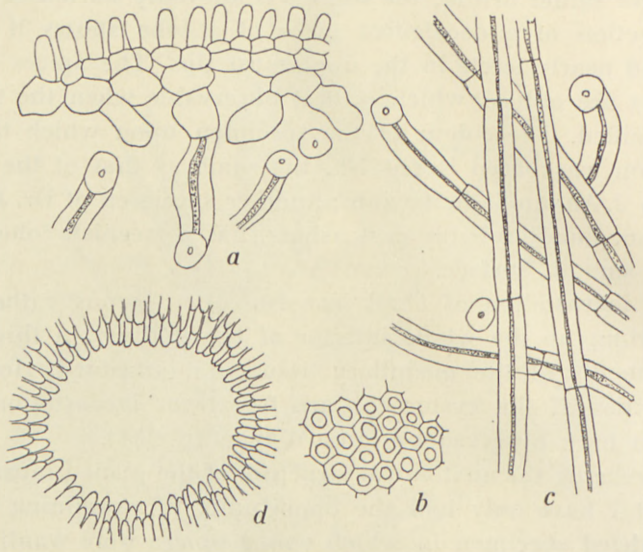

Fig. 11. *Titanophora Pikeana* (Dickie) Børgs. *a*, transverse section of the cortical tissue; *b*, the peripheric layer seen from above; *c*, fragments of the medullary filaments; *d*, a porus seen from above, surrounded by the papilliform cells. (× 500).

verse section; when viewed from above, the cells of this layer are subhexagonal in shape, about 7—10 μ broad (Fig. 11 *b*). But the freely projecting upper ends of the filaments are not always developed, and the surface of the thallus is then formed by the hexagonal cells only. An even transition is found from the places where the papilliform cells are developed to the bare places; for the papilliform cells gradually become shorter and at last are quite wanting, while at the same time the peripheral cells become more or less vaulted above. The lowermost cells of the peripheric layer are connected with the filaments of the medullary tissue.

In various places in the cortical layer more or less numerous glandular cells are found immersed in it (Pl. I, fig. 1). The glands

are flattened-subglobular with thick walls, about 30 μ broad and 23 μ high; they have a yellowish, clear, refractive content.

The medullary filaments (Fig. 11 *c*) are of variable breadth, about 8—12 μ thick, have thick walls and are composed of nearly cylindrical cells about 60—70 μ long; the filaments are furcated

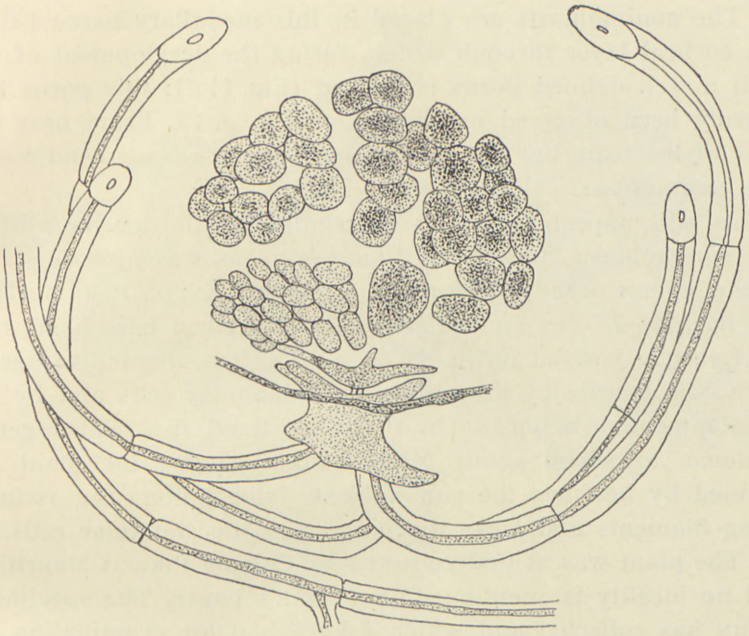

Fig. 12. *Titanophora Pikeana* (Dickie) Børgs. Transverse section of a gonimoblast. The fertilizing filament is seen in connection with the auxiliary cell. The figure is somewhat schematic. (× 700).

now and then. They traverse the interior of the thallus, running in all directions and intercrossing, but having plenty of space between them.

The specimen is a female one, having ripe or nearly ripe fruits also near the apices of the thallus. Consequently it has been impossible to follow the development of the female organs nor that of the auxiliary cells, but in several cases I have been able to observe the fertilizing filaments (Verbindungsfäden Berthold, 1884) in fusion with the auxiliary cells (compare Fig. 12 and Pl. I, fig. 2). The auxiliary cell is large and irregularly polygonal, its corner having often long prolongations. Towards the

periphery the first gonimoblast cell is formed. This cell is large and flattened. Several gonimoblast filaments are developed from it, the cells of which become carpospores. Each of these gonimoblast filaments forms a group of carpospores, and as these groups are of different ages the groups of carpospores are in different stages of development.

The gonimoblasts are placed in this medullary tissue below the cortical layer through which during the development of the fruit a well defined porus is formed (Fig. 11 *d*); this porus has already been observed by J. AGARDH, l. c. p. 13. From near the base of the fruit, but not always, some few filaments bend round the gonimoblast.

As will appear from this description of the development of the gonimoblasts, the genus *Titanophora*, as was already stated above, comes near to *Schizymenia*; compare KYLIN, 1930, pp. 38—40, figs. 25—27 the *Turnerella pacifica* described here being like *Schizymenia pacifica* Kylin, 1932, p. 10. *Titanophora* also agrees with *Schizymenia* by the presence of glandular cells and by the development of a porus. On the other hand the related genus *Platoma*, to which genus Mme WEBER referred the plant examined by her, has the gonimoblasts lying among the assimilating filaments and lacks the porus and the glandular cells.

The plant was at first collected by Colonel PIKE at Mauritius, but no locality is mentioned in DICKIE's paper. The specimens JADIN has collected and which I have had for examination are undetermined and without locality. In his list JADIN does not mention it.

To his section *Titanophora* of the genus *Halymenia* J. AGARDH further referred *Halymenia incrustans* J. Ag. from the West Indies; not having been able to examine any specimen of this species I am unable to express any opinion about it. On the other hand, I feel sure that the plant from New Guinea which the late Mme WEBER in her Liste, p. 253, calls *Platoma Pikeana*, considering it to be the same as the plant from Mauritius, cannot be DICKIE's species but another species of *Titanophora*.

Soon after I had begun the examination of the plant from Mauritius I felt some doubts about their connection, since it seemed to me that the habit figure of the plant as well as the anatomical figures of Mme WEBER showed essential differences

from that of the species from Mauritius. I therefore enquired of the Rijks Herbarium, Leiden, where, as is well known, the herbarium of Mme WEBER is kept, if it were possible for me to see for a short time a specimen of the plant in question. On account of the war the specimen could not be lent, but Director

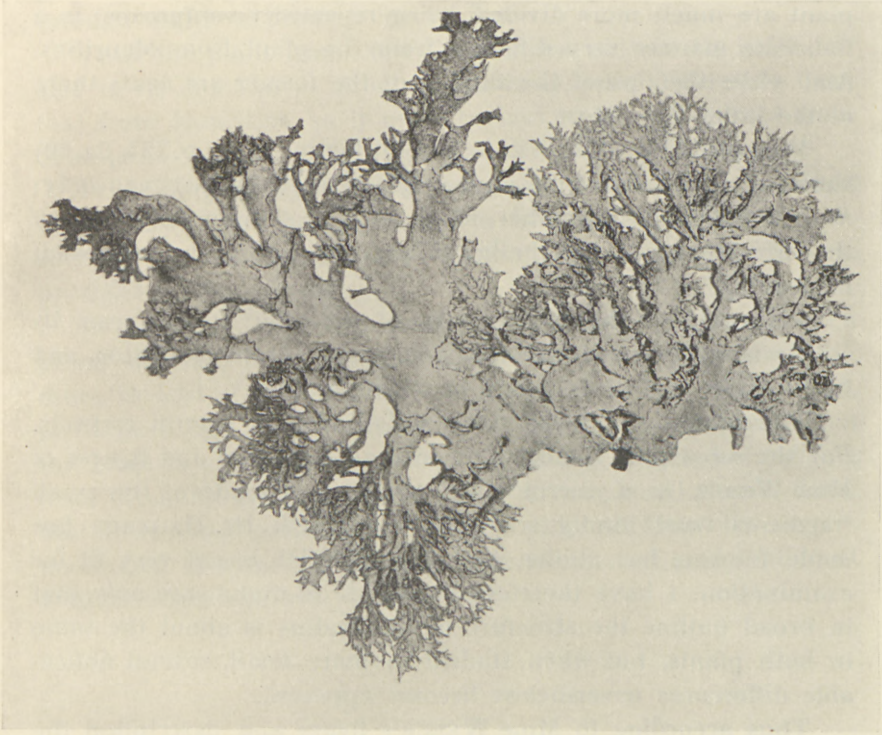

Fig. 13. *Titanophora Weberae* Børgs. A photo of the type-specimen in the Rijks Herbarium, Leiden. (About $^4/_5$ natural size).

LAM and Dr. J. TH. KOSTER had the great kindness to send me a photo of one of the two specimens of the plant found in the herbarium and further some fragments of it.

Fig. 13 gives an illustration of the plant. When compared with that from Mauritius the differences are disclosed. *Platoma Pikeana* Weber is much bigger and more robust and much more irregularly divided. Especially is the difference between the very broad main segments and the much divided upper parts of the thallus conspicuous. As is also observable in the photo of the plant,

small proliferations are present along the margins of the thallus which I have not seen in the plant from Mauritius.

The broad portions in the middle of the thallus of *Platoma Pikeana* Weber reach a breadth of up to 2 cm, thus they are much broader than those in the plant from Mauritius; on the other hand, the uppermost parts of the thallus in Mme Weber's plant are much more divided, being repeatedly subfurcated in a flabellate manner very different from the plant from Mauritius. And while the tips of the thallus of the former are acute those of the latter are obtuse.

Most probably Mme Weber's habit figure (Liste, p. 253, fig. 80) shows a small part of the upper right side of the specimen only; as her figure, on the other hand, does not show anything of the broad parts of the thallus it must be said to be somewhat misleading.

According to the small pieces of the plant I have seen, its colour is whitish red like that of the plant from Mauritius, and like this its surface is warty in a similar way.

The anatomy of both plants differs also in several respects. For the most part I must refer to the description and figures of Mme Weber, as a microtomical preparation made of the small fragments most kindly sent me, and which Dr. Hagerup has made for me, has shown that the material is not very fit for examination. I have therefore been able to make sure only that in broad outline the structure of the thallus is about the same in both plants, but when studied in more detail, several noticeable differences nevertheless become apparent.

Thus according to Mme Weber's figure and description the peripheric tissue is much more developed, consisting of well marked short filaments several times divided.

And the papilliform free ends of the assimilating filaments found in *Titanophora Pikeana* are not mentioned by Mme Weber; neither have I found them, but the epidermal cells are often much elongated, attaining a length twice that of their breadth.

Glandular cells very like those in the plant from Mauritius as to size and shape are also found in the cortical layer of the plant from New Guinea; their occurrence is not mentioned in the description of Mme Weber.

According to Mme Weber, the auxiliary cells originate from one of the lowermost cells in the assimilating filaments; compare her figure 82. The auxiliary cells are roundish and differ by this from the very irregularly shaped auxiliary cell of the plant from Mauritius. Fig. 83 of Mme Weber shows that the gonimoblast lies in the medullary layer below the cortical one, through which a porus is developed.

Thus the plant from New Guinea, not only in its habit but also in its anatomical structure shows essential differences from that from Mauritius, so it must be said to be well-defined from this species.

In memory of Mme Weber who first collected and described this stately plant I propose to call it ***Titanophora Weberae*** nov. spec., Syn. *Platoma Pikeana* Weber p. p., Liste Alg. Siboga, pp. 253—55, figs. 80—83.

In connection with the description of this species Mme Weber points out that most probably the *Halarachnion calcareum* Okamura in List of Mar. Alg. collect. in Carol. and Marian Isl., 1916, p. 13, pl. I, figs. 19—21 is the same as her *Platoma Pikeana*. That the plant is a *Titanophora* seems very likely to me, but judging from the habit figure and description of the plant I should think that it is most probably specifically different from the above-mentioned species; but to make sure of this an examination of authentic material will be necessary.

Fam. 2. Solieriaceae.

Sarconema Zanard.

1. Sarconema filiforme (Sonder) Kylin.

Kylin, *Gigartinales*, 1932, p. 22. Børgesen, Indian Rhodophyceæ, 1934, p. 11, fig. 7. — *Dicranema filiforme* Sonder in Bot. Zeitung, 1845, p. 56.

In the collection of Dr. Jadin there is a small specimen of a *Sarconema* from Réunion which agrees quite well with Indian specimens. The only difference I have found is that a transverse

section of the plant shows that the cells of the medullary layer are a little smaller than those in the Indian plant; compare my figure 7 l. c.

Réunion: Without locality, F. JADIN no. 176.
Geogr. Distr.: West Australia, India.

Solieria J. Ag.

Solieria natalensis (Reinb.) Børgs. nov. comb.

Rhabdonia natalensis Reinbold in TYSON, New South African Marine Algæ, 1912, p. 199.

Several undetermined specimens in JADIN's collection are, I think, referable to this species; but I have not been able to confirm the determination by means of authentic material.

The figure (Fig. 14) gives the habit of one of the specimens. The frond is terete, perhaps with the exception of the basal parts of the main branches which in the dried condition may reach a breadth of about 8 mm; higher up the main filaments are about 1 mm thick, tapering very little upwards.

As the figure shows, the plant has a rich but irregular pseudodichotomous ramification, forming a dense tuft. The branches are upward directed, the axes in the lower parts rounded, higher up more acute. The tips of the branches are obtuse, often also acute. Short spinelike adventitious branchlets are often given out; this was especially the case in the tetrasporic specimen.

The consistency of the dried plant is very firm and cartilaginous; its colour is dark red-brownish.

An examination of the tips shows that the development of the thallus takes place in accordance with the fountain type, characteristic of the Fam. *Solieriaceae*, compare KYLIN, 1932, p. 13. The structure of the thallus is upon the whole that known from other species of *Solieria*.

The large specimen (Fig. 14) is sterile, but one of the smaller ones is a female plant; the cystocarps occur scattered about the surface of the thallus. They are very protruding with an uneven wartlike surface; a transverse section shows that they are built

up in conformity with KYLIN's figure of that from *Solieria chordalis* (1932, p. 19, fig. 3 B).

Another specimen is tetrasporic; the zonately divided sporangia are formed in the cortical layer and are spread over the thallus.

Fig. 14. *Solieria natalensis* (Reinb.) Børgs. Some filaments from a large specimen prepared separately. (× 1).

REINBOLD points out that the habit of the plant comes near to KÜTZING's figure of *Gigartina flagelliformis* (Tab. Phycol., vol. 18, pl. 5, fig. c) and to *Trematocarpus elongatus* (vol. 18, pl. 4, fig. d). The plant from Mauritius, too, shows a very great resemblance to these figures.

JADIN in his list, p. 164, mentions a *Rhabdonia* spec. from Mauritius (no. 442); a small specimen of his has this number and is this species; on the other hand all the other specimens are unnumbered.

Mauritius: Mahébourg, Sept. 1890, JADIN no. 442.
Geogr. Distr.: South Africa.

Eucheuma J. Ag.

The late Mme WEBER, in her monograph on the Malayan Algæ, submitted the species of this very polymorphic genus to a thorough examination and found that as regards their anatomy the presence or absence of a central axis in the thallus is of much systematic value. As stated by Mme WEBER, J. AGARDH (Spec. Alg., vol. II, p. 624) had already observed this central axis in *Eucheuma muricatum* Gmel. (= *Euch. spinosum* J. Ag.).

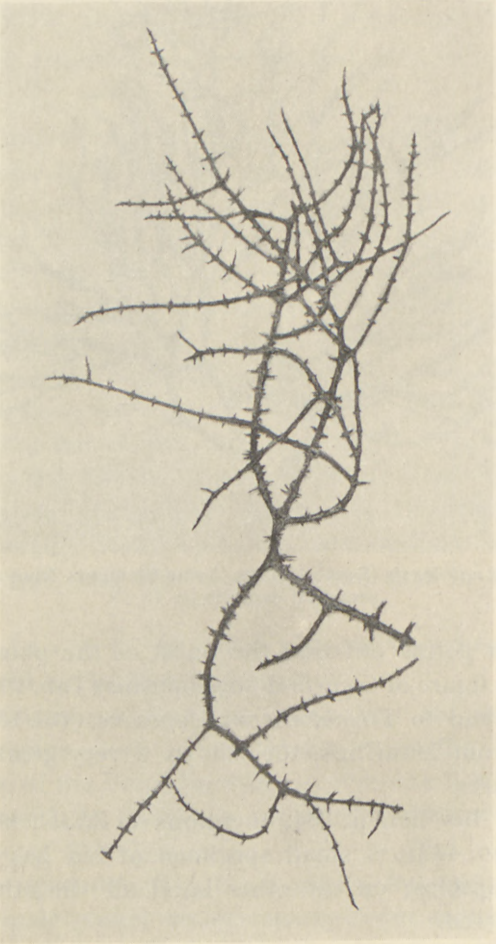

Fig. 15. *Eucheuma serra* J. Ag. Habit of a specimen (no. 854) near the typical form (× $^2/_3$).

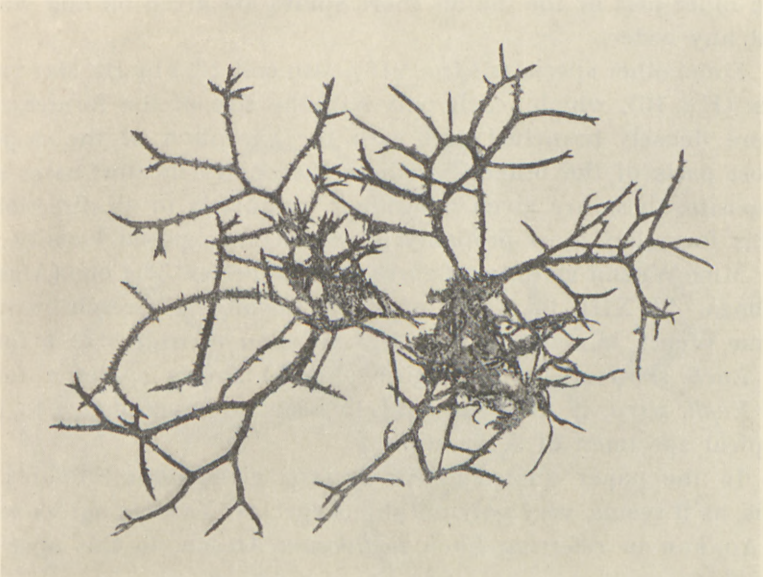

Fig. 16. *Eucheuma serra* J. Ag. A more densely branched and more densely spined form (no. 916) most probably from an exposed place. (× 2/3).

The species of *Eucheuma* from Mauritius which I have examined have all had a central axis with the exception of the specimens which I have with much doubt referred to *Eucheuma chondriforme* J. Ag.

Sectio I. Anaxifera.

1. Eucheuma serra J. Ag.

Agardh, J., Spec. Alg., II, p. 626; Epicr., p. 601. Weber-van Bosse, Algues Siboga, p. 411, pl. XIII, figs. 4–5. Kylin, *Gigartinales* p. 24, pl. 10, fig. 21. Yamada, Y., The species of *Eucheuma* from Ryûkyû and Formosa, 1936, p. 120, figs. 1–2, pl. 21–22.

In Dr. Mortensen's collection some specimens reaching a height of about 16 cm are found; they agree quite well with the original specimen of J. Agardh according to a photo published by Kylin, *Gigartinales*, 1932, pl. 10, fig. 21. In the specimens (nos. 853—54) (Fig. 15) the spines are nearly all opposite or, when more than two are present, subverticillate[1], lower down in

[1] Compare J. Agardh, Till Algernes Systematik, VII, Florideæ, p. 87.

the older part of the thallus more spines are given off and without any order.

Some other specimens (no. 916), also collected by Dr. MORTENSEN (Fig. 16), which reach only half the size of the former, are more densely branched and with the exception of the uppermost parts of the branches, where the spines in most cases are opposite, these are given out rather irregularly in all directions. This form has most probably grown in an exposed locality.

Mme WEBER gives two figures of this species. The one (Algues Siboga, Pl. XIII, fig. 4) is of *Euch. nodulosum* Aresch. which Mme Weber in agreement with J. AGARDH considers as a form of *Euch. serra*; the other figure (Fig. 5) shows a sterile form of *Euch. serra*, it must be said to bear no resemblance to the typical specimen of J. AGARDH.

In the paper quoted above YAMADA gives several figures of this, as it seems, very polymorphous species; YAMADA agrees with J. AGARDH in referring *Euch. nodulosum* Aresch. to this species.

Mauritius: Cannoniers Point, 25. Oct. 1929, TH. M.
Geogr. Distr.: Malayan Archipelago, Mauritius.

2. **Eucheuma nodulosum** Aresch.

ARESCHOUG, J. E., Phyceae novae, 1854, p. 22 (348). KYLIN, H., *Gigartinales*, p. 24, tab. 10, fig. 22. — *Eucheuma serra* forma *nodulosum* Weber, Liste Algues Siboga, p. 411, pl. XIII, fig. 4.

Of this species a fine cystocarpic specimen is found in the collection belonging to the Riksmuseum, Stockholm. J. AGARDH in Epicrisis, p. 601 considers this plant as the fertile form of *Euch. serra*, but here I follow KYLIN in regarding it as a separate species.

The material was not fit for examination of the anatomical structure.

Mauritius: LJUNGGREN, no locality or date.
Geogr. Distr.: Endemic.

3. **Eucheuma horridum** (Harv.) J. Ag.

AGARDH, J., Spec. Alg., p. 625; Analecta Algologica, p. 121. WEBER-VAN BOSSE, Algues Siboga, p. 412, tab. 16, fig. 3. KYLIN, *Gigartinales*, p. 23, tab. 9, fig. 19. — *Gigartina horrida* Harv., Alg. TELFAIR, no. 12, 1834, p. 152 (non *Sphaerococcus horridus* Ag.).

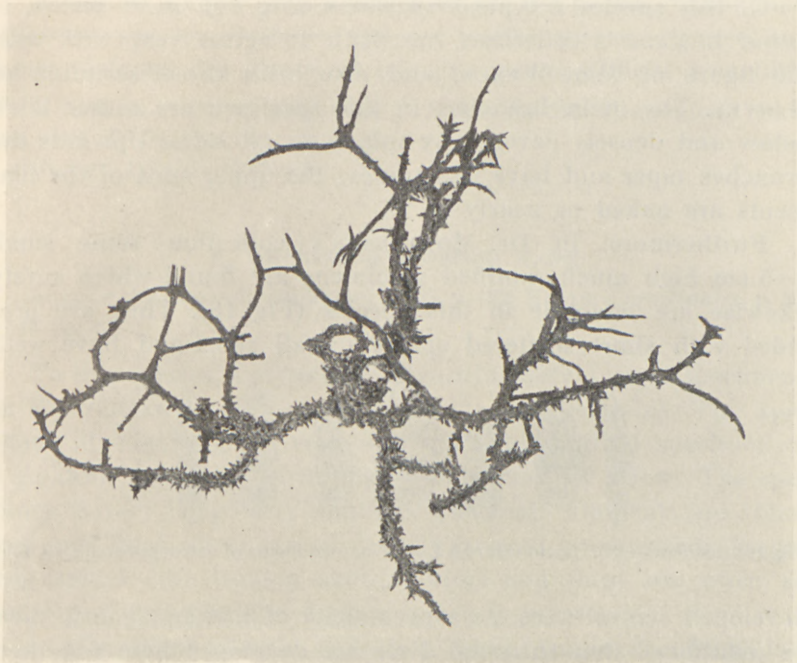

Fig. 17. *Eucheuma horridum* (Harv.) J. Ag. Habit of a most probably typical specimen, JADIN no. 316. (× 1).

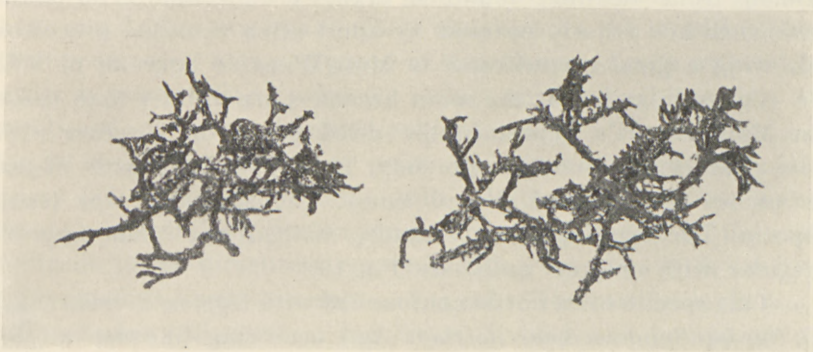

Fig. 18. *Eucheuma horridum* (Harv.) forma *radicans* nov. form. J. Ag. (× 1).

Of this species a typical specimen (Fig. 17), as it seems, is found in JADIN's collection (no. 316). It agrees very well with the figure of Mme WEBER, and also with the description of HARVEY. The main branches in this specimen are rather thick below and densely covered by spines on all sides. Upwards the branches taper and have less spines; the upper ends of the filaments are naked or nearly so.

Furthermore, in Dr. MORTENSEN's collection some small 4—5 cm high much ramified specimens are found which surely likewise are referable to this species (Fig. 18). They are provided with short scattered spines on all sides and have well-

Fig. 19. *Eucheuma horridum* (Harv.) J. Ag. Various forms of tetrasporangia. (× 350).

developed central axes. As a peculiarity of this small form must be mentioned that rhizoidal discs are developed here and there upon the thallus. Most probably this plant has formed a low dense covering upon the rocks, to which it becomes fixed by the discs; because of this peculiarity it may be called forma *radicans*.

Finally, but not without doubt, I refer to this species a specimen from Réunion found in JADIN's collection. The main branches are densely covered by short often ramified processes showing a great resemblance to Mme WEBER's figure 3, pl. XVI in Algues Siboga. But the main branches are thinner than those in Mme WEBER's figure; in the dried condition, together with the processes, about $2^1/_2$ mm only. The upper bare parts of the branches are furcated several times. The specimen was tetrasporic. The sporangia are zonately divided but often very irregular with oblique walls, see Fig. 19.

This species must not be confounded with *Hypnea horrida* (Ag.) J. Ag. (= *Sphaerococcus horridus* Ag.) occurring likewise at the island. HARVEY did so; he writes (l. c. p. 153): "In referring the specimens to AGARDH's "*Sph. horridus*", I have been perhaps a little guided by his reference, "ad insulam Franciae"."

About its habitat JADIN writes in his list p. 164: "Abondant, croissant sur les récifs en grosses touffes rosées, exposée aux lames violentes. La plante est très cassante et difficile à cuellir".

Mauritius: Flacq, July 1890, F. JADIN, no. 316. Cannoniers Point, Oct. 1929, TH. M. (forma *radicans*).

Geogr. Distr.: Endemic.

4. Eucheuma jugatum J. Ag.

AGARDH, J., Analecta algologica, 1892, p. 122. KYLIN, *Gigartinales*, p. 23, pl. 9, fig. 20.

To this species, known from Mauritius only, several specimens in MORTENSEN's and JADIN's collection are referable. The specimen of JADIN agrees very well with the original specimen of J. AGARDH according to the figure published by KYLIN. The specimen is very irregularly ramified. The main filaments are about 2—3 mm thick; they are all densely covered by shorter or longer thin spines (the longest about 3 mm) and these are often so densely placed that the thallus is nearly setose.

In Dr. MORTENSEN's collection some specimens from Isle Marianne are, I think, also referable to this species, the filaments in the older parts of the thallus being densely covered by long, thin, often curved spines. In the young parts of the thallus the spines are placed more scattered, often also opposite, and thus show some resemblance to *Eucheuma horridum*.

This species together with the above-mentioned 3 species have nearly the same anatomical structure. In the middle of the thallus a central axis composed of thin elongated cells with thick walls is found. The medullary tissue surrounding this axis is composed of large roundish-polygonal cells. These cells are largest innermost, becoming gradually smaller towards the periphery and at the same time getting thicker and thicker walls through which long prolongations from the lumen of the cell protrude in all directions, communicating by pores with similar prolongations from the neighbouring cells. The cortical layer consists of short filaments a few times divided and composed of ellipsoidal cells.

Whether the above-mentioned 4 species are actually anything but forms of a very polymorphous species is an open question

to me. I have had very little material and these species are no doubt in most cases described upon very scarce material, difficult to prepare as they are, and furthermore the variations of the plants under different external conditions have not been sufficiently taken into consideration.

Mauritius: Herb. F. JADIN (without locality). Isle Marianne, Oct. 29. TH. M. no. 788.

Geogr. Distr.: Endemic.

5. **Eucheuma odontophorum** nov. spec.

Thallus ca. 5 cm altus et ultra (?), perennis, ancipite-compressus, hic illic constrictus et subfurcatus, in marginibus dentosis.

Thallus in sicco sine dentibus ca. 3 mm latus, dentes triangulares acuti, ca. 1 mm longi.

Substantia in sicco corneo-cartilaginea.

Organa fructificationis desunt.

Mauritius: Off Flat Island, ca. 25 fathoms, Oct. 1929, TH. MORTENSEN.

In Dr. MORTENSEN's collection a small but characteristic species of this very polymorphous genus is found (Fig. 20). Most regrettably the material is sterile, but the structure is that of *Eucheuma*.

The plant is surely perennial with periodical growth which stops during the unfavourable season; when the growth begins a single or mostly two shoots are given off from the tips of the thallus from last year.

The thallus is markedly compressed, a transverse section of it being rhomboidal-lanceolate. Along the margins on both sides a row of acute teeth are present; the teeth, triangular of shape and, when dry, sharp and prickly, are nearly 1 mm long and the intervals between them about 1—1½ mm. In the dried condition the thallus without the teeth is about 3 mm broad; when saturated with water the breadth is about 6 mm and the thickness of the thallus about 2½ mm.

When dry, the colour of the plant is dark-red, and the consistence is corneous-cartilaginous, the surface being much shrivelled. And, as is mostly the case with dried *Eucheuma*, the thallus is more or less covered with salt incrustations.

A transverse section of the thallus shows the cortical layer to be composed of densely placed filaments several times furcated and consisting of oblong cells, the uppermost peripheral ones being longest and clavate of shape. Inwards the cells become gradually larger, polygonal, often with a tendency to be

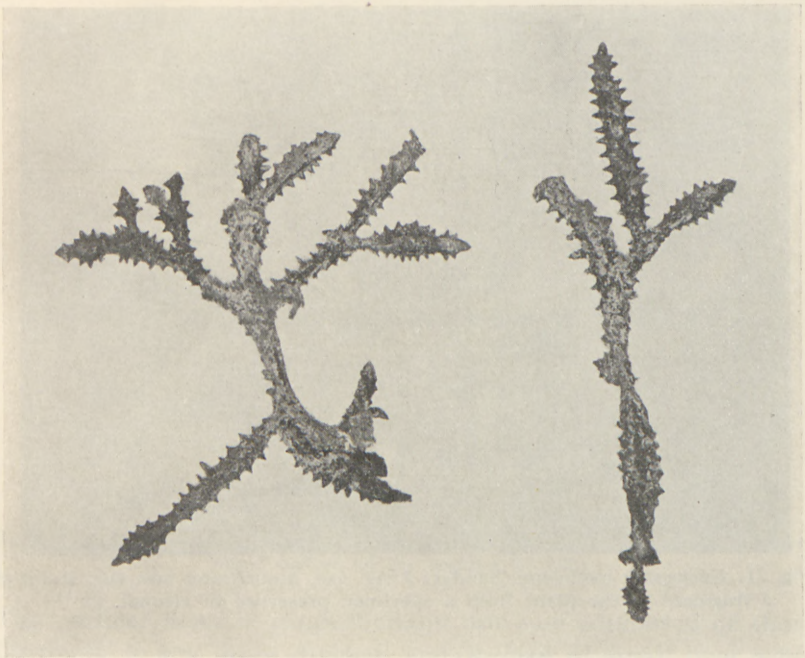

Fig. 20. *Eucheuma odontophorum* nov. spec. Two specimens. (× 1^1/$_3$).

stellate. These larger cells make an even transition to the medullary layer which is composed of roundish thick-walled cells, smallest towards the periphery, larger innermost; having a diameter of up to 200 μ. In the middle of the thallus a much compressed central axis is present, composed of densely crowded, small thick-walled cells, elongated in longitudinal section.

6. **Eucheuma speciosum** (Sonder) J. Ag.

AGARDH, J., Spec. Alg., II, p. 628; Epicr., p. 603. HARVEY, Phyc. Austr., tab. LXIV. — *Gigartina speciosa* Sonder, in Bot. Zeit., 1845, p. 55. *Gigartina ornata* Kütz., Tab. Phycol., vol. 18, pl. 6.

var. *mauritiana* n. var.

A var. *typica* præcipue differt thallo tuberculis robustis conicis plus minus dense obtecto.

Dredged near Gunner's Quoin and Flat Island at a depth of about 25 fathoms, TH. M. (Station 44), 1929.

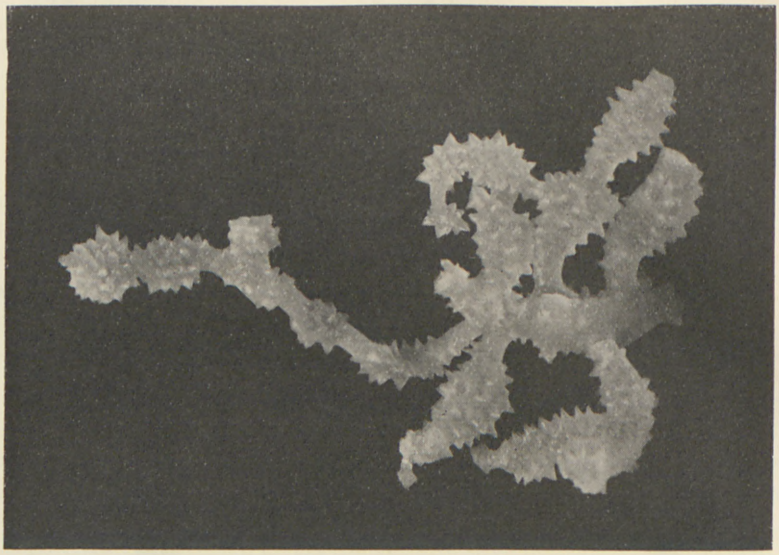

Fig. 21. *Eucheuma speciosum* (Sonder) J. Ag. var. *mauritiana* nov. var. Habit of a fragment of the plant from a specimen preserved in alcohol. (× 5/6).

A fine specimen (Fig. 21) preserved in alcohol is found in Doctor MORTENSEN's collection. In most respects it agrees fairly well with HARVEY's above-cited figure having a rather regularly constricted nodulose and somewhat compressed thallus, but when according to HARVEY's figure and description the thallus is said to be "beset on all sides with slender, setaceous, simple or branched processes", this description cannot be said to be in accordance with the plant from Mauritius. In this the thallus is densely covered by vigorous conical spines about $1^1/_2$—2 mm long together with the spines attaining a breadth of nearly 1 cm. Now and then short pieces of the thallus are left bare without spines. Slender, simple or branched processes do not occur.

A transverse section of the thallus is oval. The plant has a central axis; round this there is a medullary layer composed

of large thick-walled cells. As was the case in the above-mentioned species, the cells of the medullary layer decrease in size towards the periphery, at the same time getting very thick walls through which prolongations from the lumen of the cells communicate with those issuing from the neighbouring cells (Fig. 22).

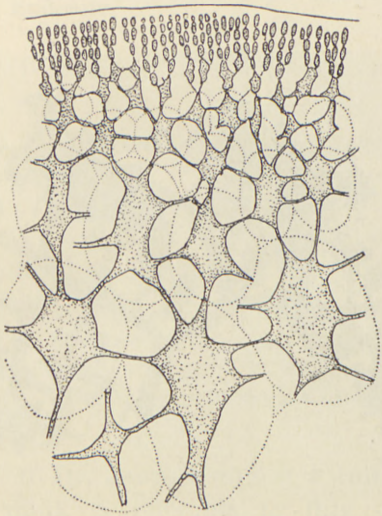

Fig. 22. *Eucheuma speciosum* (Sonder) J. Ag. var. *mauritiana* nov. var. Transverse section of the thallus. (× 225).

The cortical layer is proportionally thin and composed of short filaments, a few times divided, and formed of small oval cells.

JADIN, in his list (p. 164) mentions *Eucheuma speciosum* from Mauritius and gives as localities: Flacq and Mahébourg.

About its habitat he says: "Sur les récifs en grosses touffes rosées, mais cette espèce est assez moins abondante que la précédente" (*Eucheuma horridum*).

Geogr. Distr.: *Eucheuma speciosum* is known from Mauritius, Australia, Tasmania.

Sectio II. Anaxifera.

7. Eucheuma condriforme J. Ag.

AGARDH, J., Till Algernes System., VII, Florideae, p. 86; Analecta algol., 1892, p. 125. KYLIN, *Gigartinales*, p. 24, pl. 11, fig. 26.

This species has been described upon specimens from Mauritius by J. AGARDH. I have not seen any authentic material of the plant.

In JADIN's collection two small undetermined specimens (compare Fig. 23) are found which might show some likeness to the photo of the original specimens published by KYLIN; but essential

Fig. 23. *Eucheuma chondriforme* J. Ag. One of the specimens in JADIN's collection. (× 1).

differences are present; for instance the main sections of the thallus are much narrower than those found in the original specimens. In some respects the specimens show a fair resemblance, too, to the West Indian species *Eucheuma Gelidium* J. Ag., according to KYLIN's figure of this species, l. c., pl. 11, fig. 24.

A transverse section of the thallus shows a medullary tissue of roundish thick-walled cells reminding one very much of that of *Gracilaria*. In one specimen, that pictured in Fig. 23, the main axis is flattened, in the other subterete. No central axis is present. Whether any central axis is present in the authentic specimen is not mentioned by KYLIN. On the other hand, KYLIN points out that the construction of the cystocarps of this species seems to agree better with that of *Sarcodia*. The specimens I have seen are sterile, and as they were most probably cast ashore, they are not very fit for anatomical examination. But

as said above, the reference of the two small specimens I have seen to this species is not to be relied upon; meanwhile, since this species was originally based upon specimens from Mauritius I have mentioned it in the list. The specimens of JADIN have no locality.

Geogr. Distr.: Endemic.

Fam. 3. ***Rhodophyllidaceae.***

Gelidiopsis Schmitz.

1. **Gelidiopsis intricata** (Ag.) Vickers.

VICKERS, A., Algues Mar. Barbade, 1905, p. 61. FELDMANN, J., Remarques etc., 1931, p. 157. — *Sphaerococcus intricatus* C. Ag., Spec. Alg., p. 333. *Gelidium intricatum* Kütz., Spec. Alg., p. 767. SETCHELL, W. A., American Samoa, 1924, p. 163, fig. 31. *Acrocarpus intricatus* Kütz., Tab. Phyc., vol. 18, p. 12, pl. 35.

The collection of Dr. JADIN contains a tuft of a small *Gelidiopsis* which I think is referable to this species originally described upon a specimen from Mauritius.

However, the description of the species is rather defective

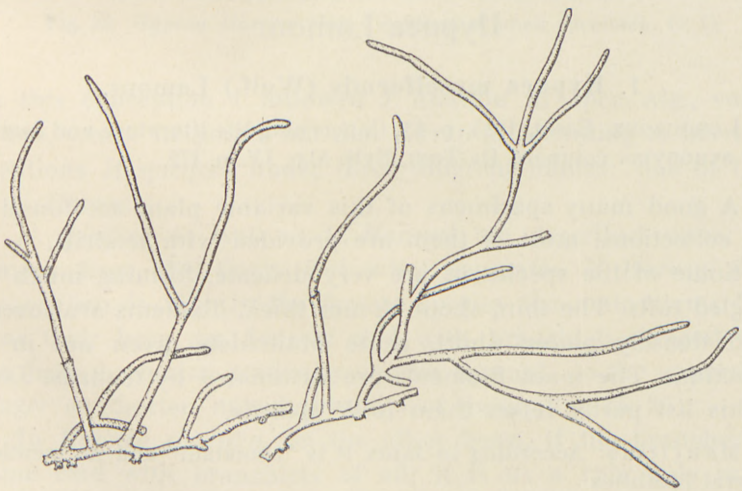

Fig. 24. *Gelidiopsis intricata* (Ag.) Vickers. Parts of the thallus. (About × 5).

and as KÜTZING's figure also is somewhat schematic I give here a figure of the plant (Fig. 24). This forms a low, about $^1/_2$—$^3/_4$ cm high, tuft formed by the densely crowded and very intricate thin filaments. The plant has creeping, decumbent filaments from which the erect ones arise. The filaments are nearly terete; their diameter is as a rule from 150—200 μ long, but may in rare cases attain up to 280 μ. The filaments are much curved and bent and the ramification is scattered and irregular.

The surface cells are rather large, about 8—12 μ long and 4—6 μ broad. This agrees with the size of the surface cells SETCHELL (l. c. p. 163) found in specimens from Tutuila Island. I have not seen any kind of fructification but SETCHELL has found tetrasporangia born on short conical branchlets; compare his figure 31.

Mauritius: Without locality in Herb. JADIN.

Geogr. Distr.: Seems to be widely distributed in the Indian and Pacific Ocean, West Indies.

Fam. 4. ***Hypnaceae.***

Hypnea Lamour.

1. **Hypnea musciformis** (Wulf.) Lamour.

LAMOUROUX, Essai, 1813, p. 43. Concerning the literature and numerous synonyms compare DE-TONI, Syll. Alg., IV. p. 472.

A good many specimens of this variable plant are found in the collections, most of them are provided with tendrils.

Some of the specimens are very delicate, forming much entangled tufts. The thin, about $^1/_2$ mm thick, filaments are covered with densely placed short, acute branchlets given out in all directions. The main filaments are terminated by tendrils. JADIN in his list partly refers them to *H. spinella*.

Mauritius: According to JADIN it is "abondant" and he mentions several localities.

Geogr. Distr.: Widely distributed in warm seas.

2. Hypnea Harveyi Kütz.

Kützing, F., Spec. Alg., p. 760; Tab. Phycolog., vol. 18, pl. 28, figs. a, b, c

Some small specimens (Fig. 25) in Jadin's collection agree very well with Kützing's figure of this species in Tab. Phycologicae. In my paper on North Indian Algae, 1934, p. 18, I referred this species as a synonym to *H. spicifera* (Suhr.) Harv.

Fig. 25. *Hypnea Harveyi* Kütz. Habit of a small specimen. (× 1).

In this conception I followed J. Agardh in Spec. Alg., vol. II, p. 445, where he points out that Kützing, according to his view, mentions *H. spicifera* under three different names; one of these is *H. Harveyi.*

H. spicifera as well as *H. Harveyi* are described upon specimens from the Cape. But what separates *H. Harveyi* from *H. spicifera* is that in the former species the main stems from near their base are densely clad with branchlets, longest near the base, becoming gradually shorter upwards to the uppermost, longer or shorter, naked summits of these (Fig. 25, 26).

In *Hypnea spicifera,* on the other hand, if the branches become clad with branchlets at all, it is as a rule only in the upper parts of the main branches that longer or shorter parts

become covered by branchlets. These branchlets are all of nearly the same length, often unilaterally placed and often interrupted by naked interstices.

Thus these species are in reality easily separated.

JADIN in his list p. 166 calls it *H. spicigera* Harv. and writes about its habitat: "Croissant sur les rochers là où la vague est très forte".

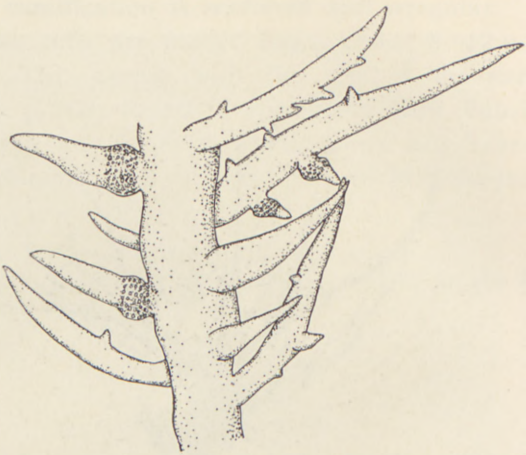

Fig. 26. *Hypnea Harveyi* Kütz. A fragment of the thallus of the plant figured in Fig. 25. (About × 12).

Mauritius: Mahébourg, Sept. 1890, JADIN no. 466. Flacq, Sept. 1890, JADIN no. 485.

Geogr. Distr.: Cape.

3. **Hypnea charoides** Lamx.

LAMOUROUX, J., Essai, 1813, p. 44, pl. 10, figs. 1—3. WEBER-VAN BOSSE, Alg. Siboga, p. 449, figs. 188, 189. TANAKA, *Hypnea* from Japan, 1941, p. 243, fig. 16. — *Halymenia seticulosa* J. Ag., Spec. Alg. II, p. 446.

I have referred to this species several specimens in JADIN's collection, basing the determination upon LAMOUROUX's above-quoted figure only; it has not been possible for me to compare the specimens with any authentic material. The specimens also show much likeness to Mme WEBER's figure 188—89, p. 450 of a form which she calls var. *indica*, but they are often still more densely ramified. This for instance was the case in one of the

specimens (Fig. 27), the branches and branchlets forming nearly compact roundish bundles up along the main filaments. In Fig. 28 a small piece of a branch from such a bundle is pic-

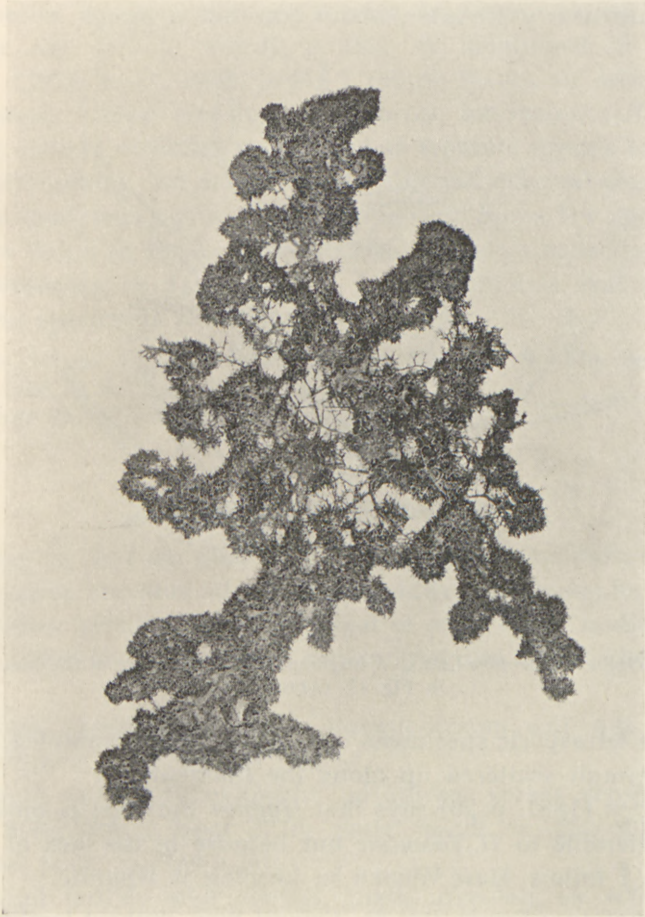

Fig. 27. *Hypnea charoides* Lamx. Habit of a specimen. (× 1).

tured; it shows the irregularly alternating ramification and the more or less divaricate branchlets.

This much ramified form is surely the same as that which HARVEY refers to in Alg. TELFAIR, p. 153 as *Hypnea musciformis* Lamour., β *ramulosa*. Compare also J. AGARDH, Spec. Alg., vol. II, p. 448. I base this statement upon a specimen from Mauritius

collected by Colonel PIKE and found in the collection belonging to the Riksmuseum, Stockholm. This specimen was determined by DICKIE as *Hypnea divaricata*.

The most densely ramified specimens are female. The cystocarps are nearly globular, about 500—600 μ broad.

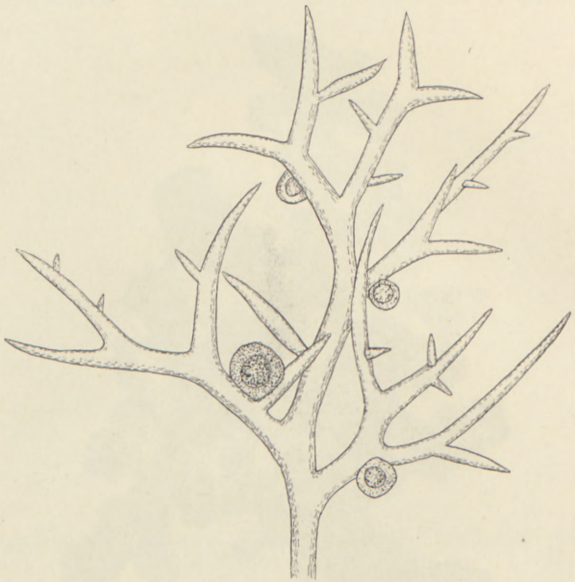

Fig. 28. *Hypnea charoides* Lamx. A fragment with cystocarps of the plant figured in Fig. 27. (About × 10).

The tetrasporic specimens are not so densely ramified, having short ramuli scattered up along the filaments.

HAUCK (1887, p. 20) says that *Hypnea charoides* is most probably referable to *H. Valentiae* but because of the lack of stellate bulbils I follow Mme WEBER in keeping it separate.

Mauritius: Cassis, Dec. 21., Colonel PIKE. Without locality, Herb. JADIN.

Geogr. Distr.: Indian Ocean, Australia, Japan etc.

4. **Hypnea Valentiae** (Turn.) Mont.

MONTAGNE, C., Plantes cellulaires. 1840, p. 161. AGARDH, J., Spec. Alg., vol. 2, p. 450. HAUCK, Ueber einige von J. M. HILDEBRANDT etc., 1886, p. 20. BØRGESEN, Contributions, I, 1937, p. 47. — *Fucus Valentiae* Turner, Fuci, pl. 78. *Hypnea musciformis* γ *Valentiae* Harvey, Alg. TELFAIR, p. 153.

Of this variable species Dr. MORTENSEN has collected several large specimens. They form much ramified bushes up to 30 cm high, with numerous branches and branchlets, all with acute tips. All over the thallus stellate small bulbils characteristic of this species are present in great numbers; compare my fig. 387 in Mar. Alg. D.V.I., vol. II, p. 382. As mentioned in former papers (1934, p. 17; 1937, p. 47) I follow HAUCK in referring to this species several closely related forms, for instance *H. hamulosa, H. cornuta* etc. often considered as separate species but most probably nothing but forms due to different external conditions.

The specimens are sterile or tetrasporic. Some few specimens are also found in Dr. JADIN's and Dr. VAUGHAN's collections, and it is mentioned in JADIN's list p. 165, where it is said to be a common species at the island.

Mauritius: Cannoniers Point, Oct. 1929, TH. M. Flic en Flacq, 31. Dec. 1938, R. E. V., no. 258.

Geogr. Distr.: Most warm seas.

5. **Hypnea bryoides** nov. spec.

Frons ca. 2—3 cm alta, ex filamentis decumbentibus, repentibus, hapteris brevibus ad substratum adfixis et filamentis erectis, simplicibus, teretibus, ramuliferis aut in parte basali nudis, prope basem 1 mm latis, ad apicem versus gradatim tenuioribus composita.

Ramuli sparsi, irregulariter quoqueversum orti, aut steriles aut fertiles.

Ramuli steriles subcylindrici ad apicem versus sensim attenuati, 600—1800 μ longi et in parte basali ca. 250—300 μ, in superiori parte ca. 150 μ lati.

Ramuli fertiles inter steriles mixti, urceolati, ca. 400—500 μ lati, tetrasporangia per totam superficiem plus minus abundanter praesentia.

Cystocarpia et antheridia non visa.

Mauritius: Flat Island, 17. Oct. 29, TH. MORTENSEN legit.

This handsome, delicate *Hypnea* (Fig. 29), of which I have seen only a single specimen, forms a low tuft about $2^1/_2$ cm high. It is fixed to the substratum by means of decumbent creep-

Fig. 29. *Hypnea bryoides* Børgs. Habit of the plant. (× 2).

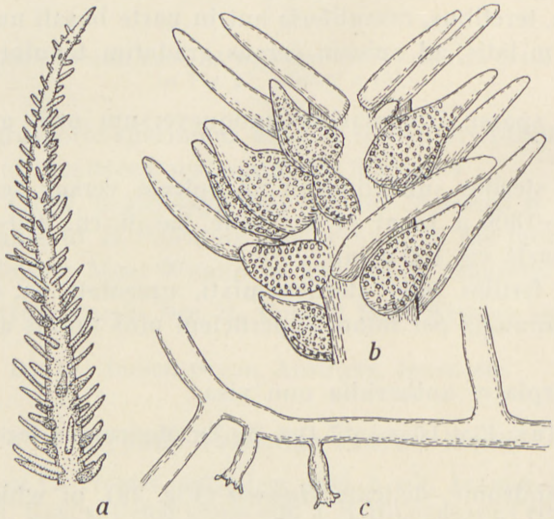

Fig. 30. *Hypnea bryoides* Børgs. *a*, upper part of a filament. *b*, fragment of the thallus with stichidia. *c*, part of a creeping filament. (*a* and *c* × 6, *b* × 30).

ing filaments from which short hapters are given out downwards and erect shoots upwards (Fig. 30 *c*). The erect shoots are terete, unbranched, stemlike and rather straight. Near the base their diameter is up to about 1 mm long; upwards they taper slowly to the summits.

The erect filaments are densely clad all round with short sterile branchlets intermingled with sporangiferous ones (Fig. 30 *a*, *b*). The sterile branchlets are subcylindrical, about 600 to 1800 μ long; near the base their breadth is about 250—300 μ, tapering gradually upwards to about 150 μ. Near the base they are a little narrowed and their tips are obtuse. They are in most cases unbranched, only a very few times have I seen a branchlet provided with a short side-branch.

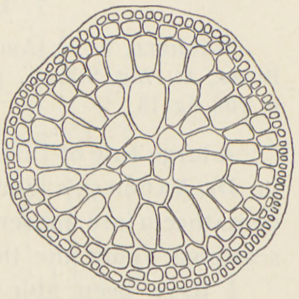

Fig. 31. *Hypnea bryoides* Børgs. Transverse section of erect filament. (× 60).

The sporangiferous bodies (Fig. 30 *b*) are short, bottle-like, and more or less oblique; from the base their breadth increases very quickly to about 400—500 μ, whereupon they taper gradually, ending in a slender neck about 150 μ broad.

A transverse section of the thallus shows in the middle some few cells with a narrow lumen surrounded by larger cells which gradually decrease in size towards the periphery. The cells of the medullary layer are rather thick-walled.

I do not know any species of *Hypnea* resembling this little plant.

6. **Hypnea pannosa** J. Ag.

Agardh, J., Alg. Liebmann, p. 14; Spec. Alg., II, p. 453. Kützing, Tab. Phycol., vol. 18, pl. 27. Weber-van Bosse, Algues Siboga, p. 455. — *Hypnea musciformis* λ *cornuta* Harv., Alg. Telfair, 1834, p. 154.

Some specimens collected by Dr. Mortensen quite agree with the type-specimen of this species in the Botanical Museum, Copenhagen. Besides these specimens I have seen a well-prepared tuft in Dr. Vaughan's collection, and a small one from Réunion (no. 63) in Dr. Jadin's collection.

It is mentioned in Jadin's list of Algae p. 165, and about its

occurrence he writes: "Forme un gazon de coloration rose-irisé sur les rochers et sur les fonds sablonneux, dans les rigoles creusées dans les coraux".

Mauritius: Cannoniers Point, Oct. 1929, TH. M. Barkly Island, Aug. 1939, R. E. V. no. 335.

Geogr. Distr.: Pacific and Indian Oceans.

7. **Hypnea nidulans** Setch.

SETCHELL, W. A., American Samoa, 1924, p. 161, fig. 30. WEBER-VAN BOSSE, A., Alg. Siboga, p. 455, fig. 192. TANAKA, *Hypnea* from Japan, p. 246, fig. 18.

A large tuft of this alga intermingled with *Ceramium* is found in Dr. VAUGHAN's collection. The specimen is tetrasporic. The nemathecia are present in great number as saddlelike cushions scattered about the thallus.

I have been able to compare the specimen with a cotype specimen from Tutuila (no. 1084) which Professor SETCHELL has most kindly sent me and found that the plant from Mauritius agrees perfectly with the Samoan plant.

SETCHELL has pointed out that HARVEY's Friendly Island Alga no. 44 distributed as *Hypnea pannosa* is in reality this species. An examination of a specimen of HARVEY's plant found here in the Botanical Museum shows that this plant has some more short spines scattered upon the thallus than is the case in the specimens from Mauritius and Tutuila Island.

Mauritius: Pointe aux Sables, Aug. 1939, R. E. V. no. 342.

Geogr. Distr.: Samoa, Friendly Islands, Japan, Malayan Archipelago etc.

8. **Hypnea (?) horrida** (Ag.) J. Ag.

AGARDH, J., Nya Alger fr. Mexico, 1847, p. 14; Spec. Alg., vol. II, p. 454; Epicrisis, p. 565. — *Sphaerococcus horridus* Ag., Spec. Alg., 1821, p. 322; Systema Alg., 1824, p. 237. *Gigartina horrida* Grev., Alg. Brit., 1830, p. LIX.

Of this plant some specimens, most probably cast ashore, are found in JADIN's collection. As usual the specimens are sterile, this species has not yet been found fruiting, and its systematic position is therefore uncertain.

So as to be able to examine the anatomical structure of the plant I saturated some pieces of the thallus in water and I then noticed that the thallus appeared to have distinct transverse stratifications. A longitudinal section (Fig. 32 *b*) of the thallus showed that this peculiarity was due to the fact that the nearly cylindrical cells of the medulla are all of the same length and arranged in layers above each other. A transverse section of the

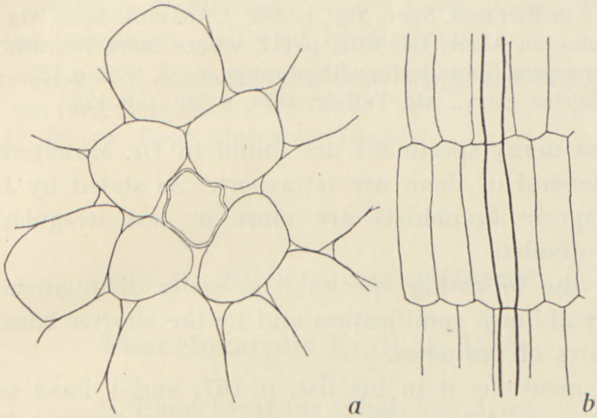

Fig. 32. *Hypnea horrida* (Ag.) J. Ag. *a*, transverse section of the thallus. *b*, longitudinal section of the same. (*a* × 60, *b* × 25).

thallus (Fig. 32 *a*) shows that the cells are roundish, and decrease in size from the middle towards the periphery of the thallus. In the middle of the thallus a slender central axis is found, composed of a single filament, the cells of which have the same length as those of the medullary layer. The walls of the central axis are somewhat undulated and rather thick.

In the two specimens I have been able to examine the length of the cells was different, in one specimen about 1300 μ, in the other about 1000 μ; their diameter was about 200—300 μ. The central cell had a diameter of about 100 μ.

Hypnea horrida is mentioned in JADIN's list p. 166. About its habitat at the island he writes: "Croît en grosses touffes très cassantes sur les récifs. Souvent au voisinage des *Eucheuma*".

Mauritius: Flacq, Sept. 1890, JADIN no. 438, 462 bis.
Geogr. Distr.: Endemic.

Fam. 5. Plocamiaceae.

Plocamium (Lamour.) Lyngb.

1. **Plocamium Telfairiae** Harv.

Harvey in Kützing, Spec. Alg., p. 885. J. Agardh, Spec. Alg., II, p. 400. Yendo, Notes on Algae, III, 1915, p. 111, where more literature is mentioned. Børgesen, Some Indian Rhodophyceae, 3, 1933, p. 123. — *Thamnophora Telfairiae* Harv.. Alg. Telfair, 1834, p. 149, tab. 125.

A good many specimens are found in Dr. Mortensen's collection. Several of them are tetrasporic. As stated by J. Agardh the tetrasporic branchlets are more or less irregularly, often stellately divided.

From the following species it is easily distinguished by its more corymbiform ramification and by the shorter basal pinnule in the pairs of branches.

Jadin mentions it in his list, p. 167, and I have seen some few specimens of his collection. About its habitat at the island he writes: "Croissant soit sur des récifs soit sur des rochers avançant en pointe au niveau des récifs; toujours exposé aux vagues très violentes".

As is well known, Mauritius is the type-locality of this species, which is widely distributed in the Indian Ocean.

This species is no doubt common at the island. In Jadin's and Mortensen's collections I have seen specimens from Flat Island, Isle Marianne, Flacq and Mahébourg.

Geogr. Distr.: Mauritius, New Zealand, Tasmania, New Holland, Japan, India (Karachi).

2. **Plocamium cornutum** (Turn.) Harv.

Harvey, Nereis Australis, 1847, p. 123. J. Agardh, Spec. Alg., II, p. 404. — *Fucus cornutus* Turner, Fuci, pl. 258. *Thamnocarpus cornutus* Kütz., Phycol. Gener., p. 450, tab. 59, fig. 3; Tab. Phycol., vol. 16, tab. 55.

Several specimens are found in Dr. Mortensen's collection. From the above-mentioned species, with which it agrees in having two pairs of branchlets alternating up along the main

stems on both sides, it differs by its more robust habit, its more erect main branches, which keep nearly the same breadth upwards due to the fact that the uppermost ramified branchlet have all nearly the same length, and finally because the lowermost undivided branchlets have a length nearly double that in *Ploc. Telfairiae*. KÜTZING's above-quoted figure in Tabulae gives a good illustration of the plant.

This species is mentioned in JADIN's list, p. 167; I have seen two of his specimens, collected by DARUTY.

Mauritius: Isle Marianne and Flat Island, Oct. 1929, TH. M. Ilôt Gabriel, Mai 1874, DARUTY.

Geogr. Distr.: Cape, Mascarene Islands.

Fam. 6. ***Sphaerococcaceae.***

Phacelocarpus Endl. & Dies.

1. **Phacelocarpus tristichus** J. Ag.

AGARDH, J., Till Algernes System., VII. *Florideae*, p. 57.

Some small specimens are found in the collections. This is the smallest and most graceful of all known *Phacelocarpus* (Fig. 33).

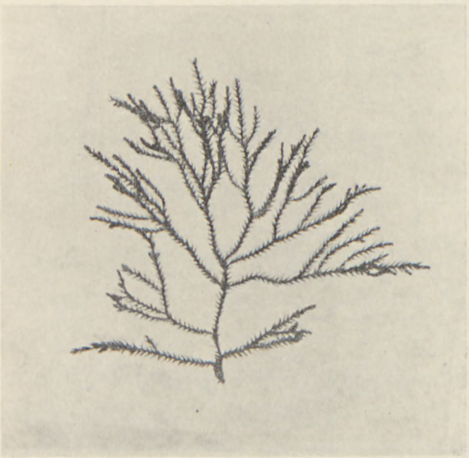

Fig. 33. *Phacelocarpus tristichus* J. Ag. Habit of the plant. (× 1).

The conical and somewhat incurved pinnae are tristichously arranged and are longer than the breadth of the stem-like part of the thallus.

The specimens are sterile.

Mauritius: Ilôt Brocus, "washed into the lagoon", Aug. 1938, R.E.V. Mahébourg, Sept. 1890, JADIN no. 463.

Geogr. Distr.: Endemic.

Fam. 7. Sarcodiaceae.

Sarcodia J. Ag.

1. Sarcodia ceylanica Harv.

HARVEY, W. H., Alg. Ceylon Exsicc., no. 27. J. AGARDH, Spec. Alg., vol. III, p. 431. KÜTZING, Tab. Phycol., vol. 19, p. 12, pl. 33 a, b. KYLIN, Gigartinales, 1932, p. 56. BØRGESEN, Alg. Ceylon, 1936, p. 85.

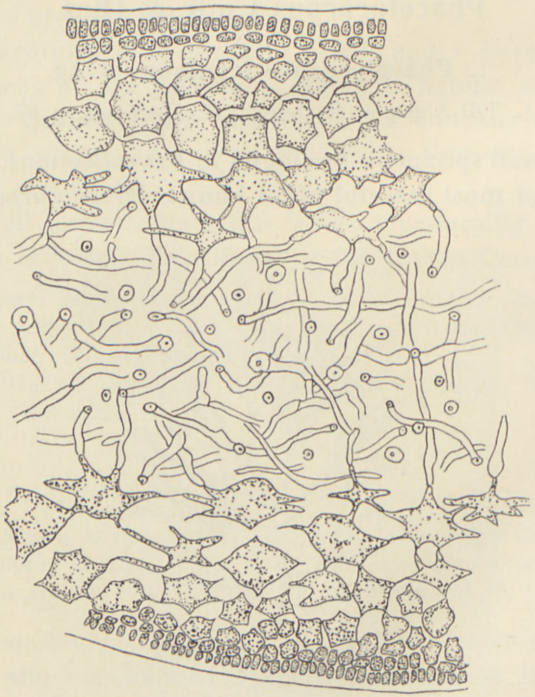

Fig. 34. *Sarcodia ceylanica* Harv. Transverse section of the thallus. (About × 200).

The collections contain several specimens. A transverse section of the thallus shows that in the peripheric part of the medullary layer the cells found there are stellate (Fig. 34), a peculiarity which, as pointed out by KYLIN, is found also in *S. Montagneana* and *S. Gattyae.*

The elongated transversely divided sporangia are developed in the cortical layer.

In the gonimoblasts their base and interior is composed of a parenchymatic tissue of rather large stellate cells in direct connection with the stellate cells in the medullary layer; from this tissue the gonimoblastic filaments radiate in all directions. The much protruding fruits have a thick wall with a well-developed porus above. In Icones of Japanese Algae, vol. IV, no. VI, 1921 OKAMURA in Plate 178, fig. 10 gives a figure of a longitudinal section of a cystocarp of *Sarcodia Montagneana* which agrees quite well with that of the plant I have examined.

About its habitat JADIN, p. 164 writes: "Assez abondante; cette plante croît sur les rochers volcaniques recevant de grosses lames ou soumis à des courants violents. La plante est toujours recouverte, même aux marées basses".

Mauritius: Pointe aux Roches, Febr. 7, 1939, R. E. V. no. 262. Flacq, 1890, JADIN no. 204, 207.

Geogr. Distr.: Ceylon, Japan etc.

Fam. 8. ***Gracilariaceae.***

Corallopsis Grev.

1. **Corallopsis Opuntia** J. Ag.

AGARDH, J., Epicr., p. 409. — *Corallopsis Cacalia* Harv., Alg. Ceylon, exsicc. no. 30.

In JADIN's collection some few typical specimens are found and further a single one which has only very scarce annular constrictions and therefore reminds one of *Gracilaria crassa* (Harv.) J. Ag. As I have pointed out in my paper on algæ from Ceylon

(1936, p. 86) I do not think it very improbable that *Corallopsis Opuntia* and *Gracilaria crassa* are forms of the same species influenced by different external conditions.

In this connection I may mention that when visiting the Kew Herbarium shortly before the war I examined the 3 specimens of *Corallopsis Cacalia* Harvey, Alg. Ceyl. exsicc. no. 30 found in the algal collection. In my opinion the two specimens of these are more poorly developed specimens of *Gracilaria crassa*, most probably plants which have grown out of the optimum of the occurrence of this species; the third one, on the other hand, is a bleached, most probably washed-up specimen, quite unlike the two above-mentioned specimens. This latter one answers very well to J. Agardh's description of *Corallopsis Cacalia*.

Among the material of *Gracilaria crassa* in the Kew Herbarium, on the other hand, some specimens of Harvey (Alg. Ceylon exsicc. no. 29) are quite typical. But some other specimens from Ceylon (Fergusson, Alg. Ceyl. no. 121) referred to this species are more poorly developed and more like the two specimens of Harvey mentioned above, which I think are intermediate forms between the two species in question. But to be able to decide the above mentioned supposition finally an examination of living material in situ is necessary.

In his list p. 164 Jadin mentions this species; about its habitat he writes: "Recueilli sur la plage après un gros temps; doit être assez rare".

Mauritius: Flacq, July 1890, Jadin no. 245. The specimens I have seen were collected by Daruty in 1894.

Geogr. Distr.: Ceylon, Malay Archipelago, Mauritius etc.

Gracilaria Grev.

1. Gracilaria lichenoides (L.) J. Ag.

Agardh, J., Spec. Alg., p. 588; Epicr., p. 412. — *Fucus lichenoides* L., Turner, Hist. Fucorum, tab. 118 a.

Dr. Vaughan's collection contains a single but typical specimen; it is a female specimen with cystocarps scattered about the thallus. A transverse section of the thallus shows a rather

thick cortical layer of small cells becoming gradually larger innermost, and surrounding a medulla of large cells.

Some undetermined specimens in JADIN's collection are, I think, also referable to this species.

Mauritius: Flic en Flacq, R. E.V., no. 249, Dec. 31., 1938, "attached to coral debris in lagoon". Without locality, JADIN.

Geogr. Distr.: Red Sea, Indian Ocean.

2. **Gracilaria arcuata** Zan.

ZANARDINI, J., Plant. Mar. Rubr., 1858, p. 57, tab. III, fig. 2. FELDMANN, J., Note sur quelq. Alg. mar. de Tunisie, 1931, p. 14, figs. 4–6.

var. *Snackeyi* Weber, Liste Alg. Siboga, 1928, p. 430, fig. 173.

A small much bleached and most probably washed-up specimen in Dr. JADIN's collection is, I presume, referable to the above-mentioned variety of this species.

It forms a dense much ramified and intricate roundish tuft. The filaments are arcuate and the branches issue more or less secundly; towards the summits of the filaments the ramification becomes irregularly furcated and divaricate; the apices of the branchlets are acute. Mme WEBER's figure gives a good illustration of the plant.

The specimen is a female plant with some few cystocarps. ZANARDINI has not found fruiting specimens; but in the Mediterranean Sea FELDMANN (1931, p. 14, figs. 4, 6) has collected cystocarpic specimens of a plant which he refers to ZANARDINI's species, and points out as a character of this species that the cystocarps are large and ballshaped; in the plant from Mauritius the few and surely rather young cystocarps are also rather large.

As to the anatomical structure of the plant from Mauritius, a transverse section of the thallus shows much likeness to that of FELDMANN. The peripheric cells of the cortical layer are radially elongated like those of the plant from the Mediterranean Sea, but about 18 μ long only, thus somewhat smaller than those of FELDMANN's plant; on the other hand, the cells of the interior of the medullary layer had a diameter of about 500—600 μ, the same size that FELDMANN gives for his plant. For the rest, when the transverse section of the plant from the Red Sea (comp. ZANARDINI's figure 2 a) is considered, the cells of the

medullary layer seem to be smaller than those of the plant from Mauritius and the Mediterranean Sea. Mme WEBER does not mention the anatomy of the Malayan plant.

In his list p. 165 JADIN mentions *Gracilaria radicans* Hauck as occurring at the island. In his collection I have seen two small specimens (nos. 218 and 424) referred to this species. As

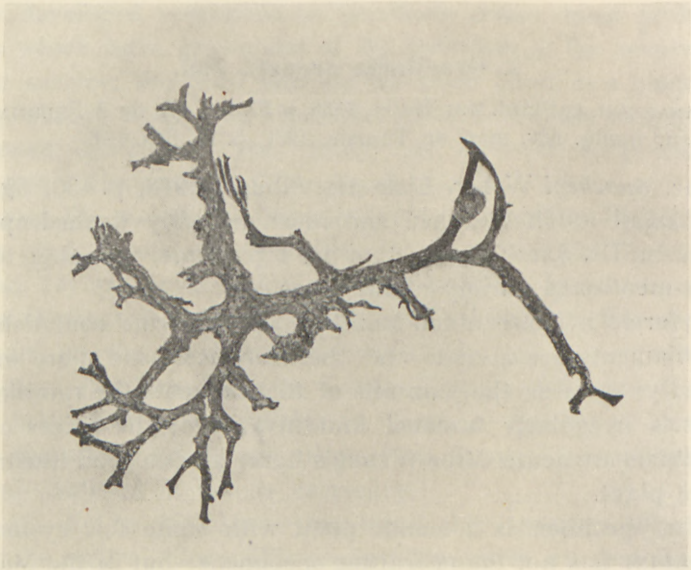

Fig. 35. *Gracilaria arcuata* Zan. var. *Snackeyi* Weber forma *rhizophora* Børgs. (× 1).

some material of HAUCK's species, collected at Madagascar by HILDEBRANDT (no. 94), and determined by HAUCK (1886, p. 165) is found in my herbarium I have been able to compare the specimens of JADIN with authentic material and found that JADIN's specimen cannot be referred to HAUCK's species. On the other hand, as the material of JADIN's plant I have seen, though rather poor, seems to bear a great resemblance to the above-mentioned variety of *Gr. arcuata* I do not hesitate to refer the specimens to it, as also the anatomical structure is rather alike. The reason why JADIN has referred this plant to HAUCK's species is no doubt that from the lower part of the filaments rhizoids are given out, by means of which the filaments become rooted to the substratum.

Because of this peculiarity I propose to call this special form forma *rhizophora* (Fig. 35).

In this connection I should further like to point out that the plant which in Contributions, III, 1938, p. 221 I referred, though with much doubt, to *Gracilaria debilis* (Forssk.) Boergs. is this form; the only specimen I have agrees with this form not only as to its ramification but is also provided with rhizoids.

Mauritius: Herb. JADIN, without locality, DARUTY legit 1892; forma *rhizophora*: Flacq, June 1890, JADIN no. 218; Mahébourg, September 1890, JADIN no. 424.

Geogr. Distr.: Red Sea, India, Malayan Archipelago etc., Mediterranean Sea.

3. **Gracilaria corticata** J. Ag.

AGARDH, J., Spec. Alg., II, p. 602; Epicr., p. 423.

A small specimen in JADIN's collection is referable to this species.

It is already known from Mauritius as the *Chondrus multipartitus*, β *foliifer*, Grev. which HARVEY mentions in Alg. TELFAIR, p. 147, according to J. AGARDH, l. c., is this species; J. AGARDH refers to it as a separate variety: var. *linearis*.

The specimen of JADIN has no number and is undetermined but in his list p. 165 the species is mentioned. About its habitat at the island JADIN writes: "Croissant en assez grande abondance; en buissons d'un joli rose; recouvert à marée basse de cinquante centimètres d'eau environ".

Mauritius: Fort Georges à Port-Louis, Aug. 1890, JADIN no. 349.
Geogr. Distr.: Red Sea, Indian Ocean.

4. **Gracilaria foliifera** (Forssk.) Børgs.

BØRGESEN, F., Revision FORSSK. Alg., 1932, p. 7, fig. 1, where the chief literature of the species is mentioned. — *Fucus foliifer* Forssk., Flora Ægyptiaco-arabica; 1775, p. 191.

A specimen of this highly variable plant has been collected by Dr. VAUGHAN. The plant is more subdichotomously divided and fastigiate than is usually the case in this species and in some respects recalls *Gracilaria corticata*. Two small specimens

in JADIN's collection with rather narrow and proliferous thallus also seem referable to this species.

Mauritius: Barkly Island, R. E. V. no. 336, Aug. 1939. Without locality, JADIN.

Geogr. Distr.: Warmer parts of Atlantic Ocean, Mediterranean Sea, Red Sea, Indian Ocean.

5. **Gracilaria Millardetii** (Mont.) J. Ag.

AGARDH, J., Till Alg. Syst., IV, VII, 1884, p. 64. — *Rhodymenia Millardetii* Mont., in MONTAGNE et MILLARDET, Algues, 1862, p. 9, pl. XXV, fig. 3.

Of this elegant and characteristic small species, originally described upon specimens from the Mascarene Islands and hitherto known from these alone, I have seen three small specimens only, two of which are from Mauritius and one from Réunion. Two of these specimens are antheridial and one is tetrasporic.

AGARDH describes 3 forms of this species: the typical form, forma *Millardetii* with a thallus divided into broad segments

Fig. 36. *Gracilaria Millardetii* (Mont.) J. Ag. forma *Millardetii* J. Ag. Habit of a male plant. (× 2).

(MONTAGNE l. c. fig. 3) and two with narrow lobes: forma *crenulata* and forma *linearifolia*.

The small antheridial plant of which Fig. 36 shows a picture

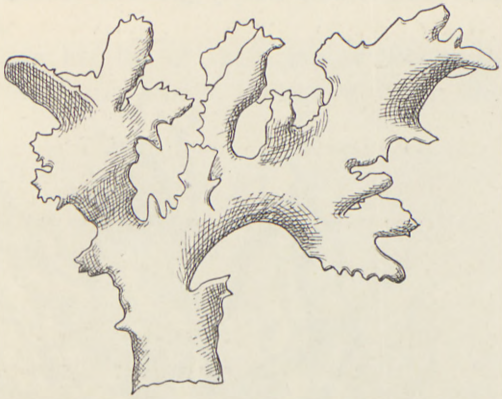

Fig. 37. *Gracilaria Millardetii* (Mont.) J. Ag. forma *Millardetii* J. Ag. A small piece of the plant shown in Fig. 36 drawn on an enlarged scale. (× 5).

is, I think, referable to forma *Millardetii*. Fig. 37 shows a small piece of this plant on an enlarged scale. When compared with MONTAGNE's figure of a cystocarpic specimen the antheridial plant has much narrower lobes, about 2—3 mm broad. The thallus is

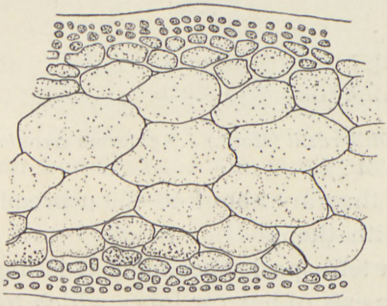

Fig. 38. *Gracilaria Millardetii* (Mont.) J. Ag. Transverse section of the thallus (About × 300).

flat and the segments are several times irregularly flabellately divided; the margins are provided with short processes in some places, in others not.

A transverse section (Fig. 38) of the thallus shows a cortical layer composed of small roundish cells. The medullary layer

consists of roundish or oblong cells, smallest nearest the periphery, larger towards the middle where they have a breadth of up to 100 μ. The whole thallus is about 300 μ thick.

The small antheridial nearly globular caves are formed in

Fig. 39. *Gracilaria Millardetii* (Mont.) J. Ag. forma *crenulata* J. Ag. (× 1^1/$_3$).

the cortical layer; they are about 40—50 μ broad and the surface is closely beset with them.

Fig. 39 shows a tetrasporic specimen. It is higher, about 6 cm, and the thallus consists of narrow lobes which towards the apices become more irregularly divided and crenulated; this plant, I think, is referable to forma *crenulata* J. Ag.

The specimen from Réunion, like the one from Mauritius, is antheridial. It has a very narrow, linear thallus and is most probably referable to forma *linearifolia* J. Ag. (Fig. 40).

Having now become acquainted with this small *Gracilaria*, it strikes me that the small *Gracilaria* I described in Contributions,

II, 1937, p. 327, fig. 3 as a new species: *Gracilaria pygmaea* most probably, like the above-mentioned, is forma *linearifolia* of this species. The only specimen I had for examination was female. To decide the question definitely more material than I have seen will be required.

Mme WEBER, in her list of the algae of Siboga p. 432, has figured a small *Gracilaria* which, though with a ?, she refers to

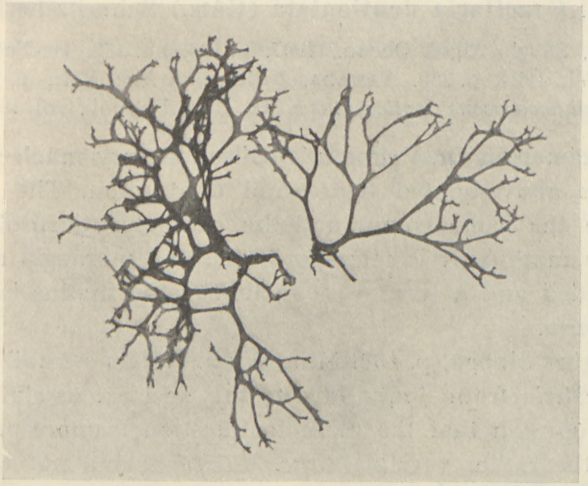

Fig. 40. *Gracilaria Millardetii* (Mont.) J. Ag. forma *linearifolia* J. Ag. (× 1 1/3).

Gracilaria denticulata (Kütz.) Schmitz, syn. *Sphaerococcus denticulatus* Kütz., Tab. Phyc., vol. XIX, 1869, p. 19, tab. 51. The plant of Mme WEBER was collected at Java and from her description, and especially from her figure, I am much inclined to suppose that the plant in question is a form of *Gracilaria Millardetii.*

In addition to this I would further point out that the well-known Chinese algologist, Professor C. K. TSENG, in a letter received from him from America just before the postal connection became interrupted, has written to me that near Hong Kong he has collected a small *Gracilaria* which he presumes to be like *Gracilaria pygmaea.* Most probably the plant in question is a form of *Grac. Millardetii.*

It seems rather likely therefore that this small species is actually widely distributed in the Indian Ocean.

As to the habitat of *Grac. Millardetii* JADIN, l. c. p. 165, writes:

"Abondant. La plupart des exemplaires ont été recueillis rejetés sur la plage sauf le numéro 369 qui croissait sur des débris de grosses coquilles a l'ilôt Barclay dans une eau calme".

Mauritius: llôt Barclay à Port-Louis, Aug. 1890, JADIN no. 369.
Geogr. Distr.: Mascarene Islands; most probably also distributed throughout the Indian Ocean.

6. **Gracilaria denticulata** (Kütz.) Schmitz herb.

MAZZA, Saggio Algol. Ocean., 1907, p. 138, no. 172. DE-TONI, Sylloge Alg., vol. VI, 1924, p. 265. YAMADA, Notes Jap. Alg., VIII, p. 125, pl. 25, fig. 2. — *Sphaerococcus denticulatus* Kütz., Tab. Phycol., vol. 19, tab. 51.

A specimen in Dr. VAUGHAN's collection very much resembles KÜTZING's above-quoted figure and description. The specimen has quite the same colour and the characteristic proliferations along the margin. It is tetrasporic and the sporangia are cruciately divided and a transverse section of the thallus is like that of *Gracilaria*.

In Algues Siboga, p. 432, Mme WEBER refers a small specimen of a *Gracilaria* from South Java to this species; as stated above, I am of opinion that the plant in question is more probably a form of the rather variable *Grac. Millardetii* and not this much bigger species of KÜTZING.

Most probably the plant from Mauritius is the same as that which A. MAZZA, l. c. p. 172 (compare also what is said by DE-TONI in Sylloge Algarum, vol. VI, p. 265), found in SCHMITZ's herbarium under the above-mentioned name. This specimen originates from The Kowie in South Africa and this species might therefore very well occur at Mauritius also.

Mauritius: Barkly Island, "cast up by waves", R. E. V. no. 355.
Geogr. Distr.: New Caledonia, Timor, South Africa, Japan.

Fam. 9. Mychodeaceae.

Mychodea Harvey.

Mychodea chamaedoridis nov. spec.

Frons epiphytica, teretiuscula, in *Chamaedoride Delphinii*, caespitosa, intricata, ca. 3—4 cm alta et $^1/_4$—$^1/_2$ mm lata, irregulariter sparse ramosa. Rami quoqueversum egredientes, plus minus curvati, apicibus acutis et saepe hamatis.

Color thalli exsiccatione purpureo-fuscus. Fructificatio ignota.

Mauritius: Flat Island, 17. Oct. 1929, Th. Mortensen legit.

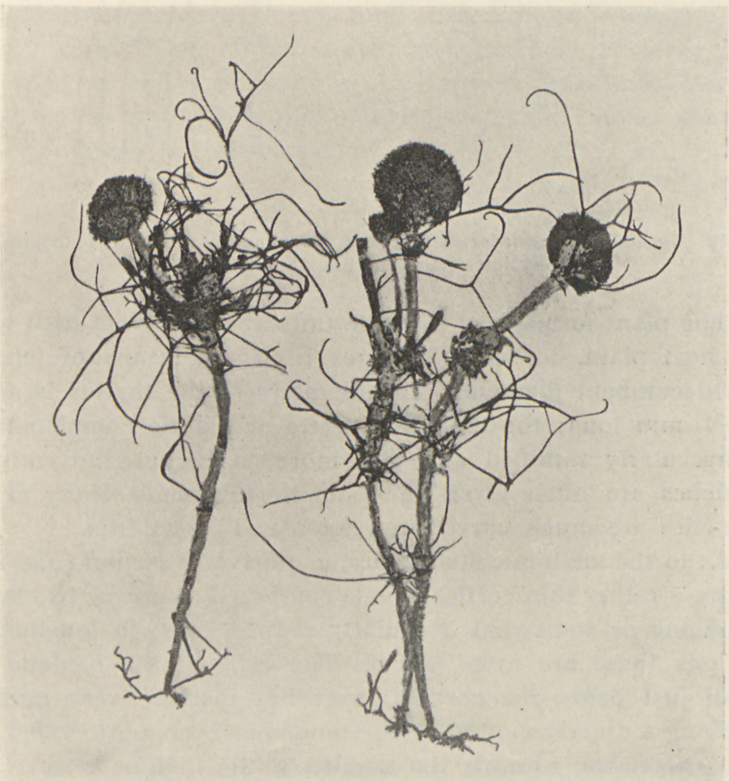

Fig. 41. *Mychodea chamaedoridis* Børgs. Filaments of the plant epiphytic upon *Chamaedoris Delphinii* (Hariot) Feldm. et Børgs. (× 1).

As an epiphyte upon *Chamaedoris Delphinii* (Har.) Feldm. and Børgs. a small *Mychodea* is found in MORTENSEN's collection (Fig. 41). It is very regrettable that all the specimens are sterile but even so, I think it justifiable to regard the plant as a new species since it cannot be referred to any of the species of this genus hitherto described. In appearance it shows much likeness to forms of *M. hamata* J. Ag. but it scarcely attains half the size of this species and is also very different in its anatomical structure.

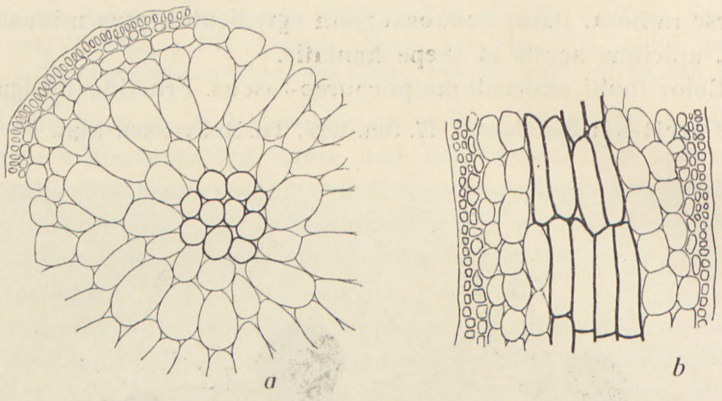

Fig. 42. *Mychodea Chamaedoridis* Børgs. *a*, transverse section and *b*, longitudinal section of the thallus. (About × 60).

The plant forms very intricate tufts about 3—4 cm high upon the host plant, to which it fixes itself by means of tendrils and decumbent filaments. The diameter of the thallus is about $^1/_2$—$^1/_4$ mm long; the thallus is terete or a little complanate. It is irregularly ramified and still more so because adventitious branches are often given out from the filaments. Many of the branches are much curved and often end in tendrils.

As to the anatomical structure, a transverse section (Fig. 42 *a*) shows a rather thin cortical tissue composed of one or two layers of oblong or somewhat irregularly shaped cells; in longitudinal sections these are more square. The cells of the medulla are small just below the cortical layer but increase very quickly, reaching a diameter of 50—100, sometimes even up to 200 μ and then decreasing towards the middle of the thallus. Here a not very marked axis is found, formed of up to about ten cells; in transverse section these cells are roundish or a little edged; they

have rather thick walls and a diameter of about 20—30 μ. In longitudinal sections (Fig. 42 *b*) they are found to be subcylindrical, about 180 μ long with more or less oblique transverse walls.

As is mentioned by KYLIN, 1932, p. 62, the *Mychodea* species grow by means of an apical cell; in some species this is easily observable, in others difficult to see because of the crowded cells at the tips of the thallus. In the *Mychodea* species from Mauritius a good many cells surround densely the apical cell; but being a little larger than those nearest to it, it is in most cases discernible without difficulty.

As was said above, the plant from Mauritius bears some likeness to *M. hamata*, but it is much smaller and poorer developed in all respects. The same applies to the cells of the medullary tissue, which are about $^1/_2$—$^1/_3$ smaller than those in *M. hamata*; and the central axis, which is very prominent in the latter species, is often rather difficult to observe in a transverse section of the thallus of *M. Chamaedoridis*. Also the cortical layer is much more developed in *M. hamata*.

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with some of the more important of the synonyms, the latter printed in italics.

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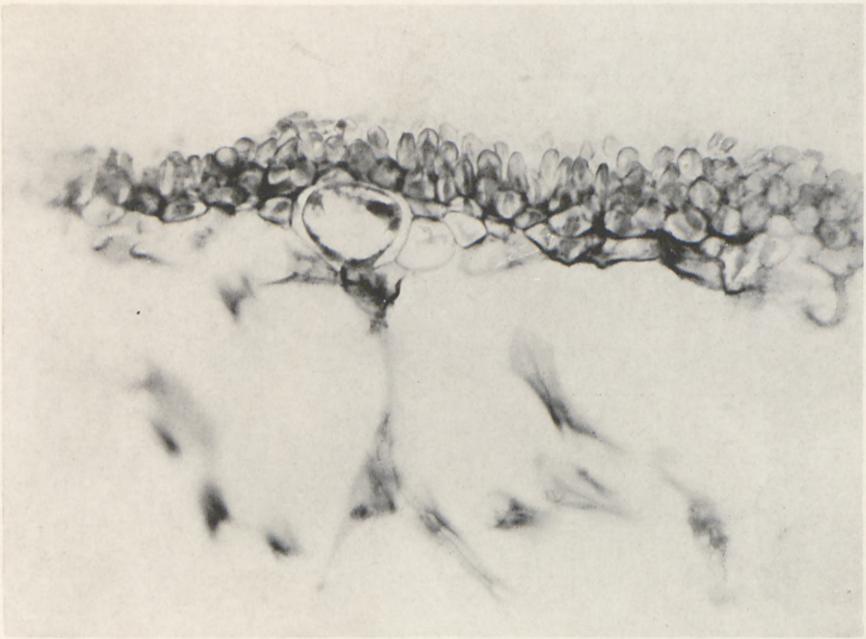

Fig. 1. *Titanophora Pikeana* (Dickie) Børgs. Transverse section of the thallus showing the cortieal layer with the papilliform, saboylindrical cells; in the middle a gland-cell and below fragments of the filaments traversing the interior of the thallus. (× 530).

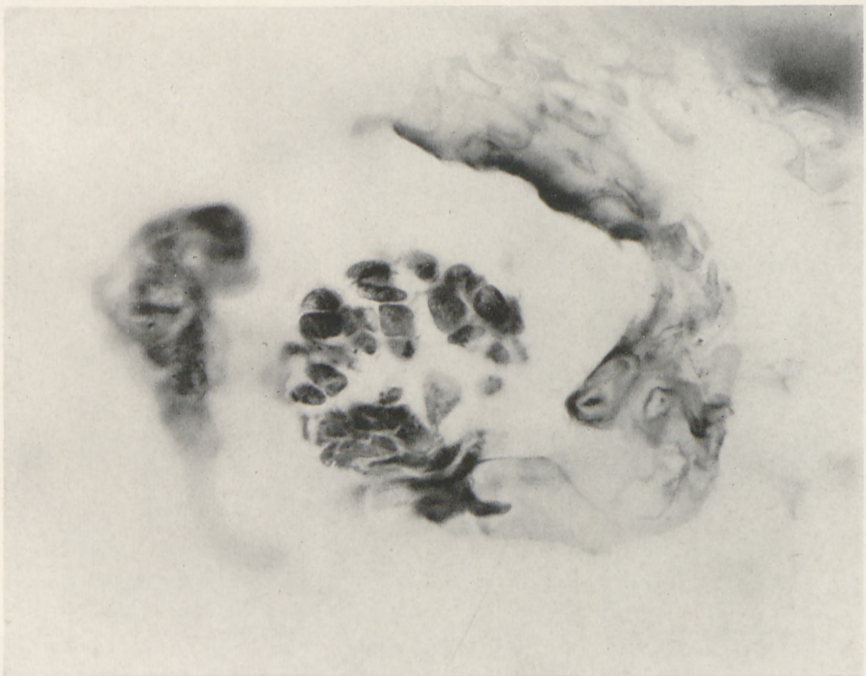

Fig. 2. *Titanophora Pikeana* (Dickie) Børgs. A gonimoblast. The fertilizing filament is visible crossing over the auxiliary cell. (× 530).

DET KGL. DANSKE VIDENSKABERNES SELSKAB
BIOLOGISKE MEDDELELSER, Bind XIX, Nr. 2

POSTEMBRYONALE ENTWICKLUNG UND BIOLOGIE DER RHEOPHILEN KÖCHERFLIEGE *OLIGOPLECTRUM MACULATUM* FOURCROY

VON

ANKER NIELSEN

KØBENHAVN
I KOMMISSION HOS EJNAR MUNKSGAARD
1943

INHALT

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Einleitung.

Diese Arbeit wurde nach den gleichen Grundsätzen wie meine frühere Arbeit über die himmerländischen Quelltrichopteren (10) ausgeführt, auf die auch hinsichtlich der angewandten Terminologie sowie der für *Oligoplectrum* und andere Köcherfliegen gemeinsamen Züge verwiesen sei. Die Beobachtungen im Freien wurden in und an himmerländischen Flüsschen angestellt, wo die Art in kolossalen Mengen vorkommt. An günstigen Stellen kann ihre Dichte — bestimmt durch die Anzahl der Individuen, die sich fertig entwickeln — bis zu 10.000 pro qm betragen. — Bezüglich der Abbildungen sei bemerkt: Wo nichts Anderes angegeben ist, sind die Bilder so angeordnet, dass ihr oberer Rand dem oralen Ende des Tieres oder (wenn das dargestellte Objekt von vorn oder hinten gesehen ist) seiner Dorsalseite entspricht. Auf den mit * bezeichneten Abbildungen wurde versucht, die Farbzeichnung durch verschieden intensive Punktierung wiederzugeben; auf allen anderen morphologischen Larven-Abbildungen bedeutet Punktierung weichhäutige Partien.

Die ausgewachsene Larve und die Puppe wurden von KLAPÁLEK (3), ULMER (14 und 15) und LESTAGE (4) beschrieben. Ich selbst habe früher (9) den Laich und das erste Larvenstadium geschildert. SILTALA (13) gab eine kurze Darstellung der Borstenverhältnisse in den beiden letzten Larvenstadien der nahestehenden Gattung *Brachycentrus* CURTIS.

Obwohl der Larvenköcher seit PICTET (12) bekannt ist, wusste man bisher nur äusserst wenig über die Biologie der Art; immerhin war es der Aufmerksamkeit nicht entgangen, dass sie auf schnellfliessendes Wasser angewiesen ist. So gibt KLAPÁLEK (3, S. 63) Gebirgsflüsse als ihren Biotop an; LESTAGE (4) schreibt: »Elle vit dans les eaux rapides des ruisseaux des régions montagneuses«. DELPÉRÉE (1) fand die Art in dem belgischen Fluss Ourthe an einer Stelle mit so starker Strömung, dass man in dem knietiefen Wasser kaum feststehen konnte; zugleich stellte er fest, dass an den ruhigeren Stellen die Larven fehlten.

Der Köcher wird als schlank konische, gerade, aus sehr feinen Sandkörnchen gebaute Röhre beschrieben. KLAPÁLEK gibt ihre Dimensionen folgendermassen an: Länge bis zu 20 mm, Breite vorn 1,8, hinten 0,86 mm. »Die hintere Öffnung des Gehäuses ist durch eine ring-

förmige, sehmale Membran nur etwas verkleinert.« DELPÉRÉE (1) fügt hinzu, dass man im vordersten Teil des Köchers oft auch Baumrinden- und Wurzelstückchen sieht, die fast stets der Quere nach angebracht sind. Nach Angabe von MEYER-DÜR (7) sind die Köcher an Steinen befestigt, und zwar oft in so grosser Anzahl, dass man an einem einzigen Stein Tausende von ihnen finden kann. Diese Angabe wird von DELPÉRÉE bestätigt und ergänzt: Die Köcher sind mit dem Vorderende gegen die Strömung auf dem Teil des Steines befestigt, der der Strömung am stärksten ausgesetzt ist. Hier sind sie so dicht angebracht, »que, souvent, les fourreaux chevauchaient l'un sur l'autre«. Er veröffentlicht schöne Photographien der »colonies larvaires«. Schliesslich habe ich selbst (9) die Biologie des ersten Larvenstadiums kurz geschildert und dabei u. a. nachgewiesen, dass der Köcher bereits in diesem Stadium am Substrat befestigt wird.

»Das Nymphengehäuse wird auf 13—16 mm verkürzt, vorne durch eine gelbbraune, feste Membran verschlossen, welche in der rotbraunen Mitte mit 7—15 kleinen Öffnungen versehen ist. Der Vorderrand wird durch eine braune Membran erweitert. Die hintere Öffnung ist wie im Larvengehäuse. Die Gehäuse werden mit dem Vorderrande auf Steine und die Fontinalis befestigt« (KLAPÁLEK, 3, S. 63). Diese Beschreibung wird von ULMER (14, S. 319) und LESTAGE (4, S. 877) wiederholt, nur erwähnen diese Verfasser nicht die »braune Membran«, durch die der Vorderrand »erweitert wird«. LESTAGE (4) gibt an, dass der Köcher durch Abschneiden des vorderen Stückes auf 9—11 mm verkürzt wird, wohingegen DELPÉRÉE (1) schreibt: »... quand elle assure la fixité de sa maison, avant la nymphose, la larve ne regarde pas à jeter par ci, par là, des fils d'attache supplémentaires, et il n'est pas rare de rencontrer des fourreaux, qui adhérent au support par plus de la moitié de leur face antérieure«. Im übrigen gibt auch er an, dass die Köcher verkürzt werden, zuweilen bis zu einer Länge von nur 4 mm.

Etwas besser sind wir durch die Arbeit amerikanischer Forscher über die Larvenbiologie der verwandten Form *Brachycentrus nigrosoma* BANKS unterrichtet. So schreibt LLOYD (5, S. 367): »The larva fastens its case by a stout silken attachment to the top of some current-swept boulder and then rests with legs outspread as indicated in figure 217 in a receptive attitude, waiting for whatever organic materials the current may bring within its grasps«. Und MURPHY (8) etwas ausführlicher: Die ersten 6 Wochen ihres Lebens verbringen die Larven an ruhigen Stellen des Wasserlaufes, wo sie rasch über Steine und Pflanzen hinkriechen. Die ersten beiden Wochen leben sie ausschliesslich von Diatomeen, zu denen später Grünalgen und Fragmente von Samenpflanzen kommen. Wenn die Larven 6 Wochen alt sind und fast die Hälfte ihrer endgültigen Grösse erreicht haben, wandern sie nach den stark fliessenden Stellen des Baches und befestigen den Vorderrand des Köchers so mit der einen Seite (diese Gattung baut vierseitige Köcher) auf der Unterlage, dass sein Vorderende gerade gegen die Strömung gerichtet ist. »With head thrust slightly forward, prothoracic legs extending straight ahead, mesothoracic legs upward, and meta-

thoracic legs at the sides (fig. 1, 2), they wait for food. — From a purely herbivorous diet obtained by active searching, they now become mainly carnivorous, waiting in a most receptive attitude for whatever may come within their powerful grasp«. Die beigefügten Abbildungen zeigen Mittel- und Hinterbeine ziemlich stark gebogen. Diese Stellung nehmen die Larven auch in ruhigem Wasser ein, jedoch bemerken sie Beute nur dann, wenn diese von der Strömung gegen ihre Beine geführt wird. MURPHY beobachtete, dass dem Wasser zugesetzte Karminkörnchen von den dichtstehenden Dornen auf Mittel- und Hinterschenkeln aufgefangen werden, und dann »the short spines and row of long strawcolored hairs on the inner edge of the femur of the prothoracic legs (fig. 5) were used to scrape off the particles and transfer them to the mouth-parts.... When a quantity of material, such as bits of plant tissue, pieces of wood, bark, or silt was introduced into the stream, the larvae would rear themselves out of their cases far enough to expose the entire thorax and proceed to comb it with the mesothoracic legs in a single swift stroke. Then the prothoracic legs were used to remove the material and convey it to the mouth-parts, where it was eagerly chewed as though it were the daintiest of morsels.... it seems probable, that the primary reason for this action is to keep the passage way open for a good stream of water through the case, and that the foodgetting is a secondary matter and quite incidental«. Um sich zu verpuppen »the larvae fasten their square cases firmly to the stones. Then they feverishly set about spinning a silken sheet of lining, that is perforated in the center, at both ends«. In einer späteren Arbeit referiert LLOYD (6, S. 82) diese Untersuchungen und fügt hinzu: »From time to time larvae confined in aquaria detached their cases and moved from place to place. It seems probable that the larvae in the streams, also, at times move about in search of building material, for it is unlikely that chance would place enough suitable case-building material within their reach«. Ferner berichtet LLOYD, dass die anale Puppenmembran im hinteren Drittel des Köchers angebracht ist.

Die vorliegende Arbeit wurde mit Unterstützung des CARLSBERG FONDS ausgeführt, und der RASK-ØRSTED FONDS hat die Kosten der Übersetzung getragen; den beiden Fonds sei hier mein herzlicher Dank ausgesprochen. Ebenso danke ich Dr. phil. OLGA KUTTNER für die sorgfältige Übersetzung der Abhandlung.

Der Laich.

In einer früheren Arbeit (9) habe ich Laich und Eiablage beschrieben und werde mich daher auf eine kurze Zusammenfassung, ergänzt durch ein paar spätere Beobachtungen, beschränken.

Vor der Eiablage trägt das Weibchen den Eiklumpen in einer grossen Vertiefung am Hinterleibsende. Der Eiklumpen ist intensiv grün, eiförmig, jedoch sagittal etwas zusammengedrückt, und

etwa 2,5 × 2 × 1,2 mm gross. Seine Oberfläche erscheint durch die dicht gedrängten Eier etwas uneben. Ihre Anzahl beträgt etwa 400, ihre Grösse (nach der Ablage) etwa 0,45 × 0,33 mm. An Weibchen in Gefangenschaft wurde beobachtet, dass sie des Nachts unter Wasser kriechen, wobei die Eiklumpen stark anschwellen und am Substrat festkleben; die Weibchen bleiben an den Klumpen hängen und sterben. Die abgelegten Eiklumpen sind etwa 5 × 4 × 2 mm gross. Die farblose Gallerte enthält in der äusseren, 0,2—0,5 mm dicken (am stumpfen Ende am dicksten) Schicht keine Eier; ihre alleräusserste Schicht ist von zäherer Beschaffenheit als die übrige Gallerte. Während des Anschwellens wird der Umriss der Klumpen häufig etwas unregelmässig; die Eier behalten ihre grüne Farbe.

Im Freien findet man die Eier an ruhigen Stellen am Ufer der Wasserläufe (Tafel I, Abb. 2), an Schilf u. dergl. befestigt, unter Wasser, jedoch meist recht nahe der Oberfläche. Die Klumpen sitzen bald mit der Breitseite, bald mit der Schmalseite fest, am häufigsten aber wohl mit der ersteren; ihre Oberfläche ist oft fast ganz von angeklebten, feinen Detritusteilchen bedeckt. Entsprechend der grossen Zahl von Tieren finden sich auf dem Substrat zuweilen so zahlreiche Eiklumpen, dass es fast vollständig von ihnen bedeckt wird (Tafel II, Abb. 3).

Ich hatte mehrmals Gelegenheit zu beobachten, dass an regnerischen Tagen fast alle Weibchen Eiklumpen tragen; noch weit häufiger konnte ich feststellen, dass man an trockenen Tagen keine Weibchen mit Eiklumpen findet. Man ist daher zu der Annahme gezwungen, dass die Bildung der Eiklumpen von einer gewissen Luftfeuchtigkeit abhängig ist. Bei unmittelbarer Betrachtung des weiblichen Abdomens und des noch nicht abgelegten Eiklumpens erhält man auch den Eindruck, dass dieser schon während seiner Entstehung Wasser aufsaugt. Weibchen, die Eiklumpen tragen, sind sehr träge.

Die Larve.

Die Larve (Abb. 1) ist lang und sehr schlank. Bei der ausgewachsenen Larve ist das Längenverhältnis von Kopf, Thorax und Abdomen wie 10 : 16 : 100. Wie man sieht, ist der Thorax im Verhältnis zu dem langen Abdomen sehr kurz. Die Breite

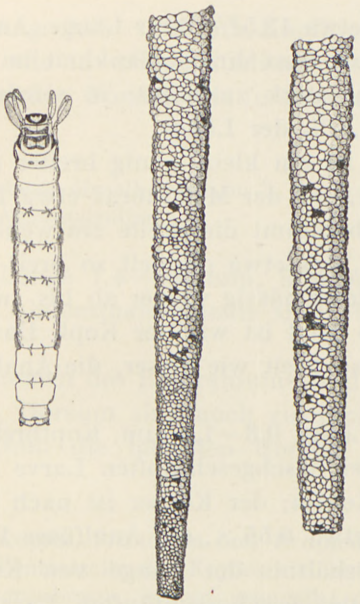

Abb. 1*. Ausgewachsene Larve (Borsten weggelassen) und 2 Köcher des 5. Stadiums. 4/1.

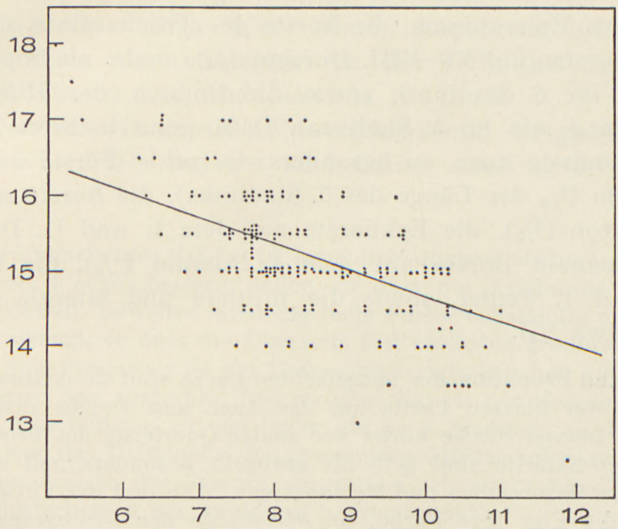

Abb. 2. 5. Larvenstadium. Graphische Darstellung des Verhältnisses von Breite und Länge der Larve. Abszisse: Länge der Larve in mm. Ordinate: Breite in % der Länge.

der Larve beträgt etwa 13,5 % ihrer Länge. Aus dem Diagramm (Abb. 2) ersieht man, dass ihre Schlankheit im Verlauf des letzten Larvenstadiums stark zunimmt; in seinem Beginn beträgt die Breite etwa 17,5 % der Länge.

Der Prothorax ist ein klein wenig breiter als der Kopf, der Mesothorax etwa 1,6 ×, der Metathorax etwa 1,9 × so breit wie dieser. Von hier ab nimmt die Breite ein wenig zu bis zum III. Abdominalsegment, das etwa doppelt so breit ist wie der Kopf; dann nimmt sie gleichmässig wieder ab bis zum VIII. Segment, das etwa 1.7 × so breit ist wie der Kopf. Das IX. Segment ist ungefähr 1,3 mal so breit wie dieser, die Analfüsse fast ebenso breit.

1. Stadium. Länge 0,8—1,4 mm, Kopfbreite etwa 0,18 mm. Der Metathorax der frischgeschlüpften Larve hat etwa die 1,3 fache Breite des Kopfes; der Körper ist nach hinten stark verschmälert. IX ist etwa 0,55 ×, die Analfüsse 1,2 × so breit wie der Kopf. Das Verhältnis der Länge von Kopf, Thorax und Abdomen ist bei der frischgeschlüpften Larve wie 10 : 11 : 24, am Ende des Stadiums wie 10 : 14 : 50.

Abgesehen von den lateralen Borsten auf III—VII sind die folgenden durch besondere Länge ausgezeichnet: die dorsalen Borsten des Epicraniums, die Borste des Trochantinus und die grossen Borsten auf VI—VIII. Dorsum (alle mehr als doppelt so lang wie im 5. Stadium), sowie die Borsten der Mittelhüfte (3 × so lang wie im 5. Stadium). Dafür sind mehrere andere verhältnismässig kurz, so besonders die orale Borste auf dem Mesonotum ($^1/_3$ der Länge des 5. Stadiums), die Borste auf dem Mesepimeron ($^2/_5$), die Eckborste auf dem I. und II. Dorsum, die medioanale Borste auf dem I. Dorsum ($^1/_6$), die laterale Borste auf I. Venter, sowie die mittlere und laterale auf II. Venter (etwa $^1/_3$).

Kopf und Pronotum der ausgefärbten Larve sind dunkelbraun, mit Ausnahme der blassen Partie um das Auge und einiger zusammenfliessender blasser Flecke hinter der analen Querleiste des Pronotums. Die übrigen Sklerite sind gelb bis graugelb, besonders hell sind die Sklerite des Mesonotums und Metadorsums. Während der Entwicklung verlieren die Eier ihre Farbe; die Weichteile der frischgeschlüpften Larve sind daher farblos, abgesehen zuweilen von etwas grünem Inhalt des Darmkanals. Im Verlauf des ersten Stadiums nehmen die Weichteile einen grünlichen Ton an.

2. Stadium. Länge 0,95—1,85 mm, Kopfbreite 0,23—0,26 mm. Dieselben Borsten wie im ersten Stadium sind besonders lang, ebenso sind die gleichen Borsten wie in diesem Stadium verhältnismässig kurz.

Die Farbe ist nahezu dieselbe wie im 3. Stadium; die Femora mit oder ohne dunklen Aussenstreifen.

3. Stadium. Länge 1,4—3,5 mm, Kopfbreite 0,305—0,345 mm. Die vorderen Abdominalsegmente sind etwa 1,6 × so breit wie der Kopf.

Die dorsalen Borsten des Epicraniums und die grossen Borsten auf VI.—VIII. Dorsum sind noch ziemlich lang, die Borste des Trochantinus und die lateralen Borsten auf I—II länger als im 5. Stadium.

Dorsalseite des Kopfes, Pronotum und Propleuron sind einfarbig braun, Lateral- und Ventralseite des Kopfes viel heller graubraun. Mesonotum vorn hell graubraun, hinten graugelb; dieselbe Farbe hat das laterale Sklerit des Metadorsum, während das mediane sehr hell und undeutlich ist. Farbe von Mittel- und Hinterschenkel wie im 4. Stadium.

4. Stadium. Länge 2,2—7,6 mm, Kopfbreite 0,435—0,53 mm. Am Ende dieses Stadiums ist das Verhältnis der Länge von Kopf, Thorax und Abdomen wie 10 : 15 : 85.

Die dorsalen Borsten des Epicraniums und die grossen Borsten auf VI.—VIII. Dorsum sind immer noch länger als im 5. Stadium.

Die Grundfarbe des Kopfes ist erheblich dunkler als im 5. Stadium, die dunklen Flecke dagegen heller, so dass die Zeichnung weniger stark hervortritt. Zuweilen wird die helle Farbe vollständig durch die dunkle verdrängt, so dass die Oberseite fast einfarbig graubraun wird. Auch auf dem Pronotum ist der Unterschied zwischen hell und dunkel nicht so gross wie späterhin. Das mediane Sklerit des Metadorsum ist sehr hell. Das Labrum ist viel heller als im 5. Stadium; an den Mandibeln ist nur das Distalende des proximalen Gliedes schwarz gefärbt. Auf der Aussenseite der Femora ein dunkel schwarzbrauner Streifen, auf den Vorderbeinen am wenigsten hervortretend.

5. Stadium. Länge 4,8—11,6 mm, Kopfbreite 0,68—0,81 mm. Die kleinste Ruhelarve war 5,8 mm lang.

Die Grundfarbe von Kopf, Pronotum und Beinen ist strohgelb. Der Kopf ist am hellsten auf der Dorsalseite und besonders auf dem Teil der Lateralseite, der das Auge umgibt. Auf dem Frontoclypeus findet sich vorn ein dunkelbrauner, parabolischer Fleck, der bis zum Vorderrand, jedoch nicht bis zur epicranialen Sutur reicht, hinten ein oralwärts eingebuchteter, analwärts zweispitziger, dunkelbrauner Fleck, der bis zur epicranialen Sutur reicht. Die beiden Flecke sind oft durch einen unpaaren, hell bräunlichgrauen Streifen mit einander verbunden. Dorsal auf der hinteren Hälfte des Epicraniums ein dunkelbrauner Längsfleck, der vom analen Kiel begrenzt wird, jedoch nur vorn die epicraniale Sutur erreicht. Er setzt sich nach vorn in einen viel helleren braunen Fleck fort, der die vordere Hälfte der Dorsalseite des Epicraniums ganz ausfüllt. Gula und Ventralseite des Epicraniums dunkelbraun. Längs der Basis der Mandibel ist der Oralrand dorsal dunkelbraun, ventral schwarzbraun; längs der Gularsutur erstreckt sich ein vorn breiterer, hinten schmalerer, schwarzbrauner Streifen, der sich ganz schmal längs des Foramen occipitis fortsetzt. Auf dem vorderen Fleck des Frontoclypeus sieht man ein Paar dunkle, etwas vertiefte Punkte, auf dem hinteren Fleck einige undeutliche, hellere Punkte. Auf dem hinteren Fleck des Epicraniums 3 ziemlich deutliche, hellere Punkte, auf der Occipitalseite und analwärts auf der Lateral- und Ventralseite undeutliche, hellere Punkte. — Das Labrum ist schwarzbraun, Mandibeln und Cardo sind schwarz, die ersteren auf der Lateralseite proximal dunkelgelb, die Ventralseite des Mentums schwarzbraun, die übrigen Sklerite des Maxillolabiums gelbbraun bis hellbraun, die distalen am hellsten. — Der hintere Teil des Pronotums (hinter dem Querkiel) ist graubraun mit grossen, aber undeutlichen, helleren Punkten (auch hinter der analen Querleiste). Der Vorderrand ist dunkelbraun, anale Querleiste, Hinterrand und Ventralseite der Hinterecke sind schwarz. Das Pleuron ist etwas dunkler als der vordere Teil des Notums; die Vorderseite des Trochantinus ist schwarz, ebenso ein Fleck auf dem Episternum am Coxalgelenk. — Die Sklerite des Meso- und Metadorsums sind hell graubraun, am hellsten das laterale Sklerit des Metadorsums. Die lateralen Sklerite (beider Dorsa) besitzen eine feine, braune Längsfurche, deren nächste Umgebung gelb gefärbt ist. Auch ein mehr oder weniger grosses Stück der Lateral- und Analkante des mesodorsalen Lateralsklerits ist gelb, zuweilen in so grosser Ausdehnung, dass das ganze lateroanal von der Furche liegende Stück gelb erscheint. Mitten auf dem medianen Sklerit grosse, gelbe Punkte, die meist (indem auch ihre Umgebung sich gelb färbt) so sehr mit einander verschmelzen, dass die graubraune Färbung in eine orale und eine anale Partie geteilt wird. Mitten auf der Mediankante der lateralen Sklerite beider Dorsa ein gelber Punkt. Metapleuron hell graubraun, Mesepisternum braun, Mesepimeron dunkelbraun. Die Ventralkante beider Episterna sowie eine mehr oder weniger grosse Strecke des Vorderrandes (dorsal niemals der ganze Rand) schwarzbraun bis schwarz, Mesepimeron ventral und dorsal schwarzbraun, die anale Hälfte oder

mehr von der Ventralkante des Metepimerons dunkelbraun bis schwarzbraun. Am Dorsalende der Furche ein brauner Fleck, Rest der Furche schwarz, am breitesten ganz ventral. Deutliche Punkte sind nicht zu sehen. — Die proximale Partie der Hüften und die Aussenseite des Femur haben einen schwach bräunlichgrauen Anstrich, Tibia und besonders Tarsus und Klaue sind klar bräunlich. Proximalrand der Coxa breit schwarz; der lateroorale Kiel auf den Vorderhüften fast in ganzer Länge schwarz; proximal auf der Vorderseite von Mittel- und Hinterhüften ein breiter, schwarzer Längsstreifen (auf den Mittelbeinen längs der oralen Dornenreihe). Der Proximalrand des Trochanters der Vorderbeine ist braun. Abgesehen von einem weissen Punkt im Grunde der Längsfurche der Vorderhüfte finden sich keine deutlichen Punkte; am deutlichsten ist ein Punkt auf der Hinterseite der Coxa, nahe am Proximalrand. — Analschild und Sklerite der Analfüsse sind strohgelb bis sehr hell graubraun, Spitze und Ventralspitze der Klaue braun. »b« trägt an der Gelenkverbindung mit »c« einen kleinen braunen Fleck; der Dorsoproximalrand von »c« ist schwarzbraun, in der Mitte am dunkelsten. Keine deutlichen Punkte. — Die Weichteile sind intensiv grün gefärbt, was (jedenfalls in der Hauptsache) auf der Farbe des Fettkörpers beruht. Wie später gezeigt wird, rührt diese Farbe nicht von Chlorophyll in der Nahrung her.

Die Kopfkapsel ist 0,865 mal so breit wie lang und 0,715 mal so hoch wie breit. Der Kopf (Abb. 3) ist ungefähr am analen Drittel am breitesten, nach vorn nur wenig verschmälert, und von eigentümlicher Form. Vorn ist die Lateralseite des Epicraniums von der Dorsalseite durch einen Längskiel getrennt, der ganz vorne scharf ist, mit lateral gerichteter Kante (bei Ansicht von oben kann man die Lateralseite nur eben ausserhalb des Kiels wahrnehmen). Nach hinten wird der Kiel breiter und abgerundeter und geht lateralwärts allmählich in die flache Kuppel über, auf der das Auge sitzt, während er von der Dorsalseite durch eine breite, flache, abgerundete Furche getrennt ist. (Bei Betrachtung des Kopfes von vorn oder hinten erinnert dieses Gebilde schwach an das eben hervorbrechende Horn eines Kälbchens.) Schliesslich biegt der Kiel auf die Dorsalseite um und wird ganz niedrig, breit abgerundet und undeutlich. Die hintere Hälfte der Dorsalseite ist von der Lateral- und Occipitalseite durch einen Kiel getrennt, der in die Lateral- und Occipitalseite allmählich übergeht, während er von der Dorsalseite scharf abgesetzt ist; diese liegt — besonders hinten — etwas tiefer als die Kante des Kiels. Die beiden Kiele der rech-

Borstentabelle.

		1. Stadium	2. Stadium	3. Stadium	4. Stadium	5. Stadium
Pronotum.	Vorderrandborsten median von der Eckborste	0	2—3	6—8	(7)11—14	13—16
	Vorderrandborsten lateral von der Eckborste	0	1	5—7	9—14	13—19
	Von den letzteren sind gerade	0	1	3—4	3—6	4—5
Mesonotum.	Eckborsten	1	3	6	7—10	10—15
	Grosse anale Flächenborsten	1	1	2	2—3	2—5
Metadorsum.	Eckborsten	1	3	6—8	7—10	8—15
	Grosse anale Flächenborsten	1	1	2	3—4	2—5
Mesepisternum		1	1	2	2—3	3—4
Metepisternum		1	2	3—4	(3)4—5	4—7
Metepimeron.	Orale Borsten	1	2	3	4—5	5—6
	Anale Borsten	0	2	5—8	6—9	9—14
Vorderbein.						
Coxa.	Anale Aussenseitenborsten	1	1	2	2—3	3—4
	Hinterseitenborsten	0	0	0(—1)	0—1	1—4
Femur.	Distale Aussenseitenb.	1	1(—2)	2—3	2—3	3—6
Mittelbein.						
Coxa.	Orale Aussenseitenborsten	2	2	4	5—7	7—10
	Anale Aussenseitenborsten	1	1	1	(2—)3(—4)	4
	Hinterseitenborsten	0	0	0	0—1	3—6
Femur.	Aussenseitenborsten	2	6—8	10—13	(10)14—16	17—23
	Innenseitenborsten	2	8—9	11—13	15—18	19—21
Hinterbein.						
Coxa.	Orale Aussenseitenborsten	2	2	4—5	6—9	8—12
	Proximale, anale Aussenseitenborsten	0	0	1	1—2	2—3
	Mittlere, anale Aussenseitenborsten	1	1	1	1	1
	Distale, anale Aussenseitenborsten	0	1	2	3	(3—)4
	Innenseitenborsten	1	1	2—3	4—5	5—7(11)
	Hinterseitenborsten	0	0	0	0	0—2
Femur.	Aussenseitenborsten	2	5—6	11—14	13—16	18—29
	Innenseitenborsten	2	7—9	12	16—19	20—24
IX. *Dorsum.*	Sekundäre Borsten	0	0	1(—2)	1—2	1—3
»b«.	Dorsale Borsten	4	5	(6—)7(—8)	6—8	7—9

ten und linken Seite bilden zusammen ein sehr breites U. Die Höhe der Occipitalseite beträgt etwa 29 % der Gesamthöhe des Kopfes. Ungefähr auf der Mitte der Dorsalseite findet sich ein sehr stumpfwinkliger, abgerundeter Querkiel. Die hinter diesem liegende (von dem U-förmigen Kiel eingerahmte) Partie ist flach,

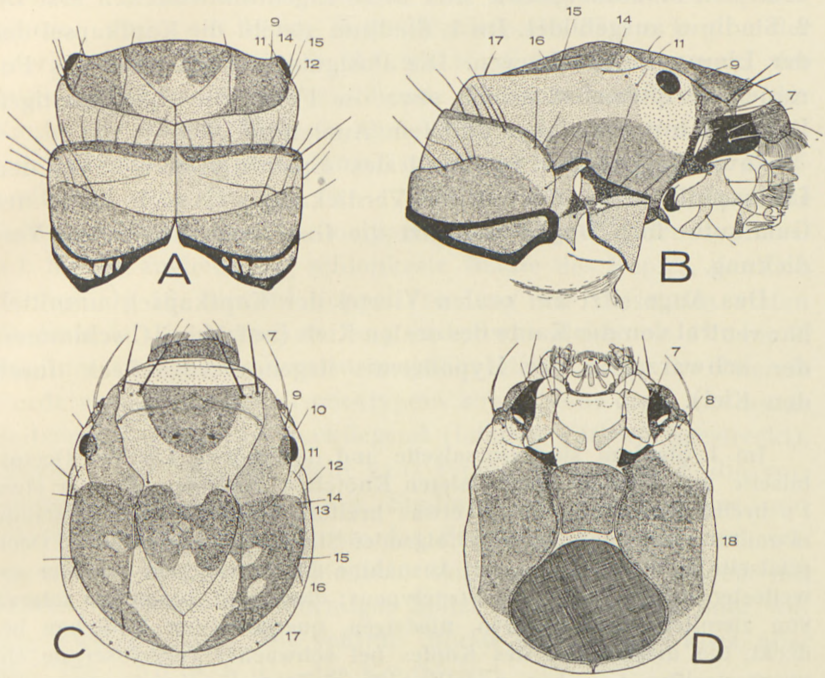

Abb. 3*. Kopf und Prothorax von der Dorsalseite (A) und von rechts (B). Kopf von der Dorsal- (C) und Ventralseite (D). $^{40}/_{1}$. Auf B ist das Vorderbein nahe der Basis abgeschnitten. Die Zahlen geben die Nummern der Borsten des Epicraniums an.

die davor liegende von einer Seite zur anderen gewölbt. Die Äste der epicranialen Sutur sind deutlich geknickt. Die Vorderecken des Postclypeus sind ausgezogen und stark abwärts gebogen. — Hinten (unter dem analen Kiel) besitzt die Lateralseite eine grosse, jedoch sehr flache Einbuchtung, die nur bei Betrachtung des Kopfes von unten oder schräg von oben her deutlich wahrnehmbar ist. Der Oralrand bildet am ventralen Mandibelgelenk einen ungewöhnlich weit vorspringenden Zapfen, der sich gegen die Lateralseite des Stipes legt. Die Postgula ist nahe am Vorderende am breitesten und reicht mit ihrem brei-

ten, schwach konkaven Hinterende bis zum Foramen occipitis. Die Prägula ist schmaler als die Postgula; da sie stark abwärts gerichtet ist, während die Postgula sich — besonders vorne — in longitudinaler Richtung wölbt, ist die Furche zwischen beiden etwas spitzwinklig. — Mit Ausnahme des Zapfens am ventralen Mandibelgelenk sind diese Eigentümlichkeiten erst im 2. Stadium ausgebildet. Im 1. Stadium gleicht die Kopfkapsel der der Limnophilinen-Larven. Die Postgula erreicht zwar das Foramen occipitis, hat jedoch etwa die Form eines gleichseitigen Dreiecks mit breit abgerundetem Analende.

Inwendig ist der Oralrand des Epicraniums wie bei den Limnophilinen verdickt; diese Verdickung setzt sich längs der Gularsutur fort. Dagegen besitzt die Gula keine inwendige Verdickung.

Das Auge sitzt am oralen Viertel der Kopfkapsel, unmittelbar ventral von der Kante des oralen Kiels (auf Abb. 3C schimmert der schwarze, in der Hypodermis liegende Augenfleck durch den Kiel).

Im 1. Stadium sind Dorsalseite und der grösste Teil der Occipitalseite mit kleinen, abgerundeten Knötchen bekleidet, die als etwa 1 μ breite, leuchtende, durch etwas breitere, dunkle Linien getrennte »Punkte« erscheinen. Auf den folgenden Stadien sind Dorsal- und Occipitalseite glatt und blank, mit Ausnahme des Anteclypeus und der am weitesten oralen Partie des Postclypeus; diese sind, besonders lateral, von ziemlich dichtsitzenden, niedrigen, quergestellten Knötchen bedeckt, die diesem Teil des Kopfes bei schwacher Vergrösserung ein quergestreiftes Aussehen verleihen. Im 1. Stadium sind Ventral- und Lateralseite des Epicraniums mit flachen, dachziegelförmig angeordneten Knoten bekleidet; auf der Ventralseite sind sie breit und quergestellt, auf der Lateralseite mit aufwärtsgerichteter Kante versehen. Vor dem Auge sind sie kleiner, stehen mehr vereinzelt und sind nicht dachziegelförmig angeordnet. In den folgenden Stadien werden sie immer mehr rückgebildet. Schon im 2. Stadium ist die Ventralseite glatt; im 5. Stadium finden sich dachziegelförmig angeordnete Knoten (bis 22 μ breit) nur auf den beiden hinteren Dritteln der Grenze zwischen Lateral- und Ventralseite, sowie einige vereinzelte, kleine (höchstens 8 μ breite), wenig auffallende Kämme von Spitzchen vor und hinter dem Auge (an der Grenze des blassen Augenfleckes).

Mit Ausnahme der medianen und der mittleren Vorderrandborste des Frontoclypeus sind alle Kopfborsten hell. Die dorsalen Borsten des Epicraniums sind sehr kurz (jedoch verhält-

nismässig recht dick), so dass die Vorderrandborsten des Frontoclypeus und die dorsale Mandibelgelenkborste (7) die längsten Borsten des Kopfes sind ($^3/_{10}$ der Kopfbreite). Auch diese Borsten sind verhältnismässig dick. Borste 8 ist kaum halb so gross. Borste 9 und 11 stehen auf der Dorsalseite des oralen, die Borsten 14—17 auf der Dorsalseite des analen Kiels des Epicraniums, und zwar 14 an ihrem Vorderende, 17 am Beginn der querliegenden Partie und 15 und 16 dicht zusammen in der Mitte zwischen 14 und 17. Borste 13 steht gerade unter der Kante des analen Kiels, in der Nähe von 14. Von den dorsalen Borsten des Epicraniums ist 15 die längste ($^1/_5$ der Kopfbreite; $14 = {}^1/_7$, $9 = {}^1/_8$, $10-12$, 16 und 17, sowie die mittlere Seitenrandborste des Frontoclypeus etwa $^1/_{10} - {}^1/_{11}$ der Kopfbreite). Sie ist zugleich die schlankeste Borste des Kopfes, deutlich dünner als 16, obwohl doppelt so lang wie diese. Im ersten Stadium ist 15 dreimal so lang und etwas dicker als 16; im 2. Stadium sind beide Borsten ungefähr gleich dick. Die laterale Vorderrandborste des Frontoclypeus sowie seine orale und anale Seitenrandborste sind flachliegend (letztere rückwärtsgestreckt), ebenso — wenn auch nicht ganz typich — Borste 13, die vorwärtsgestreckt ist. Im 1.—4. Stadium sind die mediane und die mittlere Vorderrandborste des Frontoclypeus gelb und von normalem Bau, im 5. Stadium dagegen dunkel, jedoch mit blasser, schwach besenförmiger Spitze (diese ist in 2 oder wenige Zweige geteilt). Die ventrale Borste (18) ist sehr kurz, aber dick ($24 \times 3\,\mu$, d. h. $^1/_{33}$ der Kopfbreite).

Die dorsale Grube zwischen den Mandibelgelenkborsten hat die Form einer schmalen (etwa $13 \times 1{,}5\,\mu$), senkrechten Spalte. Schon im 1. Stadium ist sie etwas oval; ihre typische Form entwickelt sich jedoch erst auf den späteren Stadien. Am Auge findet sich nur eine Grube, die etwas innerhalb der Kante des oralen Kiels liegt. Die Grube zwischen Borste 14 und 15 liegt etwas innerhalb der Kante des analen Kiels, mitten zwischen den beiden Borsten, während die anale Grube vor Borste 17 liegt. An der Basis des Zapfens am ventralen Mandibelgelenk 4 Gruben; 3 mediane (auf Abb. 3 D von der Cardo verdeckt) und 1 laterale. Die ventrale Grube liegt ganz nahe der Gularsutur, und zwar an ihrem analen Ende. Die occipitalen Börstchen sind $7{,}5 \times 1{,}5\,\mu$ gross. Die 3 dorsalen derselben und die occipi-

tale Grube stehen in einer schrägen Reihe, letztere am weitesten median und anal, am Rand des Foramen occipitis und daher etwas schwer zu sehen.

Im 1. Stadium ist die Stellung der dorsalen Borsten und Gruben (Abb. 4 B) etwas anders als später, besonders sind die Borsten 15 und 16 viel näher an 14 herangerückt, und die anale Grube liegt medioanal von Borste 17. Im 2. Stadium liegt sie median, im 3. medio-oral von dieser Borste.

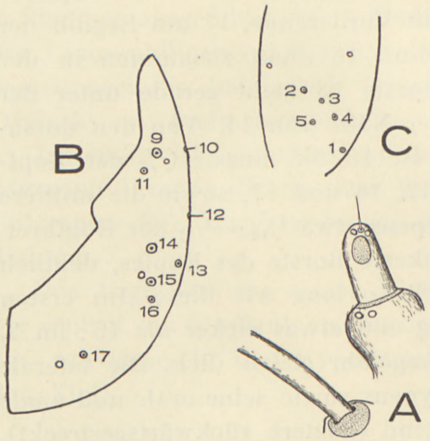

Abb. 4. A. Linke Antenne und Basis der Borste 7, von links gesehen. $^{430}/_{1}$. Orale Richtung nach links. — B. Stellung der dorsalen Borsten und Gruben des Epicraniums im 1. Stadium, schematisch dargestellt. $^{250}/_{1}$. — C. Borsten und Gruben des Pronotums im 1. Stadium, halb von der Dorsalseite und halb von rechts gesehen, schematisch dargestellt. $^{250}/_{1}$. Orale Richtung nach rechts. Die Zahlen geben die Nummern der Borsten an; bei C: 1 Eckborste, 2—5 Flächenborsten. — Vgl. Text.

Die Antenne (Abb. 4 A) sitzt etwas unterhalb des oralen Kiels und ein wenig hinter dem dorsalen Mandibelgelenk. Sie trägt die beiden gewöhnlichen Gruben. Das Sinnesstäbchen ist vom gewöhnlichen *Limnophilinen*-Typus, im 5. Stadium 24 × 11 µ gross, in den früheren Stadien verhältnismässig länger. Im 1. Stadium ist es umgekehrt konisch, 18,5 µ lang, an der Basis 4,25, distal 6 µ dick. Seine Entwicklung gleicht der bei den *Limnophilinen*, jedoch bleibt die Borste, die median steht, in allen Stadien erhalten; im 5. Stadium ist sie 10 × 0,75 µ gross. Sowohl oral wie anal von ihr sieht man eine Grube.

Das Labrum (Abb. 5) ist dadurch ausgezeichnet, dass seine rechte Seite stärker entwickelt ist als seine linke. Es ist etwa 2,4 mal so breit wie lang und etwa 4,6 mal so breit wie hoch. Sein dicker Vorderrand ist leicht, aber breit eingebuchtet, die Ventralseite schwach konkav. Das Sklerit reicht überall, ausgenommen eine schmale Partie an den Vorderecken, bis zum Rand und erstreckt sich an der Einbuchtung des Vorderrandes sogar ein Stück weit auf die Ventralseite. Hier ist es (sowohl

dorsal wie ventral) inwendig verdickt, ebenso längs des Seitenrandes. Der Rand ist bei der vorderen Randborste geknickt, jedoch wird dieser Knick einigermassen durch eine wagerechte, sklerotisierte Lamelle verdeckt, die — von der Ventralseite gesehen — den Ursprung der Borste verbirgt. Die Tormae sind recht schlank.

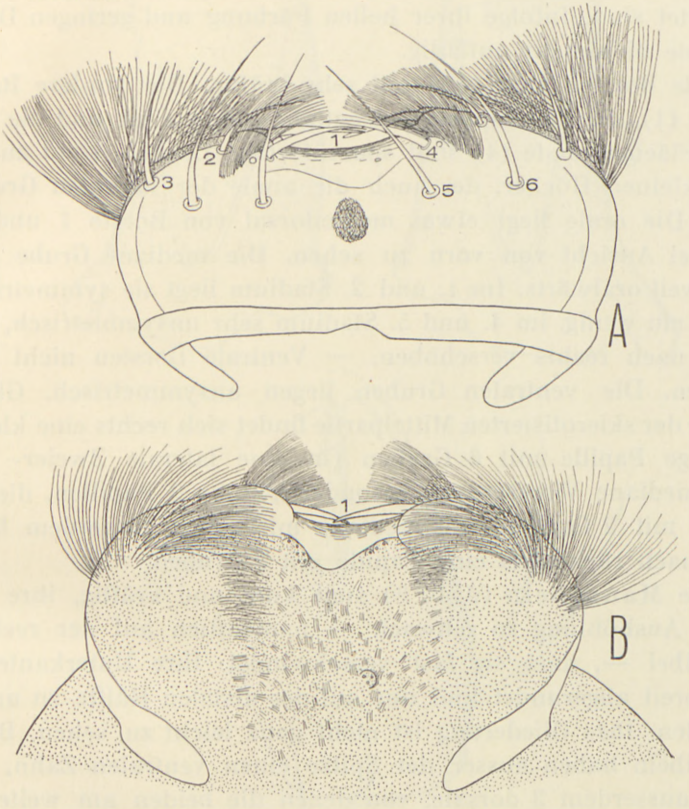

Abb. 5. Labrum von der Dorsal- (A) und Ventralseite (B). $^{160}/_{1}$. Bei B ist gleichzeitig der Anteclypeus dargestellt. Die Zahlen geben die Nummern der Borsten an.

Auf der Dorsalseite findet sich oral — von der Basis der Borste 1 bis zur Mitte zwischen dieser und Borste 6 — ein breiter Streifen von 82×1 µ grossen Haardornen, die so dicht stehen, dass sie sich am Grunde beinahe berühren. Diese Haardornen fehlen auf dem 1. und 2. Stadium. Auf den Vorderecken findet sich sowohl dorsal (auf dem weichen Stück) wie ganz besonders ventral ein breiter Besatz ebenso dichtstehender Haardornen,

die lateral 100 × 1,2 μ gross, median nur 45 μ lang sind. Hier setzt sich das Feld in einem Längsbesatz kürzerer (28 μ), einwärts gerichteter Haardornen fort, der auf der rechten Seite stärker entwickelt ist als auf der linken. Analwärts gehen die Haardornen in Längsreihen sehr feiner, etwa 18 μ langer Spitzchen über; diese bekleiden auch den mittleren Teil der Lippe, wo sie rückwärts gerichtet sind. Infolge ihrer hellen Färbung und geringen Dicke sind sie nicht sehr auffällig.

Alle Borsten sind gelb und sehr kräftig. Die vordere Randborste (1), die den Lippenrand entlang gebogen ist, und die vordere Flächenborste (4) sind sporenförmig. Letztere steht auf einem kleinen Höcker, der auch die anale der lateralen Gruben trägt. Die orale liegt etwas mediodorsal von Borste 1 und ist nur bei Ansicht von vorn zu sehen. Die mediane Grube liegt sehr weit oralwärts. Im 1. und 2. Stadium liegt sie symmetrisch, im 3. ein wenig, im 4. und 5. Stadium sehr unsymmetrisch, und zwar nach rechts verschoben. — Ventrale Borsten nicht vorhanden. Die ventralen Gruben liegen unsymmetrisch. Gleich hinter der sklerotisierten Mittelpartie findet sich rechts eine kleine, niedrige Papille mit 6 Gruben (in eine laterale Zweier- und eine mediane Vierergruppe gesondert), links 2 Papillen, die laterale mit 2 Gruben, die mediane mit einer; ausserdem links weit nach rückwärts eine Papille mit 3 Gruben.

Die Mandibeln (Abb. 6) sind kurz und kräftig, ihre mediane Aushöhlung so schwach — namentlich auf der rechten Mandibel —, dass sie fast verschwindet. Ihre Unterkante ist sehr breit abgerundet und nur auf der distalen Hälfte zu unterscheiden. Ihre Gliederung ist nicht ganz leicht zu sehen. Beide Mandibeln haben ausser der Spitze einen ventralen Zahn, die linke ausserdem 3 dorsale, von denen die beiden am weitesten dorsalen sehr kurz, abgerundet und wenig deutlich sind; die rechte Mandibel trägt 2 dorsale Zähne, die beide deutlich, wenn auch stumpf sind. Die Zähne sind häufig stark abgenutzt. Die Innenseite der linken Mandibel trägt distal 3 Längsrippen, die von der Spitze, dem ventralen und dem einen dorsalen Zahn entspringen.

Auf den späteren Stadien erscheint die Aussenseite des proximalen Gliedes (ausgenommen ganz proximal) matt durch dichtsitzende, 5,5 μ breite, flache Knötchen. Ferner findet sich eine

dorsale Innenbürste, bestehend aus einem ganz schmalen Besatz (etwa 2 Reihen) von Haardornen. Merkwürdigerweise fehlt die Innenbürste im 1. Stadium. Die Rückenborsten stehen ziemlich

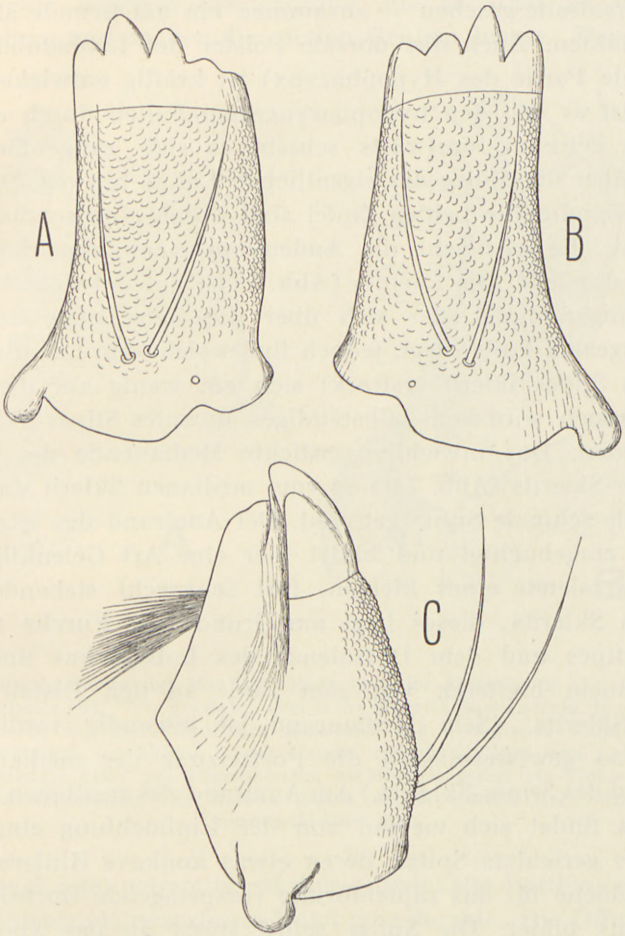

Abb. 6. Mandibeln. Linke (A) und rechte (B) von der Lateralseite, linke (C) von der Ventralseite. $^{160}/_{1}$.

proximal, ungefähr über einander, die dorsale jedoch am weitesten distal; beide sind kräftig und blass. Die Grube findet sich nahe am dorsalen Gelenk.

Das Maxillolabium erinnert seiner äusseren Form nach sehr an das der *Limnophilinen*, jedoch ist die Lateralseite des Stipes breit abgerundet und die proximale Partie des Hypopharynx

sehr stark entwickelt, wie ein Paar hohe, nur oberflächlich getrennte Längsfalten (Abb. 8). Vorn werden sie von einem Paar (etwas geknickter) gelbbrauner Sklerite eingerahmt, die — von der Dorsalseite gesehen — zusammen ein am Grunde abgeflachtes U bilden. Auch das dorsale Polster des Labiallobus (d. h. die orale Partie des Hypopharynx) ist kräftig entwickelt. Analwärts ist es von den hypopharyngealen Falten durch eine tiefe Furche getrennt, oralwärts schiebt es sich zungenförmig ein wenig über die Spitze des eigentlichen Lobus hinweg. Die Zunge ist zweizipflig, die beiden Zipfel sind durch eine schmale Partie getrennt, die vielleicht die Andeutung eines unpaaren Mittelzipfels darstellt. Die Lacinia (Abb. 7) ist gut entwickelt als zungenförmiger Zipfel, der sich über die Lateralseite der hypopharyngealen Falten legt, jedoch findet sich kein Lacinia-Sklerit.

Das Stipes-Sklerit erstreckt sich ein wenig auf die Dorsalseite, jedoch wird kein selbständiges, dorsales Stipes-Sklerit ausgeschieden. Das inwendig verdickte Medianende des lateralen Palpifer-Sklerits (Abb. 7 B) ist vom medianen Sklerit durch eine ziemlich schmale Sutur getrennt. Der Analrand des letzteren ist lateral eingebuchtet und bildet hier eine Art Gelenkfläche für das Dorsalende eines kleinen, fast senkrecht stehenden, dreieckigen Sklerits; dieses liegt am Grunde der Furche zwischen dem Stipes und dem Dorsalende des Labiallobus und grenzt mit seinem breiteren ventralen Ende an den Distalrand des Stipes-Sklerits. (Sein Medianrand ist inwendig verdickt und bildet so gewissermassen die Fortsetzung der medianen Verdickung des Stipes-Sklerits.) Am Analrand des medianen Palpifer-Sklerits findet sich median von der Einbuchtung eine grosse, abwärts gerichtete Spitze, deren etwas konkave Hinterseite eine Gelenkfläche für das zapfenförmig vorspringende Dorsalende des Mentums bildet. Die Spitze selbst stösst an das knopfförmig verdickte Analende des kleinen, stäbchenförmigen, dorsoanalen Sklerits auf dem Labiallobus. Schliesslich legt sich das mediane Ende des Palpifer-Sklerits über das hypopharyngeale Sklerit hin. Auf diese Weise wird eine komplizierte Gelenkverbindung zwischen den verschiedenen Teilen des Maxillolabiums hergestellt. Das 1. und 2. Glied des Maxillarpalpus sind median offen. Das Sklerit der Galea ist ziemlich rückgebildet und bedeckt nur die Lateralseite und etwa die proximale Hälfte der Ventralseite.

Auf dem Labiallobus (Abb. 8) findet sich ausser den gewöhnlichen Skleriten ein unpaares ventrales Stäbchen, das nicht ganz so deutlich ist wie die paarigen. Der Analrand des Mentums trägt ventral eine unpaare Einbuchtung, die so breit ist wie der Labiallobus, und in der die analen Gruben liegen. Das Sklerit

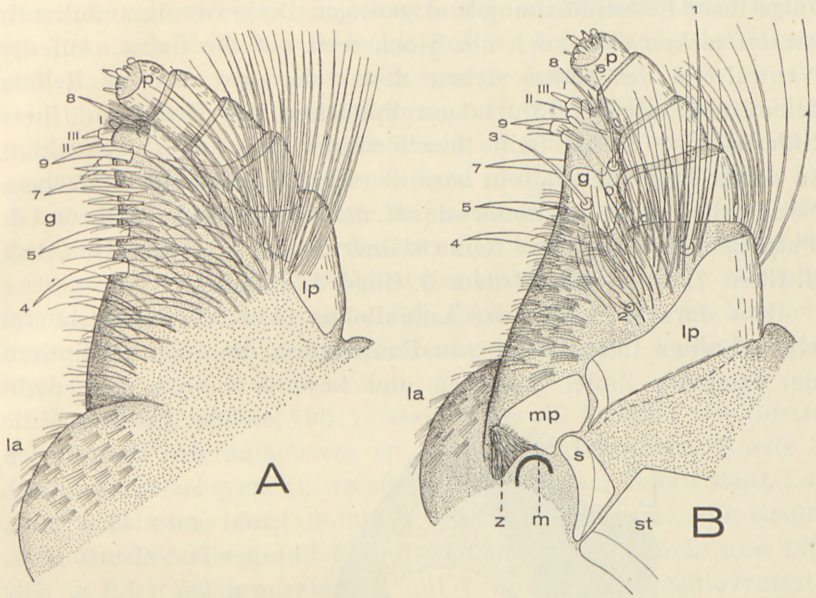

Abb. 7. Distale Partie der Maxille, rechte von der Dorsalseite (A), linke von der Ventralseite (B). $^{340}/_{1}$. g = Galea (bei B ihr Sklerit), la = Lacinia, lp = laterales Palpiferskleri t, m = Dorsalende des Mentums, mp = medianes Palpiferskleri t, p = 4. Palpalglied, s = Sklerit in der Furche zwischen Stipes und Labiallobus, st = mediodistale Ecke des Stipesskleri ts, z = abwärts gerichtete Spitze des medianen Palpiferskleri ts. Die Zahlen geben die Nummern der Borsten und Sinnesstäbchen an.

besitzt anal zwei getrennte, stärker entwickelte Partien, eine unpaare ventrale (schwarzbraun) und eine dorsale. Die Öffnung der Spinndrüsen ist von feinen, konzentrischen Furchen umgeben.

Die weiche Dorsalseite des Palpifer (Abb. 7 A) und die proximale Hälfte der Dorsalseite der Galea sind von Haardornen bekleidet, die längs einer schrägen Linie proximal vom Palpus besonders kräftig (median 64, lateral 92 × 2,5 µ) und dichtgestellt sind. Im übrigen sind sie median am kürzesten und dicksten (46 × 1,2 µ), lateral am längsten und dünnsten, proximal klein und dünn. Median auf der Galea findet sich eine Reihe von be-

sonders kräftigen, fast dornförmigen Haardornen (28 × 3 µ). Auf der weichen Ventralseite des Palpifer (Abb. 7 B) stehen proximal vom Palpus Haardornen, die distal am grössten (66 × 1,2 µ), proximal am kleinsten (46 × 0,6 µ) sind; median stehen in kleinen Längsreihen etwa 18,5 µ lange, haarförmige Spitzchen. Diese sind infolge ihrer hellen Färbung und geringen Dicke wenig auffallend; sie erstrecken sich auch ein Stück weit auf die Galea. Auf der Ventralseite der Galea stehen distal ein paar schräge Reihen feiner, aber steifer, 13 µ langer Spitzchen, am Distalrand ihres Sklerits eine dichte Reihe haarförmiger (16 × 0,5 µ) Spitzchen; in dieser Reihe ventral ein besonders kräftiges, steifes Spitzchen. Schliesslich steht mediodorsal auf dem Distalrand des 2. und 3. Palpalgliedes eine kurze Reihe kleiner, steifer Spitzchen (auf dem 2. Glied 7,5 × 0,4 µ, auf dem 3. Glied 4,5 µ lang).

Das dorsale Polster des Labiallobus (Abb. 8) besitzt lateral einen breiten Längsbesatz von Haardornen, die auch die Spitzen der lateralen Zipfel bedecken und besonders anal sehr dicht stehen. Auf der Mitte sind die Haardornen am grössten (82 × 1,2 µ), anal nur 46 µ lang, aber fast ebenso dick, oral 55 × 0,6 µ. Die Analenden dieser Besätze sind durch einen (anal konkav) gebogenen Querbesatz schlanker Spitzchen verbunden, die auf der Mitte am stärksten (28 × 1,2 µ) sind. Lateral werden sie bedeutend dünner, ganz lateral auch viel kürzer.

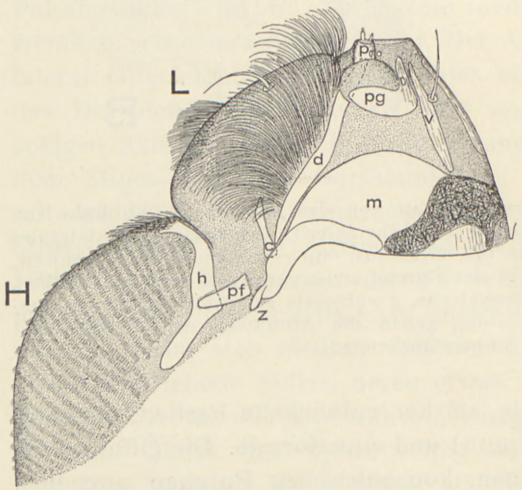

Abb. 8. Labiallobus (L) und hypopharyngeale Falten (H) von rechts gesehen. 160/1. c = dorsoanales Sklerit, d = dorsales Stäbchen, h = hypopharyngeales Sklerit, m = Mentum, pf = (eingezeichnetes) medianes Ende des medianen Palpifersklerits, p = Palpus, pg = Palpiger, v = ventrales Stäbchen, z = zapfenförmig vorspringendes Dorsalende des Mentums, das eine Art Gelenkverbindung mit der abwärts gerichteten Spitze des medianen Palpifersklerits bildet.

Die hypopharyngealen Falten sind mit rückwärts gerichteten Spitzchen in fast ungebrochenen Querreihen

bekleidet. Die mediooralen Spitzchen sind die grössten (22 × 1 μ), anal- und lateralwärts werden sie rasch kleiner. Die Lacinia trägt kleine schräge Reihen von 13 μ langen, sehr feinen, aber steifen Spitzchen.

Die Ventralseite von Submentum und Stipes ist vor den Skleriten mit vereinzelten, blassen kleinen, undeutlichen, körnchenförmigen Knötchen bedeckt; auf der Lateralseite des Stipes sind sie grösser, aber auch hier sehr undeutlich.

Alle Borsten des Maxillolabiums sind hell. Die Borste des Submentums und die laterale Borste des Stipes, die beide auf dem Rand der betr. Sklerite stehen, sind die grössten (etwa $^1/_5$ der Kopfbreite); die mediane Stipesborste ist etwas kleiner. Die Cardo trägt nur eine Borste, die kurz ($^1/_{11}$ der Kopfbreite), aber sehr kräftig und etwas besenförmig ist (im 1. Stadium normal); sie steht am Lateralende des Sklerits. Die Grube des Stipes ist länglich oval und liegt auf der Lateralseite, nahe dem Analrand des Sklerits.

Auf dem Palpifer (Abb. 7) stehen sowohl Borste 1 wie Borste 2 vor dem Sklerit; letztere ist ein kleines Börstchen. Borste 3 ist auf die Galea gerückt, wo sie median auf dem Sklerit steht. Lateral auf diesem steht Borste 6 (die 3 gleicht) oral, die Grube anal. Die Borsten 4 und 5 sind weit oralwärts gerückt und bilden auf der Galea zusammen mit 7 und 9 eine medioventrale Längsreihe von 4 kräftigen Sporen. Borste 8, die laterodistal von dieser Reihe steht, ist gleichfalls spornförmig, jedoch nicht so kräftig. Auf dem 1. Palpalglied liegt die eine Grube lateral, die andere, viel kleinere, medioventral; beide liegen proximal. Auf dem 2. Glied findet sich eine ventrale Grube am Proximalrand, auf dem 3. eine medioventrale, proximale Grube; die Grube auf dem 4. Glied liegt laterodorsal. Die zweigliedrigen Sinnesstäbchen mit langem Basalglied besitzen ein kurzes Distalglied. Das eine ungefähr mitten auf dem Distalende des Palpus stehende Stäbchen ist grösser als die beiden anderen. Die Stäbchen mit kurzem Basalglied besitzen lange, zylindrische Distalglieder mit abgerundeter Spitze. Das laterale eingliedrige Stäbchen ist bläschenförmig, die 5 anderen stäbchenförmig. Das laterale der dorsalen und das mediane der ventralen Stäbchen sind bedeutend grösser als die übrigen. Von den Sinnesstäbchen der Galea ist das laterale I grösser als das mediane; II hat kurze Proximal-

glieder und ziemlich lange Borsten, III lange zylindrische Proximalglieder und schlanke, zapfenförmige Distalglieder.

Beide Borsten auf dem Labiallobus (Abb. 8) sind klein und dick. Die dorsale Borste ist stark rückwärts gebogen. Die Grube des Palpus liegt lateroventral. Das dorsale der zweigliedrigen Sinnesstäbchen ist erheblich grösser als das ventrale; das eingliedrige (medioventrale) ist sehr klein.

Das Pronotum (Abb. 3 A, B) ist in seiner ganzen Länge ungefähr gleich breit. Die Vorderecke ist so stark abgerundet, dass sie fast verschwindet, die Hinterecke nur klein. Die anale Querfurche, die nicht sehr tief ist, wird der ganzen Länge nach von einer inwendigen Leiste begleitet, die jedoch auf der Dorsalseite niedrig, breit und stark abgerundet ist. Auf der Dorsalseite wird die vordere, tieferliegende Partie durch einen halbmondförmigen (oral konkaven) Querkiel von der hinteren, höherliegenden getrennt. Ganz medial ist der Kiel nicht so hoch wie weiter lateral, da sich dort die orale Partie der Dorsalseite etwas gegen ihn aufbaucht (auf Abb. 3 B wird dies durch die weiter lateralen Partien der Leiste verdeckt). Inwendig macht sich dieser Kiel nur dadurch bemerkbar, dass die Innenseite auf einem breiteren Stück etwas verdickt ist. Im 1. Stadium ist der Kiel noch nicht entwickelt, dagegen — ungewöhnlicherweise — bereits in diesem Stadium die unpaare Längssutur.

Der Trochantinus bedeckt die ziemlich schmale Vorderseite des Pleurons, erstreckt sich jedoch nicht auf die Medianseite. Ventral ist er in ein kurzes, schnabelförmiges Horn ausgezogen, das auf der Hinterseite (oder Ventralseite) weich ist. Der Trochantinus ist mit dem Episternum verschmolzen; beide zusammen sind — von der Seite gesehen — ungefähr trapezförmig (vorn am breitesten) und weit grösser als das kleine, dreieckige Epimeron.

Das Mesonotum (Abb. 9 B) ist wohl entwickelt, lässt jedoch grosse Teile des Dorsum frei, dessen Rand es nur an den Vorderecken erreicht. Der laterale, abwärts gerichtete Zapfen, der sich bei den *Limnophilinen* findet, fehlt hier ganz. Jede Hälfte des Notums wird durch eine im 1. Stadium noch fehlende Längssutur in ein grösseres medianes und ein kleineres laterales Sklerit geteilt; eine äusserst feine, in der Mitte braune Furche auf dem letzteren deutet dessen Teilung in eine grössere medio-

orale und eine kleinere lateroanale Partie an. So viel ich sehen kann, ist die unpaare Längssutur bereits im 1. Stadium ausgebildet und obendrein um ein Mehrfaches breiter als später.

Das Metadorsum, dem die halbmondförmige Querfurche

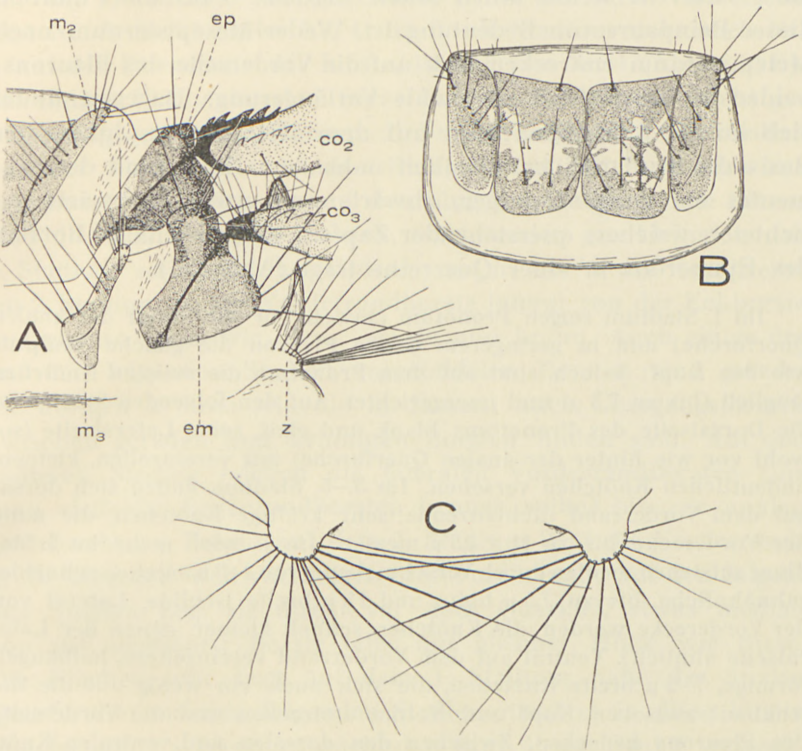

Abb. 9*. A. Meso- und Metathorax von rechts gesehen. Beine nahe der Basis abgeschnitten. B. Mesodorsum von der Dorsalseite. C. Zapfen, auf denen die analen Borsten der Metepimera stehen, von vorn gesehen. $^{40}/_{1}$. co_2 = proximaler Teil der Mittelhüfte, co_3 = proximaler Teil der Hinterhüfte, em = Metepimeron, ep = Mesepisternum, m_2 = Lateralsklerit des Mesonotums, m_3 = Lateralsklerit des Metadorsums, z = Zapfen, der die analen Borsten des Metepimerons trägt.

fehlt, besitzt wie bei den *Limnophilinen* ein Paar laterale und ein Paar medioanale Sklerite, von denen letztere länglich sind und quer stehen; dagegen fehlen medioorale Sklerite. Auf dem lateralen (Abb. 9 A) findet sich eine feine, braune Furche, die vollständig der auf dem Mesonotum entspricht.

Auf dem Mesopleuron, noch mehr auf dem Metapleuron (Abb. 9 A) verläuft die Furche zwischen Episternum und Epi-

meron sehr schräg. Hierdurch sowie dadurch, dass Metadorsum und der ziemlich scharfe Vorderrand des Metapleurons sich etwas über das Mesopleuron hinwegschieben, erhalten die Mittel- und noch mehr die Hinterbeine eine ziemlich orale Stellung, die — wie wir weiter unten sehen werden — für die Funktion dieser Beinpaare von Bedeutung ist. Weder Mesepisternum noch Metepisternum erstrecken sich auf die Vorderseite des Pleurons; beiden Epimera fehlt die anale Verlängerung. Dagegen findet sich im 3.—5. Stadium anal auf dem Metapleuron, hinter der Basis des Hinterbeines, ziemlich nahe dem Hinterrand des Segmentes ein zungenförmiger, abwärts und etwas rückwärts gerichteter, weicher, querstehender Zapfen, der die analen Borsten des Epimerons in einer Querreihe trägt (Abb. 9 C).

Im 1. Stadium zeigen Pronotum (sowohl vor wie hinter der analen Querfurche) und in geringerem Grade Pleuron die gleiche Skulptur wie der Kopf; jedoch sind auf dem Pronotum die meisten Knötchen länglich (bis zu 7,5 µ) und quergerichtet. Auf den folgenden Stadien ist die Dorsalseite des Pronotums blank und glatt, seine Lateralseite (sowohl vor wie hinter der analen Querfurche) mit vereinzelten, kleinen, undeutlichen Knötchen versehen. Im 3.–5. Stadium finden sich dorsal auf dem Vorderrand dichtsitzende, sehr kräftige Knötchen, die nahe der Vorderecke bis zu 11 × 4,5 µ messen. Im 4., noch mehr im 5. Stadium entstehen median durch Verschmelzung von 2–3 Knötchen schneidezahnähnliche, bis zu 7,5 µ hohe und 11 µ breite Gebilde. Lateral von der Vorderecke werden die Knötchen schnell kleiner (denen der Lateralseite ähnlich). Ventral auf dem Vorderrand vereinzeltere, halbkugelförmige, 7–9 µ breite Knötchen, die sich auch ein wenig auf die Gelenkhaut zwischen Kopf und Notum erstrecken und die Vorderseite des Pleurons bedecken. Zwischen den dorsalen und ventralen Knötchen (jedoch nicht lateral von der Vorderecke) findet sich ein sehr schmaler, aber dichter Besatz von äusserst feinen, haarförmigen Spitzchen (22 × 0,4 µ). Diese treten bereits im 2. Stadium in Gestalt einiger weniger Querreihen mit je etwa 4 Spitzchen auf. Im 4. und 5. Stadium ist die weiche Hinterseite des Propleurons mit vereinzelten Kämmen aus vorwärts und abwärts gerichteten, blassen Spitzchen (3,5 × 0,4 bis 5,5 × 0,7 µ) versehen. Die Kämme erstrecken sich auch auf das Epimeron, sind aber hier als flache Knötchen ohne Spitzchen ausgebildet.

Die längsten Borsten des Pronotums sind die Eckborste und die mediane Flächenborste (etwa $^3/_{10}$ der Kopfbreite); beide sind braun, während die anderen primären Borsten blass sind. Diese sind im 1. Stadium (Abb. 4 C) in einem ungefähr gleichseitigen Dreieck laterooral von der medianen Flächenborste

(Nr. 2) angeordnet. Von ihnen ist Nr. 3 flachliegend (für eine Thoracalborste sehr ungewöhnlich), nach innen gebogen, Nr. 4 ungefähr ebenso lang wie Nr. 2, jedoch viel dünner, Nr. 5 ganz klein ($^1/_{12}$ der Kopfbreite). Im 2. Stadium stehen Nr. 2 und 4 auf der Dorsalseite der Querleiste, Nr. 3 auf ihrer Vorderseite und Nr. 5 direkt hinter Nr. 4. Im 3.—5. Stadium steht auch Nr. 5 auf der Dorsalseite der Leiste, lateral von Nr. 4 (Abb. 3A, B). Sekundäre Flächenborsten (oder Eckborsten) werden nicht gebildet, dagegen vom 2. Stadium ab Vorderrandborsten. Die median von der Eckborste stehenden Borsten sind gelb und einwärts gekrümmt (am stärksten auf den jüngeren Stadien); von diesen sind die medianen am grössten, die lateralen am kleinsten. Im 5. Stadium schwankt ihre Grösse von $^1/_{12}$ bis $^1/_5$ der Kopfbreite. Im 2. Stadium ist die Vorderrandborste lateral von der Eckborste fast ebenso gross wie diese, gerade und braun. Auch einige der auf den folgenden Stadien ausgebildeten Borsten sind gerade, die übrigen krumm wie die medianen; auch Übergangsformen zwischen geraden und krummen Borsten finden sich. Auf den späteren Stadien reichen die lateralen Vorderrandborsten (die man vielleicht Seitenrandborsten nennen sollte) bis zur analen Querfurche. — Schon auf dem 1. Stadium liegt die Vorderrandgrube ziemlich weit rückwärts vom Vorderrand; auf den folgenden Stadien liegt sie direkt vor dem Querkiel, etwas median von der medianen Borste oder auf gleicher Höhe wie diese. Die Flächengrube liegt auf dem 1. Stadium nahe der analen Querfurche; auf den folgenden Stadien rückt es in diese hinein und ist daher schwer wahrnehmbar (aus diesen Grund verwechselte SILTALA — 13, S. 542 — die Vorderrandgrube von *Brachycentrus* mit der Flächengrube). Im übrigen sind Unregelmässigkeiten in der Anordnung der Borsten nicht selten, so dass sie gelegentlich im 4. Stadium noch ungefähr dieselbe Stellung haben können wie im zweiten.

Abnormitäten: Bei einem Exemplar im 4. Stadium stand Borste Nr. 5 auf dem Rand der Alveole von Nr. 4, median von dieser Borste; bei einem anderen Exemplar des gleichen Stadiums war eine der medianen Vorderrandborsten als kurzer Sporn entwickelt.

Die Börstchen des Trochantinus stehen auf dem Medianrand des Sklerits, das eine auf der Mitte, das andere ventral. Letzteres ist im 5. Stadium 27 × 1,4 µ gross, das dorsale nur halb so gross;

beide sind braun. Die Borste des Trochantinus steht ziemlich ventral auf der Vorderseite des Sklerits; ihre Länge beträgt im 1. Stadium $^3/_{10}$ der Kopfbreite; während der folgenden Häutungen nimmt ihre relative Länge beträchtlich ab (vom 1. zum 2. Stadium sogar auch ihre absolute Länge), so dass sie im 5. Stadium nur $^1/_8$ der Kopfbreite beträgt. Die Borste des Epimerons steht ventral nahe der Furche; ihre Länge beträgt in allen Stadien etwa $^1/_8$ der Kopfbreite. Auf dem 1. Stadium ist die Borste von normaler Form, auf den folgenden Stadien schwach besenförmig, ihre Spitze in zwei oder wenige Äste verzweigt.

Im ersten Stadium besitzt das mediane Sklerit des Mesonotums auf seiner oralen Hälfte eine ähnliche (wenn auch viel weniger deutliche) Skulptur wie der Kopf, während der übrige Teil dieses Dorsums glatt ist. Im 2. Stadium fehlt diese Skulptur, jedoch sieht man auf dem weichen Teil vor den Skleriten sehr feine, blasse, körnchenförmige Knötchen. Im 3.–5. Stadium finden sich zwischen diesen grössere, halbkugelige Knötchen von 5,5 μ oder — bei Verschmelzung zweier Knötchen — grösserer Breite. Sie bekleiden auch das allervorderste Stück der Sklerite; auf dem lateralen Sklerit sind sie niedrig konisch (9 μ hoch), jedoch mit abgerundeter Spitze. In der Verlängerung der medianen Sutur des Mesonotums sieht man auf der weichen Partie einen glatten Streifen. Das Metadorsum ist auf allen Stadien glatt.

Die beiden hinteren Pleuren sind auf dem 1. und 2. Stadium glatt. Auf den folgenden Stadien sind die weichen Innenseiten der Episterna und die weiche Hinterseite des Mesopleuron mit kleinen, blassen Spitzchen (5,5×0,75 μ) versehen; diese erstrecken sich ein Stück weit auf das Epimeron, wo sie nach vorwärts gerichtet und zu Kämmen mit bis zu 7 Spitzchen verbunden sind; (im 3. Stadium finden sich nur die Kämme auf dem Mesepimeron).

Auf den späteren Stadien rücken die präsegmentalen Börstchen des Mesodorsums (Abb. 9 B) so dicht zusammen, dass sie im 4. und 5. Stadium in einer Gruppe vor der lateralen Sutur des Notums stehen. Das eine (laterale) ist 44 × 3,2 μ gross (d. h. $^1/_{17}$ der Kopfbreite), die beiden anderen 27 × 2,5 μ. Auf dem Metadorsum sind sie 16,5 μ lang. Die Börstchen der Pleuren stehen ziemlich weit hinter einander auf der weichen Innenseite der Episterna (das orale wohl eher auf der Vorderseite); sie sind 31 × 2 μ gross ($^1/_{25}$ der Kopfbreite).

Die Eckborsten der beiden Dorsa (Abb. 9 A, B) stehen auf dem Vorderende der lateralen Sklerite. Auf dem Mesodorsum stehen die orale Borste nahe dem Vorderrande des medianen

Sklerits, die analen Borsten und die Grube anal auf diesem. Auf dem Metadorsum stehen die analen Borsten und die Grube auf dem medioanalen Sklerit. Auf allen Stadien sind Eckborste und medioanale Flächenborste lang und dunkel, auf dem Mesodorsum betragen sie knapp die Hälfte, auf dem Metadorsum etwa $^3/_4$ der Kopfbreite. Auf dem 1. Stadium ist die orale Borste sehr klein und blass, aber verhältnismässig dick; auf dem Metadorsum behält sie diese Form, so dass auf dem 5. Stadium ihre Länge nur $^1/_{16}$ der Kopfbreite beträgt. Auf dem Mesodorsum dagegen wächst sie während der folgenden Häutungen stark und erscheint im 3.—5. Stadium als kräftige, braune Borste ($^1/_3$ der Kopfbreite). Die beiden anderen analen Borsten, die ebenso lang sind wie die orale Borste des Metadorsums, aber dünner als diese, stehen auf dem ersten Stadium hinter einander und lateral von der grossen Borste; die Grube liegt median von dieser. Auf den folgenden Stadien rücken die kleinen Borsten und die Grube auf dem Mesonotum etwas oralwärts, so dass sie auf den späteren Stadien weit vor der grossen Borste stehen. Gleichzeitig ändert sich gewöhnlich die Stellung der kleinen Borsten zu einander, so dass sie nun neben einander zu stehen kommen. Auch auf dem Metadorsum rücken sie etwas oralwärts und stehen auf den späteren Stadien auf der lateroooralen Ecke des Sklerits, während die Grube ungefähr auf der Mitte seiner Vorderkante liegt. Neben der Eckborste und der grossen medioanalen Borste entstehen sekundäre Borsten, die ungefähr ebenso gross sind wie die primären. Die Eckborsten stehen auf dem Vorderende der lateralen Sklerite und von hier aus in einer unregelmässigen Reihe bis zum analen Drittel des Medianrandes; die analen Borsten stehen in einer Querreihe, and zwar auf dem Mesodorsum längs des Hinterrandes des medianen Sklerits, auf dem Metadorsum auf dem medioanalen Sklerit. Vom 3. Stadium ab tritt konstant eine kleine, blasse Eckborste (nicht grösser als die orale Borste des Metadorsums) auf, die ziemlich oral auf dem Lateralrand der Sklerite steht und gerade auswärts gerichtet ist. Sie fehlt zuweilen auf dem Mesonotum (bei etwa 4 % der Larven) und noch häufiger auf dem Metadorsum (16 %). Die beiden Seiten ein und desselben Tieres können sich in dieser Beziehung verschieden verhalten.

Die Borste des Episternums steht ungefähr mitten auf dem

Vorderrand des Sklerits, die des Epimerons anal auf der Ventralkante. Die Borsten beider Episterna sowie des Metepimerons sind auf allen Stadien lang und schwarz (Länge: Mesepisternum $^1/_3$—$^2/_5$, Metepisternum $^3/_5$, Metepimeron $^3/_4$ der Kopfbreite). Im ersten Stadium ist die Borste des Mesepimerons klein und blass ($^1/_8$ der Kopfbreite, vgl. orale Borste des Mesonotums), während sie auf den folgenden Stadien an Länge zunimmt, so dass sie im 4. und 5. Stadium ebenso lang ist wie die des Episternums; zugleich wird sie dunkel. Auf beiden Episterna und auf dem Metepimeron werden sekundäre Borsten gebildet (Abb. 9 A. Sehr selten sieht man — auf dem 5. Stadium — eine sekundäre Borste auf dem Mesepimeron). Die sekundären Borsten sind ungefähr ebenso lang wie die primären; jedoch ist die auf dem Metepisternum zuerst gebildete (2. Stadium), die dorsal von der primären Borste steht, nur halb so lang wie diese (auf den jüngeren Stadien verhältnismässig kürzer), aber ziemlich dick. Die anderen sekundären Borsten auf dem Metepisternum sowie alle auf dem Mesepisternum stehen in einer Reihe anal und ventral von der primären. Auf dem Metepimeron stehen die oralen Borsten in einer Reihe vor der primären, längs der analen $^3/_5$ der Ventralkante des Sklerits. Im 2. Stadium stehen die analen Borsten neben einander auf der weichen Hinterseite des Pleurons. Auf den folgenden Stadien bildet die Partie, auf der sie stehen, einen zungenförmigen, querstehenden, abwärts gerichteten Zapfen, der vom eigentlichen Pleuron abgesondert ist; die Borsten bilden auf ihm eine Querreihe (Abb. 9 C). Sie sind lang und kräftig, die medianen am längsten, and zwar länger als die Kopfbreite. die lateralen wesentlich kürzer. Die medianen Borsten sind einwärts gerichtet und kreuzen sich daher mit den Borsten der anderen Seite; die mittleren Borsten sind abwärts, die lateralen auswärts und vorwärts gerichtet. Die hintersten der oralen Borsten sind abwärts gerichtet und legen sich über die letzteren. Die Borsten des Metepimerons bilden zusammen ein Gitterkörbchen, das das Lumen des Köchers ventral absperrt. Ihre Spitzen sind ziemlich kräftig.

Median von der Basis des Vorderbeins trägt der Proventer auf den späteren Stadien ähnliche Spitzchen wie Meso- und Metapleuron; im übrigen sind die Thorakalventres glatt. Sie tragen die gewöhnlichen Börstchen; das mediane der präsegmentalen Börstchen des Proventers ist $27 \times 2{,}75$ µ (d. h. $^1/_{25}$ der Kopfbreite), die anderen nur $22 \times 1{,}2$ µ gross.

Die Beine (Abb. 10) kann man im Verhältnis zur Gesamtlänge nicht als lang bezeichnen, da die Länge der Mittelbeine nur knap $^{3}/_{10}$ der Gesamtlänge beträgt; bedenkt man indessen, dass das Abdomen unverhältnismässig langgestreckt ist, so ist

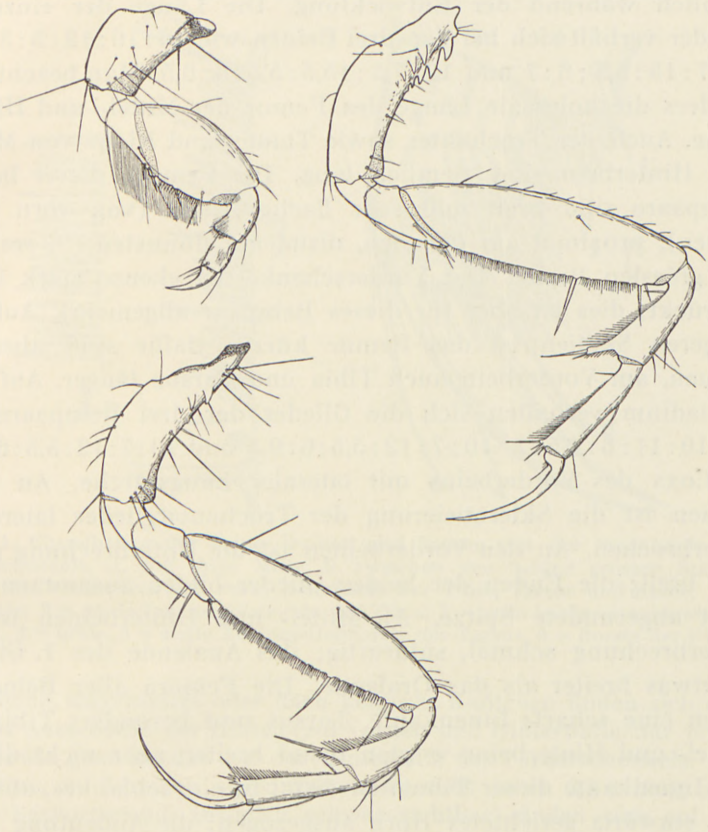

Abb. 10. Beine der rechten Seite von hinten gesehen. $^{40}/_{1}$.

die Länge der Beine keineswegs unbeträchtlich, die der Mittelbeine 4,1 × Kopfbreite. Auf dem 1. Stadium sind die Beine im Verhältnis zum Kopf kürzer (3,1 × Kopfbreite), aber — infolge des verhältnismässig kürzeren Abdomens — im Verhältnis zur Gesamtlänge länger, nämlich fast $^{2}/_{5}$ derselben. Das Verhältnis der Beinlänge zur Kopfbreite ändert sich während der Entwicklung schrittweise, jedoch am meisten vom 1. zum 2. Stadium. Auf dem 5. Stadium verhält sich die Länge der drei Beine zu einander wie 48 : 100 : 102; die Vorderbeine sind also erheblich

kürzer, aber verhältnismässig kräftiger als Mittel- und Hinterbeine. Auf den jüngeren Stadien ist der Unterschied weniger gross; im 1. Stadium verhalten sich die Vorder- zu den Mittelbeinen wie 63,5 : 100. Dieses Verhältnis ändert sich ganz allmählich während der Entwicklung. Die Länge der einzelnen Glieder verhält sich bei den drei Beinen wie 10 : 10 : 12 : 5 : 3 : 4,5, 10 : 7 : 15 : 5,5 : 6 : 7 und 10 : 7,5 : 15,5 : 5,5 : 6 : 6,5. Man beachte besonders die kolossale Länge des Femur der Mittel- und Hinterbeine. Auch der Trochanter sowie Tarsus und Klaue von Mittel- und Hinterbein sind ziemlich lang. Die Femora dieser beiden Beinpaare sind breit und sehr flachgedrückt (von vorn nach hinten), proximal am dicksten, distal am dünnsten ($^1/_2$ resp. $^2/_5$ der grössten Breite. Der Vorderschenkel ist ebenso stark flachgedrückt; dies ist aber für dieses Beinpaar allgemein). Auf den jüngeren Stadien ist das Femur kürzer, dafür sind aber die Klauen, am Vorderbein auch Tibia und Tarsus länger. Auf dem 1. Stadium verhalten sich die Glieder der drei Beinpaare wie 10 : 10 : 11 : 6 : 4,5 : 7, 10 : 7 : 12 : 5,5 : 6 : 9,5 und 10 : 7 : 12 : 5,5 : 6 : 9,5.

Coxa des Vorderbeins mit lateraler Längsfurche. An allen Beinen ist die Sklerotisierung der Trochanterglieder laterooral unterbrochen. An den Vorderbeinen ist die Unterbrechung ziemlich breit; die Enden der beiden Glieder bilden zusammen eine breit abgerundete Spitze. An Mittel- und Hinterbeinen ist die Unterbrechung schmal, suturartig; das Analende des 1. Gliedes ist etwas breiter als das Oralende. Die Femora aller Beine besitzen eine scharfe Innenkante. Tarsus und besonders Tibia der Mittel- und Hinterbeine werden distal breiter, aber nicht dicker. Die Innenkante dieser Tibien ist distal in ein schlankes, abwärts und einwärts gerichtetes Horn ausgezogen; die Andeutung eines solchen findet sich auch auf dem Tarsus. Im 1. Stadium ist die Form der Beine normaler, so fehlen u. a. die Verlängerungen an Tibia und Tarsus der Mittel- und Hinterbeine; der Tarsus hat auch im 2. Stadium noch keine Verlängerung. Am Vorderbein sind die distalen Glieder (Trochanter — Klaue) im Verhältnis zur Coxa etwas gedreht, so dass die Innenkante des Femur ein klein wenig nach vorwärts zeigt. Die Klauen sind ziemlich gerade, am Vorderbein recht kräftig, an Mittel- und Hinterbein an der Wurzel von normaler Dicke, aber sonst äussert schlank.

Proximal auf der Aussenseite der Mittelhüfte eine orale Längs-

reihe sehr starker, etwas abwärts gerichteter Dornen, deren meist proximaler niedrig und knotenförmig ist, und eine anale Längsreihe nicht ganz so starker Dornen. Letztere findet sich auch auf der Hinterhüfte, während hier die orale Reihe fehlt.

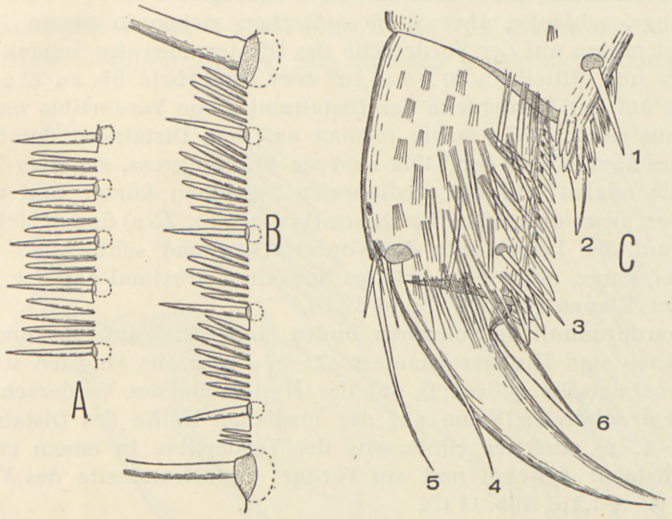

Abb. 11. Einzelheiten der Beine. Dornen und Sporen von der Innenkante des Mittelschenkels, A. von ihrer Mitte, B. zwischen den beiden grossen Sporen. C. Rechtes Vorderbein von vorn, Distalende der Tibia, Tarsus und Klaue. $^{285}/_{1}$. 1 = orale, 2 = anale Innenseitenborste der Tibia, 3 = orale Innenseitenborste des Tarsus, 4 = orale, 5 = anale Aussenseitenborste des Tarsus, 6 = Borste der Klaue.

Kleine, abgerundete oder flach konische Knötchen finden sich oral auf der Aussenseite der Hüften (auf Vorder- und Hinterhüfte nur proximal) sowie ganz median auf der Hinterseite des Vorderschenkels.

Feine, in Kämmen angeordnete Spitzchen (etwa 3,5 μ lang, sehr wenig hervortretend, zuweilen schwer sichtbar) finden sich auf der Hinterseite und proximal auf Vorder- und Innenseite der Vorder- und Mittelhüfte, auf der Vorderseite der Hinterhüfte, auf der medianen Hälfte der Vorderseite des ersten Trochantergliedes, auf dem Vordertrochanter ausserdem auch medioproximal auf der Vorderseite des 2. Gliedes, auf der Hinterseite des ersten Gliedes des Vordertrochanters und beider Glieder von Mittel- und Hintertrochanter (auf letzterem spärlich), auf den beiden distalen Dritteln der Innenseite des 2. Gliedes von Mittel- und Hintertrochanter, auf Vorder- und Hinterseite des Femur (auf dem Vorderschenkel nur auf der medianen Hälfte, auf der Hinterseite des Hinterschenkels spärlich), auf der Gelenkhaut zwischen Femur und Tibia, auf Vorder- und Hinterseite der Vordertibia, auf Vorder- und Aussenseite sowie proximal auf der Hinterseite von Mittel-

und Hintertibia, proximal auf Vorder- und Hinterseite des Vordertarsus (Abb. 11 C), auf Vorder- und Aussenseite sowie proximal auf der Hinterseite von Mittel- und Hintertarsus. Diese Spitzchen sind abwärts, auf Trochanter und Femur zugleich einwärts gerichtet. Sie sind sehr wenig auffallend, zum Teil schwer sichtbar; auf der Gelenkhaut zwischen Femur und Tibia sind sie etwas kräftiger.

Lange, schlanke, aber steife Spitzchen stehen in einem kleinen Büschel mitten auf der Vorderseite des Distalrandes der Schenkel (auf Vorder- und Mittelbein 30 × 1 μ, auf dem Hinterbein bis zu 22 μ lang), median auf der Vorderseite des Distalrandes von Vordertibia und Vordertarsus (Abb. 11 C), sowie median auf dem Distalrand der Hinterseite des Tarsus. Auf der Tibia sind sie 40 × 1 μ gross, auf dem Tarsus sind die analen und die mediooralen Spitzchen kürzer und dicker. Ein paar ziemlich kräftige Spitzchen (bis zu 30 × 2,5 μ) finden sich auch distal auf der Lateralseite des Vordertarsus, und schliesslich stehen ein paar kurze, aber recht kräftige Spitzchen proximal auf der Innenseite der Klauen (Abb. 11 C und 12 B).

Haardornähnliche Spitzchen finden sich distal auf der Innenseite des Mittel- und Hintertrochanters (22–37 μ lang, die längsten sind auf der Proximalseite gefiedert), auf der Hinterseite des Vorderschenkels in einem schmalen Saum auf der medianen Hälfte des Distalrandes (37 × 1–1,5 μ), auf der Hinterseite der Vordertibia in einem grossen, mediodistalen Büschel und auf Vorder- und Hinterseite des Vordertarsus (55 × 1,2 μ, Abb. 11 C).

Wichtiger als diese Gebilde sind indessen die Dornen und Haardornen auf der Innenkante der Beine. Auf der Innenkante des Femur steht eine sehr regelmässige Reihe von Dornen, und zwar am Mittel- und Hinterbein direkt auf der Innenkante, am Vorderschenkel ein klein wenig auf die Vorderseite herumgerückt. Auf dem Vorderschenkel sind sie etwa 27 μ lang, die mittleren 9, die proximalen und distalen etwa 5,5 μ dick. Distal stehen sie so dicht, dass sie einander fast berühren; (sie gehen gleichmässig, aber schnell in die Spitzchen der Gelenkhaut über). Auf Mittel- und Hinterschenkel werden die Dornreihen durch zahlreiche (primäre und sekundäre) Sporen in Gruppen geteilt (Abb. 11 A, B). Die proximalen Gruppen enthalten 2—4, die distalen 5—11 Dornen, die bis zu 42 × 6,5 μ gross sind und abgerundete Spitzen haben. Die proximalsten und distalsten sind kleiner, ebenso die äusseren Dornen der grösseren Gruppen. Auf der Innenseite von Tibia und Tarsus des Mittel- und Hinterbeins findet sich gleichfalls eine sehr regelmässige Reihe etwas abwärts gerichteter Dornen (Abb. 12). Der distale Dorn, der auf

der Tibia an der Basis des Auswuchses sitzt, ist der grösste (auf der Tibia bis zu 100 × 10, auf dem Tarsus bis zu 60 × 8 μ); die proximalen Dornen sind kleiner, der proximalste nur etwa 13 μ lang. Das proximale Ende der Reihen biegt etwas oralwärts um. Ganz proximal keine Dornen. Auf der Lateralseite des Tibiaauswuchses steht auch eine Reihe von Dornen, von denen der distale ziemlich lang ist, die anderen wesentlich kleiner. Auf

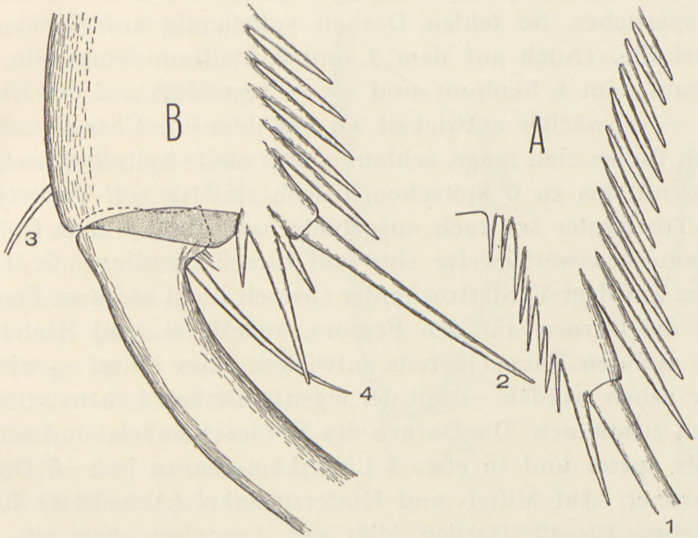

Abb. 12. Einzelheiten des rechten Mittelbeines von vorn. A. Distalende der Innenkante der Tibia, B. Distalende des Tarsus und Proximalende der Klaue. $^{285}/_{1}$. 1 = anale Innenseitenborste der Tibia, 2 = anale Innenseitenborste des Tarsus, 3 = anale Aussenseitenborste des Tarsus, 4 = Borste der Klaue.

der Lateralseite (ein wenig auf die Vorderseite gerückt) des Tarsusauswuchses ein eigentümlicher, dreispitziger Dorn (etwa 80 μ lang). Auf dem Vordertarsus (Abb. 11 C) finden sich zwei Dornreihen, eine orale und eine anale, deren Dornen jedoch bedeutend kürzer sind; der distale Dorn ist etwa 23 × 5,5 μ gross. Die anale Reihe ist kürzer und weniger regelmässig als die orale. Auf der Vordertibia findet sich nur mediooral auf dem Distalrand eine unregelmässige Gruppe sehr dichtstehender Dornen, von denen die grössten 27 × 5,5 μ gross sind. (Das grösste Gebilde, das man auf Abb. 11 C in dieser Gruppe sieht, ist der anale Sporn, dessen Alveole durch die Dornen verdeckt wird).

Ungewöhnlich lange Haardornen findet man am Vorderbein in einem schmalen Saum auf der Innenseite des 2. Trochantergliedes und des Femur (am Trochanter etwa 3 »Reihen«, am Femur stehen sie anal von den Dornen). Am Trochanter sind die Haardornen 185 × 1,2—2 μ, am Femur 90—110 × 1,2 μ gross; an ihrer proximalen Seite sind sie fein gefiedert.

Wie gewöhnlich bei Köcherfliegen ist die Ausstattung der Beine mit cuticularen Auswüchsen auf dem 1. Stadium anders und spärlicher. So fehlen Dornen vollständig auf Mittel- und Hinterhüfte. (Auch auf dem 2. und 3. Stadium fehlen sie, und noch auf dem 4. Stadium sind sie — besonders auf der Hinterhüfte — schwächer entwickelt als auf dem 5.). Überall auf den Beinen finden sich lange, schlanke, aber steife Spitzchen in Querreihen mit bis zu 6 Spitzchen, jedoch sind sie auf Vorderhüfte und -Trochanter schwach ausgebildet oder fehlen ganz (9, Abb. 5). Bemerkenswerterweise sind auf diesem Stadium die Haardornen auf dem Vordertrochanter (jedoch nicht auf dem Femur), sowie die Dornen auf den Femora, auf Mittel- und Hintertibia sowie auf den Tarsen bereits entwickelt; dies hängt — wie wir später sehen werden — mit der eigentümlichen Lebensweise der Larven zusammen. Die Dornen des Vorderschenkels sind schlanker als später und in etwa 5 Längskämmen zu je 3—5 Dornen angeordnet. Auf Mittel- und Hinterschenkel (Abb. 13 A) finden sich etwa 10—12 Dornen, die, mit Ausnahme von ein paar distalen, besenförmigen, auf der proximalen Seite gefiedert sind. Auf dem Vordertarsus wird der distalste der oralen Dornen durch einen Kamm von Spitzchen ersetzt. Auf Tibien und Tarsen der Mittel- und Hinterbeine sind — im Gegensatz zu später — Dornen auf der proximalen Hälfte oder den zwei proximalen Dritteln entwickelt; auf jedem Glied finden sich etwa 5. Ausserdem findet sich auf den Tarsen ein Paar starker Distaldornen (oral und anal) und zwischen ihnen einige schlankere Dornen oder Spitzchen. Diese beide Dornen repräsentieren vermutlich die Seitenäste des dreispitzigen Dorns der späteren Stadien.

Die Borsten der Beine sind durchweg kurz; dies trifft besonders für die Mittelbeine zu, am wenigsten für die Vorderbeine. Braun sind: alle Borsten der Coxa (mit Ausnahme der medianen Vorderseitenborste aller Beine, sowie der mediodistalen Borsten und der distalen analen Aussenseitenborsten von Mittel- und

Hinterbeinen) und die Vorderseitenborste des Femur. Alle übrigen Borsten sind blass bis gelb.

Auf der Vorderhüfte ist die orale Aussenseitenborste weit proximalwärts gerückt, auf Mittel- und Hinterhüfte hat sie im 1. Stadium die normale Stellung, rückt aber auf den folgenden Stadien auch auf diesen Beinen etwas proximalwärts, so dass sie im 5. Stadium ungefähr auf der Mitte der Aussenseite steht. Auf Mittel- und Hinterbein liegt die Grube proximal von dieser Borste, während sie auf dem Vorderbein ihre normale, distale Stellung behält. Auch die anale Aussenseitenborste von Mittel- und Hinterhüfte macht eine proximale Verschiebung durch. Im 1. Stadium steht sie am Rand der distalen Einbuchtung, im 4. und 5. Stadium etwas distal von der Mitte. Die Verschiebung ist am grössten vom 3. Stadium zum 4. Die proximale Aussenseitenborste des Vorderbeins ist lang und sehr kräftig (etwa $^2/_5$ der Kopfbreite), die orale nur halb so lang, aber auch sehr kräftig. Die anale Borste ist ebenso lang wie die proximale (im 1. Stadium länger), aber nur halb so dick; ihre Spitze ist besenförmig, auf den späteren Stadien auch die Spitze der oralen Borste. Am Mittelbein sind alle drei Borsten kurz (etwa $^1/_7$ der Kopfbreite), aber dick, besonders die beiden oralen, die als kräftige Spornborsten entwickelt sind. Die beiden oralen Borsten des Hinterbeins sind etwas länger (etwa $^1/_5$ der Kopfbreite) und dünner, die anale lang ($^2/_5$ der Kopfbreite). In der oralen Reihe des Vorderbeins werden keine sekundären Borsten gebildet; auf Mittel- und Hinterbein entsteht eine Reihe zwischen den beiden primären, und häufig auch etwas distal von der oralen. Die sekundären Borsten gleichen den primären. Anal

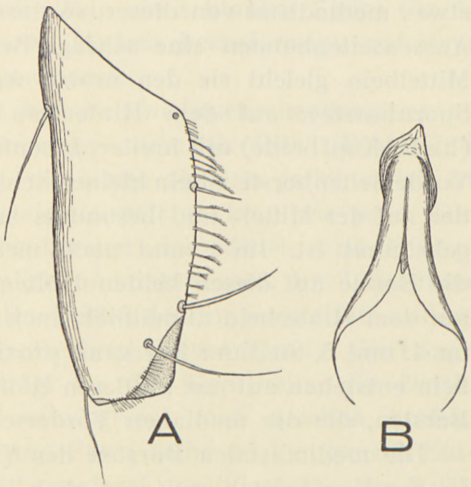

Abb. 13. A. Rechter Mittelschenkel von vorn, 1. Stadium. $^{340}/_1$. B. Grosser Doppeldorn auf III von rechts. $^{500}/_1$.

treten auf der Aussenseite sekundäre Borsten am Rand der Einbuchtung auf (d. h. distal von der primären Borste) und auf der Hinterhüfte zugleich ganz proximal. Die zuerst gebildete der letzteren (3. Stadium) ist noch länger als die primäre Borste (halbe Kopfbreite). Auf keinem der Beine kommen also Borsten auf der Oralseite der distalen Einbuchtung vor.

Die laterale Vorderseitenborste ist besenförmig. Auf dem Vorderbein gleicht sie der oralen Aussenseitenborste und steht etwas mediodistal von dieser, so dass sie mit den beiden oralen Aussenseitenborsten eine schräge Reihe bildet. Auch auf dem Mittelbein gleicht sie den oralen Aussenseitenborsten (kräftige Spornborste); auf dem Hinterbein ist sie eine lange Borste (halbe Kopfbreite) mit breiter, besenförmiger Spitze. Die mediane Vorderseitenborste ist ein kleiner, schlanker, besenförmiger Sporn, der auf der Mittel- und besonders auf der Hinterhüfte schwach gekrümmt ist. Im 2. und noch mehr im 3.—5. Stadium rückt die Borste auf diesen beiden Hüften ganz auf die Medianseite, auf dem Hinterbein allmählich auch proximalwärts, so dass sie im 4. und 5. Stadium fast ganz proximal steht. Auf dem Hinterbein entstehen auf der mittleren Hälfte der Innenseite sekundäre Borsten, die der medianen Vorderseitenborste gleichen.

Die mediodistalen Borsten des Vorderbeins sind lang (etwa $^2/_5$ der Kopfbreite) und sehr dick, die des Mittel- und Hinterbeins kurz (etwa $^1/_5$ der Kopfbreite) und dick, und zwar die orale etwas kürzer und dicker als die anale.

Schliesslich werden auf den späteren Stadien lange, dünne, sekundäre Borsten auf der Hinterseite der Hüfte gebildet, auf Mittel- und Hinterbein nur proximal oder auf dem Hinterbein überhaupt nicht.

Von den drei Börstchen des Proximalrandes ist das orale etwas auf die Gelenkhaut an der Basis der Coxa, das anale fast ganz median gerückt.

Die proximale Innenseitenborste des Trochanters ist auf dem Vorderbein lang und kräftig, auf Mittel- und Hinterbein etwas kürzer und ziemlich flachliegend, längs der Innenseite des Trochanters abwärts gebogen. Die oralen Innenseitenborsten sind kräftige Sporen; am Vorderbein steht die distale auf einem kleinen Vorsprung. Die analen sind auf dem Vorderbein normale Borsten (etwa $^1/_5$ der Kopfbreite), auf dem Mittelbein

kräftige Spornborsten oder schlanke Sporen, etwas länger als die oralen, die proximale am längsten (etwa $^1/_6$ der Kopfbreite). Auf dem Hinterbein sind sie ähnlich ausgebildet, jedoch ist die proximale 3 mal so lang wie die distale ($^1/_3$ der Kopfbreite, lange Spornborste). Auf allen Beinen steht die proximale anale Borste etwas distal von der proximalen oralen. Die Vorderseitenborste ist auf allen Beinen ein schlanker, besenförmiger Sporn. Die Hinterseitenborste ist auf dem Vorderbein eine kleinere Borste, auf dem Mittelbein ein schlanker Sporn (im 1. Stadium spitz, in den späteren Stadien besenförmig), auf dem Hinterbein eine kleinere Spornborste.

Auf dem Vorderschenkel steht die eine Innenseitenborste etwas proximal von der Mitte, die andere ziemlich distal. Beide sind spornförmig; die proximale, die etwas abwärts gerichtet ist, ist kürzer und dicker, die distale doppelt so lang wie jene. Die Entwicklung der Innenseitenborsten des Mittel- und Hinterschenkels ist sehr eigentümlich und interessant. Im 1. Stadium steht die eine am proximalen Drittel der Innenseite, die andere distal auf dieser (Abb. 13 A). Beide sind spornförmig; die Länge der proximalen Borste beträgt $^1/_{10}$ der Kopfbreite, die distale ist doppelt so lang (also sehr gross) und ziemlich kräftig. Letztere behält ihre Grösse und Form während der ganzen Entwicklung. Im 2. Stadium tritt proximal von ihr eine ganze Reihe von Sporen längs der ganzen Innenkante des Schenkels auf. Alle diese Sporen sind gleich gross, etwa $^1/_{12}$ der Kopfbreite, so dass der primäre proximale Sporn nicht mehr zu unterscheiden ist. Auf den folgenden Stadien vergrössert sich ihre Anzahl. Im 3. Stadium ist einer von ihnen ebenso gross wie der primäre distale; seine Grösse nimmt auf den späteren Stadien zu, so dass er auf dem 5. Stadium anderthalb mal so lang und fast doppelt so dick (16,5 µ) ist wie jener. Seiner Stellung nach kann er nicht die primäre proximale Borste sein, da er am distalen Drittel der Innenseite steht. Zwischen den beiden grossen Sporen der Innenkante finden sich im 3. Stadium 2 kleine, im 4. Stadium 3 und im 5. Stadium 4 (Abb. 11 B), eine sehr regelmässige Entwicklung. Die Vorderseitenborste des Vorderbeins ist ein schlanker, besenförmiger Sporn, der ungefähr auf der Mitte der Vorderseite steht. Auf Mittel- und Hinterbein steht sie im 1. Stadium distal, am medianen Drittel der Vorderseite; während der fol-

genden Stadien verschiebt sie sich längs des Distalrandes, so dass sie im 5. Stadium sehr median auf gleicher Höhe mit der distalen Innenseitenborste steht. Sie ist auf dem 1. Stadium eine Spornborste, die anderthalb mal so lang ist wie die distale Innenseitenborste; während der Entwicklung verringert sich ihre relative Grösse um die Hälfte, auf den späteren Stadien wird sie ein schlanker Sporn. Im 5. Stadium ist also das Grössenverhältnis der drei grossen Sporen auf und längs der Innenseite dieser Schenkel genau umgekehrt wie im 1. Stadium. Auf dem Vorderbein steht die Hinterseitenborste während aller Stadien sehr median, auf gleicher Höhe mit der distalen Innenseitenborste oder etwas distal von dieser; sie ist eine kräftige Spornborste (oder ein schlanker Sporn); auf Mittel- und Hinterbein steht sie sehr lateral, ungefähr auf der Mitte der Hinterseite, und ist hier ein kleiner, schlanker Sporn (auf dem Mittelbein nur $^1/_{15}$ der Kopfbreite). Im 1. Stadium steht die Borste auf diesen Beinen ziemlich distal auf der Mitte der Fläche. Auch im 2. und 3. Stadium ist sie mehr median und distal angebracht als später. Die Aussenseitenborsten sind kräftige Spornborsten; die proximale ist am längsten (etwa $^1/_6$ der Kopfbreite, im 1. Stadium noch länger) und dünnsten. Auf dem Vorderbein werden sekundäre Aussenseitenborsten nur distal ausgebildet (proximal von der primären, distalen Borste), auf Mittel- und Hinterschenkel längs der ganzen Aussenseite und dem lateralen Drittel der Vorderseite. Die oralsten dieser Borsten sind als kleine, besenförmige Sporen ausgebildet.

Siltala (13, S. 544) beschrieb die Dornen und Sporen der Femora von *Brachycentrus* im letzten Stadium; sie gleichen durchaus den oben beschriebenen. Klapáleks (3, S. 61) abweichende Schilderung der Verhältnisse bei *Oligoplectrum*, die sich auch bei Ulmer (14, S. 88, 15, S. 277) und Lestage (4, S. 874) wiederfindet, beruht vielleicht auf weniger gutem Material.

Beide Innenseitenborsten der Tibia sind als Sporen ausgebildet. Die orale, die knapp $^2/_3$ so dick ist wie die anale (diese 7,5 µ), steht auf dem Vorderbein subdistal (Abb. 11 C), auf Mittel- und Hinterbein im 1. Stadium am distalen Viertel der Innenseite, auf den folgenden Stadien fast ganz proximal und sehr median. Sie ist ziemlich schlank (4,5 µ dick); ihre Länge be-

trägt $^1/_{10}$ der Kopfbreite. Die anale Borste steht auf diesen beiden Beinpaaren im 1. Stadium auf dem Distalrand, später auf der Spitze des oben erwähnten Auswuchses. Sie ist ein sehr langer, starker Sporn (im 5. Stadium 13,5 μ dick); seine Länge beträgt im 1. Stadium $^2/_5$ der Kopfbreite; in den folgenden Stadien verringert sich seine relative Länge um die Hälfte (vom 1. zum 2. Stadium nimmt also auch seine absolute Länge ab). Die vier Aussenseitenborsten haben die für eruciforme Larven normale Stellung; die zweitanale ist die längste (am Vorderbein $^1/_7$, an den anderen Beinen $^1/_5$ der Kopfbreite) und dünnste, die orale die dickste; sie ist spornartig, am Vorderbein am stärksten, am Hinterbein am dünnsten. Auch die anale Borste ist ziemlich kräftig; am Vorderbein ist sie flachliegend, über die Hinterseite des Tarsus gebogen.

Am Vordertarsus stehen beide Innenseitenborsten subdistal. Die orale ist ein kleiner, schlanker Sporn, die anale (am weitesten distal) eine kräftige Spornborste ($^1/_{12}$ bezw. $^1/_8$ der Kopfbreite). Am Mittel- und Hintertarsus sind die Innenseitenborsten ebenso gestaltet wie auf der Tibia und die orale ($^1/_{10}$ der Kopfbreite) macht dieselbe Verschiebung durch wie dort. Die Länge der analen Borste beträgt im 1. Stadium $^1/_4$, in den folgenden Stadien $^1/_6$ der Kopfbreite, ihre Dicke im 5. Stadium 10 μ. Der Auswuchs, auf dem sie sitzt, ist erst im 3. Stadium entwickelt. Die orale Innenseitenborste dieser beiden Tibien und Tarsen scheint starker Abnutzung ausgesetzt zu sein; bei fast allen untersuchten Exemplaren war ihre Spitze abgebrochen oder abgewetzt. Auf dem Vorderbein (Abb. 11 C) stehen beide Aussenseitenborsten distal, die orale jedoch etwas proximal von der analen; auf Mittel- und Hinterbein steht die orale Borste etwas proximal von der Mitte. Am Vorderfuss sind beide kurz ($^1/_6$ der Kopfbreite) und kräftig (die orale fast doppelt so dick wie die anale) und stark abwärts gerichtet, die orale etwas flachliegend, der Klaue entlang gebogen. An Mittel- und Hinterbein ist die anale Borste klein, während die orale verhältnismässig lang, ziemlich flachliegend, der Aussenseite des Tarsus entlang gebogen ist. Die Grube liegt auf dem Vorderbein am distalen Viertel, auf Mittel- und Hinterbein am proximalen Viertel der Aussenseite.

Der Basalsporn der Klaue ist am Vorderbein kräftig (7,5 μ dick), an Mittel- und Hinterbein schlank und etwas einwärts gekrümmt.

Abnormitäten: Bei einem Exemplar im 5. Stadium waren proximale und orale Aussenseitenborste sowie die laterale Vorderseitenborste der rechten (und linken?) Vorderhüfte ganz klein (nicht grösser als das orale Börstchen), die orale Aussenseitenborste stand ausserdem mehr distal als gewöhnlich. Die anale Aussenseitenborste fehlte vollständig. Auf dem Trochanter fand sich ein überzähliger Sporn proximal von der proximalen oralen Innenseitenborste und eine überzählige Vorderseitenborste. Am Femur fehlte die proximale Aussenseitenborste; es fanden sich 2 Gruben neben einander. Schliesslich besass die Tibia eine überzählige Aussenseitenborste oral von der oralen.

Das I. Abdominalsegment besitzt keine Höcker. Dagegen finden sich an der Lateralseite von VII und VIII ein Paar flache Höcker (Abb. 1), und zwar an VII fast in ganzer Länge des Segmentes, an VIII etwas kürzer. Ihre freie Fläche bildet einen Teil einer Zylinder- oder wohl richtiger Kegelfläche. Besonders der Höcker auf VII erweist sich als recht selbständiges Gebilde; sein Vorderrand springt, von oben gesehen, als ziemlich scharfe Kante hervor, während der Höcker auf VIII mehr als Verdickung der Lateralseite des Segmentes erscheint. Diese Höcker sind jedoch erst im 2. Stadium ausgebildet. IX trägt einen ziemlich kleinen Analschild.

Kiemen finden sich nur in der Rückenreihe und zwar nur postsegmental.

Man beachte, dass die analen Kiemen früher auftreten als die oralen; sonst verlängern sich die Kiemenreihen gewöhnlich am analen Ende. Die Kiemen sind bei ihrem ersten Auftreten

Kiemenschema

	2. Stadium	3. Stadium	4. Stadium	5. Stadium
II	0	1	2—3	4—6
III	0	1	2—3	4—7
IV	0	1	2—3	3—5
V	0—1	1	2—4	3—5
VI	1	1	2	2—3
VII	1	1	2	2—3

Schema der Doppeldornen
(Die Anzahl kann vielleicht noch etwas mehr schwanken).

	2. Stadium	3. Stadium	4. Stadium	5. Stadium
III	2	7—8	7—11	25—28
IV	1—2	3—4	8—13	20—21
V	1	5	5—11	etwa 22
VI	2—4	5—6	9—14	29—41
VII	4—6	13—15	19—22	etwa 80

(2. und 3. Stadium) kurz und unverästelt; im 4. und 5. Stadium bestehen sie aus einem kurzen (etwa 90 × 90 µ) Stamm, der sich am distalen Ende handförmig verzweigt. Die Äste sind zylindrisch, mit abgerundeter Spitze, bis zu 270 × 36 µ gross, einer von ihnen häufig viel kleiner. Die Kiemen auf VI und VII sind kleiner. Trotz der starken Verästelung ist daher das Kiemenareal als klein zu bezeichnen; auf beiden Seiten zusammen ist es weniger als 1,7 mm^2.

Auf der Lateralseite von III—VII findet sich oral eine tropfenförmige (Spitze anal) Gruppe von rückwärts (auf VII zugleich etwas aufwärts) gerichteten, bräunlichen Doppeldornen. Die Länge der Gruppen beträgt auf III etwas mehr als $^1/_3$, auf VII etwas weniger als $^2/_3$ der Länge des Segmentes. Die Dornen (Abb. 13 B) entspringen von ihren Basalplatten ungefähr im rechten Winkel, sind aber an der Basis nach rückwärts gebogen. Das rückwärts gerichtete Stück ist flachgedrückt, mit abgerundeter Aussen- und scharfer Innenseite. Distal sind sie noch flacher zusammengedrückt, so dass ihre Spitze lamellenförmig wird. Da diese gleichzeitig um 90° um ihre Längsachse (einwärts) gedreht ist, so erhält man den Eindruck, als wäre der Abschluss der Dornen peitschenförmig. In den einzelnen Gruppen sind die ventralen Dornen am kleinsten (22 × 1,2 µ, äussere Hälfte »peitschenförmig«), die dorsalen am grössten (37 × 2,7 µ, äusseres Viertel »peitschenförmig«). Ausserdem findet sich in den Gruppen dorsal ein besonders grosser Dorn (im 2. Stadium 33 µ lang, im 5. Stadium 66 × 5,5—9 µ). Im 4. und 5. Stadium kann einer der anderen Dornen fast ebenso gross sein.

Die eigentliche Seitenlinie ist schwach entwickelt, dünn und grau; sie erstreckt sich vom III.—VII. Segment. Die Haardornen

stehen recht zerstreut in einer Reihe (in einem schmalen Saum ganz anal auf den Segmenten), die hinter den Gruppen der Doppeldornen beginnt. Auf IV—VI finden sich auch unter ihrer analen Hälfte einige wenige Haardornen. Die Seitenlinie ist auf VII besonders dünn und fehlt hier im 2. Stadium. Die Haardornen sind einfach gegabelt, mit ganz kurzem Schaft; die Gabeläste 130 × 1,1 μ.

Abgesehen von den Gebilden der Seitenlinie finden sich nur — auf den späteren Stadien — feine, blasse Spitzchen in sehr verstreuten Querkämmen auf dem Vorderrand der Dorsa (sehr schwer sichtbar).

Auf allen Dorsa 2, auf allen Ventres 1 präsegmentales Börstchen. Auf dem I. Dorsum stehen sie dichter zusammen als gewöhnlich. Sie sind hier 13 × 1,1 μ gross, auf VII 6,5 μ lang, auf VIII und IX sehr schwer zu sehen. Das Börstchen auf dem I. Venter ist 35 × 2,5 μ, auf II 16,5 × 1,2 μ gross, auf VII 8 μ lang.

Auf I und II ist die eine der lateralen Borsten in allen Stadien lang ($^1/_3$ der Kopfbreite) und dunkel, die andere, die direkt dorsoanal von ihr steht, ganz klein ($^1/_{20}$ der Kopfbreite) und blass. Auf III—VII sind die hinter einander stehenden, lateralen Borsten im 1. Stadium ziemlich lang; die anale ist dunkel, ihre Länge $^2/_5$ der Kopfbreite. Auf III ist die orale Borste etwa halb so lang und blass; nach rückwärts nimmt ihre Länge schrittweise zu, so dass sie auf VII fast ebenso lang ist wie die anale; hier ist sie auch dunkel. Während der 1. Häutung wird die absolute Länge der analen Borste auf die Hälfte reduziert, bei den folgenden Häutungen vermindert sich ihre relative Länge weiterhin, so dass diese im 5. Stadium nur $^1/_{10}$ der Kopfbreite beträgt. Noch stärker wird die orale Borste reduziert; im 5. Stadium beträgt ihre Länge auf allen diesen Segmenten nur $^1/_{50}$ der Kopfbreite; im 2. Stadium sind beide Borsten blass. Die orale Borste steht auf III—VI oberhalb der Seitenlinie, die anale unterhalb. Auf III stehen beide dicht zusammen, auf IV—VI länger hinter einander. Auf VII steht die orale Borste vor der Seitenlinie (unter den Doppeldornen), die anale unter der Seitenlinie. Auf VIII sind die Borsten auf allen Stadien blass und klein ($^1/_{33}$ bezw. $^1/_{25}$ der Kopfbreite). Im 1. Stadium stehen sie über einander.

Von den übrigen Borsten auf I—VIII sind die folgenden auf

allen Stadien lang und schwarz: Die medioanale Dorsalborste auf VI—VIII ($^1/_4$, $^1/_3$ und $^2/_5$ der Kopfbreite) und die mediane Ventralborste auf I ($^7/_{10}$ der Kopfbreite). Im 1. Stadium sind alle anderen Borsten blass und klein ($^1/_{25}$—$^1/_{10}$ der Kopfbreite). Im 2.—5. Stadium wird die medioanale Borste auf I. Dorsum dunkel und wächst schrittweise bis zu $^3/_5$ der Kopfbreite; im 3.—5. Stadium wächst die medioanale Borste auf II bis zu $^2/_5$ der Kopfbreite. Auch die Eckborste auf I und II wächst im 3.—5. Stadium (bis $^2/_5$ bezw. $^1/_5$ der Kopfbreite) und wird dunkel. Sie steht etwas dorsooral von den lateralen Borsten. Die laterale Borste auf I. Venter verdoppelt bei der ersten Häutung ihre relative Länge und wird dunkel; im 3.—5. Stadium beträgt ihre Länge ungefähr die Hälfte der Kopfbreite. Auch die mittlere und die laterale Borste auf II. Venter verlängern sich im 3.—5. Stadium; ihre Länge beträgt im 5. Stadium $^1/_4$ bezw. $^1/_3$ der Kopfbreite. Die mediane Borste auf II. Venter wird im 2.—4. Stadium beträchtlich dicker, so dass sie im 3. Stadium einen schlanken, im 4. und 5. Stadium einen kräftigen gelben Sporn bildet; diese Form ist für eine Abdominalborste ganz ungewöhnlich. Ausserdem wächst bei der letzten Häutung ihre relative Länge von $^1/_{10}$ auf $^1/_7$ der Kopfbreite. (Im 5. Stadium ist sie 114×13 µ gross). Die Borste steht auf dem Segment weit oral. — Auf dem 5. Stadium sind also folgende Borsten lang und schwarz: Die Eckborste auf I und II, die medioanale Dorsalborste auf I, II und VI—VIII, die mediane und die laterale Ventralborste auf I, die mittlere und die laterale auf II, während die mediane Ventralborste auf II ein kräftiger Sporn ist. In den späteren Stadien steht die mediane Ventralborste auf I und II auf winzig kleinen, nicht sehr deutlichen, gelbbraunen Skleriten und ziemlich median. Die mittlere Ventralborste auf I fehlt auf allen Stadien. Ebenso wenig gelang es mir, auf diesen Segmenten dorsale Gruben zu finden.

Auf IX. Dorsum steht eine Borste, die auf allen Stadien ganz klein ($^1/_{14}$ der Kopfbreite) und blass ist, weit lateral vom Analschild, die 4 anderen auf diesem, nahe seinem Hinterrand. Von diesen ist die mediane Borste auf allen Stadien lang ($^3/_5$ der Kopfbreite) und schwarz. Direkt laterooral von dieser Borste liegt die Grube. Die drei anderen Borsten sind kleiner und blass. Die Länge der medianen dieser Borsten beträgt auf allen

Stadien $^1/_5$ der Kopfbreite. Im 1. Stadium beträgt die Länge der mittleren Borste $^2/_5$, die der lateralen nur $^1/_{15}$ der Kopfbreite. Auf den folgenden Stadien wächst die letztere, während die erstere kleiner wird, so dass im 5. Stadium alle 3 Borsten ungefähr gleich lang sind, nämlich $^1/_5$ der Kopfbreite. Die sekundären Borsten, die der medianen von den primären Borsten gleichen, stehen ein wenig hinter deren Reihe. Eine von ihnen kann unpaar sein, d. h. mitten auf dem Hinterrand des Analschildes stehen (in der Borstentabelle als paarige Borste aufgeführt). Die mittlere der Ventralborsten ist klein und schwarz, die mediane und die laterale (letztere ganz lateral auf dem Segment) sind ganz klein und blass ($^1/_{20}$ der Kopfbreite).

Die Analfüsse (Abb. 14 A) sind ebenso gebaut wie bei den *Limnophilinen*. Der Schaft trägt ventral auf der Hinterseite, direkt median von der Klaue einen fingerförmigen, weichen, rückwärts gerichteten und etwas einwärts gekrümmten Anhang von etwa 200 μ Länge. Er ist an der Basis 55 μ dick, am proximalen Drittel auf 32 μ verschmälert; seine beiden distalen Drittel sind zylindrisch, seine Spitze abgerundet. Er ähnelt einer Kieme; jedoch mündet auf seiner Spitze eine lange tubulöse Drüse, die sich ganz in das IX. Segment hinein erstreckt. Der Zapfen fehlt auf dem 1. Stadium, während die Drüse bereits in diesem vorhanden ist. Ihre Funktion ist rätselhaft. Nach Lage der Mündung muss das Sekret anscheinend sofort durch die Wasserströmung weggeführt werden; es ist daher naheliegend, eine exkretorische (osmoregulatorische?) Funktion anzunehmen.

Der Proximalrand der Klaue (Abb. 14 B, C) ist auf der Ventralseite weit ausgebogen; er wird in seiner ganzen Ausdehnung von einer inneren Lamelle begleitet. Die weichhäutige Partie ist auf der Ventralseite erweitert, aber sonst schmal. Zwei Rückenhaken sitzen neben einander, der eine in der Mittellinie, der andere, grössere, lateral von diesem. Auf dem 1. Stadium fehlt der letztere, dafür findet sich aber ein kleinerer Rückenhaken dorsal vom ersten; dieser dorsale Haken ist noch im 4. Stadium erhalten, aber so klein, dass er kaum zu sehen ist. Auf dem 2.—4. Stadium sind also 3 Rückenhaken vorhanden.

Im 1. Stadium finden sich ventrolateral auf dem Sklerit von »b« ein paar Querreihen aus 3,5 μ langen, rückwärts gerichteten Spitzchen. Diese fehlen auf den folgenden Stadien; dafür sind aber alle weichen

Partien des Schaftes (mit Ausnahme des Anhanges) sowie »c« und das dorsomediane Ende des Sklerits von »b« von feinen, blassen 2,5 μ langen Spitzchen bekleidet, die zu Kämmen von je etwa 8 Spitzchen (auf der Medianseite ein Vielfaches hiervon) verbunden sind. Die Kämme stehen sehr dicht; trotzdem sind die Spitzchen infolge ihrer geringen Grösse schwer sichtbar. Sie sind auf der Dorsal- und Medianseite rückwärts, auf der Analseite auswärts und auf »c« vorwärts gerichtet. Auf

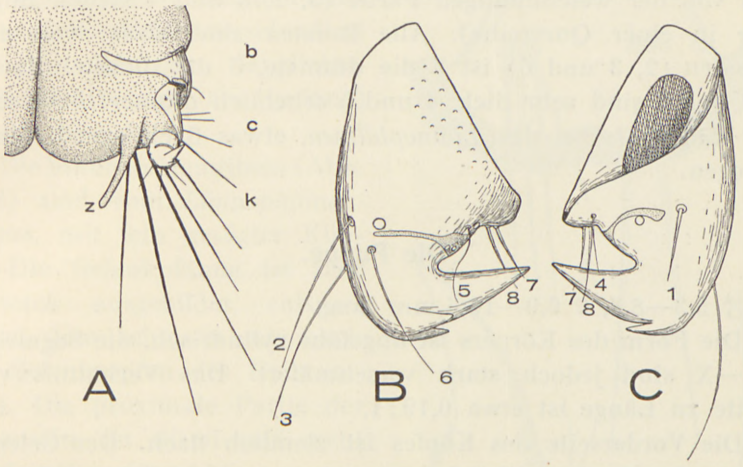

Abb. 14. A. Linker Analfuss (und angrenzende Partie von IX) von der Ventralseite. $^{40}/_{1}$. Rechte Analklaue von der Lateralseite (B) und von der Medianseite (C). $^{190}/_{1}$. b = »b«, c = »c«, k = Klaue, z = Anhang des Schaftes. Die Zahlen geben die Nummern der Borste auf der Klaue an.

der distalen Ecke von »c« gehen sie in stärkere (11 × 2,5 μ), einzeln stehende Spitzchen über. Auf der Lateralseite der Klaue sitzen proximal 2,5 μ breite, abgerundete Knötchen.

Die dorsalen Borsten von »b« stehen anal vom Sklerit in einer Querreihe. Die beiden mittleren bilden die längsten Borsten der Larve (1,25 fache Kopfbreite) und von den eigentlichen Borsten des Körpers die dicksten (13 μ). Die laterale ist etwas kleiner (etwa $^{6}/_{7}$ der Kopfbreite). Die Länge der medianen Borste beträgt im 1. Stadium die Hälfte der Kopfbreite; auf den folgenden Stadien nimmt ihre relative Länge zu, so dass die Borste im 5. Stadium ebenso lang ist wie die laterale. Die Reihe der primären Borsten wird lateral durch die sekundären fortgesetzt; von diesen ist die medianste etwas kleiner als die laterale der primären Borsten. Lateralwärts nimmt ihre Grösse ab, so dass

die Länge der lateralsten Borste nur etwa $^1/_5$ der Kopfbreite beträgt. Die laterale Borste von »b« und die Borste von »c« (ziemlich proximal) sind klein und blass ($^1/_7$ bezw. $^1/_9$ der Kopfbreite).

Die Borsten und Gruben der Klaue sind ungefähr so angeordnet wie bei den *Limnophilinen*, jedoch steht Borste 4 proximal von der weichhäutigen Partie (5, 7, 8 und 4 stehen ungefähr in einer Querreihe). Alle Borsten sind blass; von den dorsalen (2, 3 und 6) ist 3 die dünnste, 6 die dickste. Borste 1, 7 und 8 sind sehr dick, 4 und 5 erheblich dünner; 5 ist sehr viel länger als bei den *Limnophilinen*, etwas flachliegend, distal gebogen.

Die Puppe.

♂ 5,3—8,6, ♀ 6,9—10,6 mm lang.

Die Form des Körpers ist ungefähr zylindrisch, die Segmente VII—X sind jedoch stark verschmälert. Das Verhältnis von Breite zu Länge ist etwa 0,19 : 1.

Die Vorderseite des Kopfes ist ziemlich flach. Der Gelenkknoten für die Mandibel ist nur schwach entwickelt, zapfenförmig und oralwärts gerichtet. — Die vorderste Partie der Stirn und die Dorsalseite des Epicraniums sind gelblich. — Scheitel ohne Borsten. Frontoclypeus mit braunen Seitenrandborsten, von denen die mittlere am grössten ist. Die vordere ist nur halb so gross, d. h. ebenso gross wie die laterale Vorderrandborste. Die Mandibelgelenkborsten sind gelb, beide Gruben zwischen ihnen kreisrund. Die Antennen des ♂ reichen bis zur Hinterleibsspitze oder darüber hinaus, die des ♀ bis zur Mitte des V. Segmentes oder zu dessen Ende. Die Basis der Antennen trägt auf der Dorsalseite (3—)4 kurze (etwa 75 μ), aber dicke, blasse Borsten und auf der Vorderseite eine etwas grössere Borste.

Die beiden mittleren Vorderrandborsten des Clypeus (Abb. 15 A) sind lang und sehr kräftig, braun, die laterale ist kurz und blass. Die laterale Grube liegt vor den beiden grossen Borsten. Bei einem Exemplar wurde sie durch eine kleinere Borste ersetzt, die hinter den grossen Borsten stand. Das Labrum ist ungefähr halbkreisförmig; es trägt vorn eine kleine, blasse Borste und hinten 5 grosse, braune Borsten, die den grossen Borsten

des Clypeus gleichen, aber etwas kürzer sind. Sie sind in 2 Querreihen angeordnet, 2 in der vorderen, 3 in der hinteren. Letztere sind etwas grösser als die ersteren. Die mediane Grube fehlt, die laterale liegt direkt mediooral von den grossen Borsten. Die grossen Borsten auf Clypeus und Labrum sind schräg aufwärts gerichtet; im distalen Drittel sind sie stärker verschmälert und mit winzigen, länglichen Knötchen (weniger als 1 μ lang) besetzt.

Die braunen Mandibeln (Abb. 15 B) sind vom *Limnophilinen*-Typus, mit fein gesägter Klinge. Die Gelenkpfanne ist sehr schwach ausgebildet, entsprechend dem schwach entwickelten Gelenkzapfen des Epicraniums. Die proximale Partie der Innenseite ist distal mit recht vereinzelten, 10 × 0,75 μ grossen Spitzchen besetzt. Die hellen, kurzen, kräftigen Rückenborsten stehen über einander.

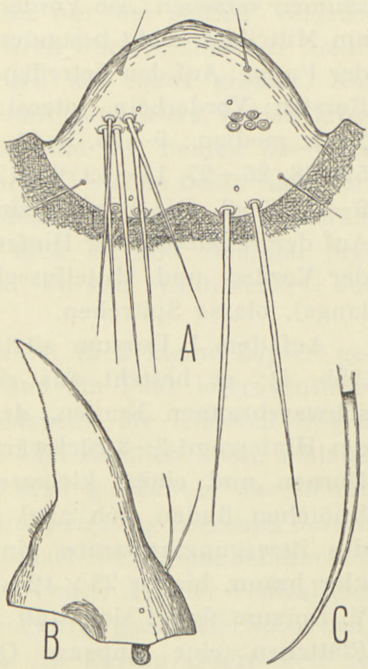

Abb. 15. Puppe. A. Anteclypeus und Labrum. Die grossen Borsten sind auf dem Clypeus links, auf dem Labrum rechts an der Basis abgebrochen. B. Rechte Mandibel von der Dorsalseite. C. Rechtes Analstäbchen von der Lateralseite. $^{112}/_{1}$.

Die Maxillarpalpen des ♀ reichen ungefähr bis zur Mitte der Vorderhüfte und sind undeutlich gegliedert; die Labialpalpen sind etwas kürzer. Die Labialpalpen des ♂ sind so lang wie die Maxillarpalpen des ♀, seine Maxillarpalpen halb so lang, sehr dick, gegen das abgerundete Distalende hin etwas verschmälert und ohne eine Spur von Gliederung.

Das Prodorsum trägt 2 (bei einem Exemplar 3) grössere, schwarze, das Mesodorsum 3 kleine, schwarze Borsten (Eckborste, orale und anale Flächenborste). Auf dem Metadorsum finden sich 2 anale Flächenborsten, während Eckborsten fehlen.

Die Flügelscheiden des ♂ reichen ungefähr bis zur Mitte von VI, die des ♀ bis zum Ende von V.

Die Vorderhüfte trägt 4, die Mittelhüfte (6) 8—12, der Vordertrochanter 2 grosse, schwarze Borsten. Vorder- und Mittelbein sind an der Tibia und den ersten vier Fussgliedern (jedoch am 3. und 4. Glied des Vorderfusses nicht median) mit Schwimmsäumen versehen. Am Vorderbein sind diese nur schwach, auch am Mittelbein nicht besonders kräftig in Anbetracht des Biotops der Puppe. Auf den betreffenden Gliedern beträgt die Anzahl der Borsten: Vorderbein, lateral: 14—15, 7—13, etwa 8, etwa 3, 2—3; median: 9—16, 7—11, etwa 2. Mittelbein, lateral: 53—71, 52—68, 26—27, 16—19, 9—12; median: 43—53, etwa 47, 18—23, 9—12, 6—7. Die Borsten sind proximal 540, distal 235 μ lang. Auf der Innenseite der Hinterhüfte und distal auf der Innenseite der Vorder- und Mittelfussglieder finden sich feine (etwa 2 μ lange), blasse Spitzchen.

Auf dem I. Dorsum sitzt lateral der Haftapparat (Tafel II, Abb. 4); er besteht aus einem sehr flachen, sklerotisierten, schwarzbraunen Knoten, dessen schuppenförmig vorspringender Hinterrand 3—8 rückwärts gerichtete, bis zu 23 × 14 μ grosse Dornen und einige kleinere, spitze Knötchen trägt. Ähnliche Knötchen finden sich anal auf der Dorsalseite. Die Plättchen des Bewegungsapparates sind gelb bis braungelb, die Dornen klar braun, bis zu 23 × 12 μ gross. Längs des Hinterrandes von V. Dorsum findet sich statt der gewöhnlichen postsegmentalen Plättchen eine unpaare Querreihe kleiner, tropfenförmiger, 35 × 12—14 μ grosser Plättchen, deren breiteres Hinterende 1(—2) vorwärts gerichtete Dornen trägt. Diese Plättchen sitzen so dicht, dass sie einander fast berühren. Die lateralen Enden der Reihe sind ein wenig nach rückwärts gebogen. Die Anzahl der Dornen auf dem Bewegungsapparat beträgt: 2—7, 2—8, 2—8 + etwa 60, 3—8, 3—7. (Die Anzahl der postsegmentalen Dornen auf V gilt für beide Seiten zusammen). Zwischen den Knoten auf I und auf der ganzen Dorsalseite von II—VI stehen rückwärts gerichtete, sehr feine (etwa 3 μ lange), blasse Spitzchen. Auf I sind die vorderen noch kleiner und in breiten Querkämmen angeordnet.

Dorsale Chitinleisten fehlen; ventral finden sich ganz schmale, gelbe auf II—VII und auf den beiden vorderen Dritteln von VIII.

Die Kiemen der Puppe gleichen denen der ausgewachsenen

Larve. Die goldbraune Seitenlinie beginnt ganz anal auf V; sie ist auf V—VII ziemlich dünn, auf VIII dicker.

I.—VIII. Dorsum tragen je eine orale Borste und 3 anale; auf den vorderen Dorsa sind jedoch die orale und die laterale der analen Borsten so klein, dass sie nur bei starker Vergrösserung zu sehen sind. Auch die anderen Borsten sind nur klein; auf den hinteren Segmenten werden sie etwas grösser. Auf II—VIII je 2 laterale Borsten, die vorn ganz klein, hinten grösser sind. Auf VI und VII steht die eine der beiden über der Seitenlinie, die andere darunter; auf VIII stehen beide über ihr. II.—VIII. Venter tragen je 3 Borsten, von denen die laterale klein, blass und verhältnismässig dick ist und ziemlich oral steht, und zwar auf II—VII lateral von der »Chitinleiste«, auf VIII median von dieser.

Das schmale Hinterende von X ist in 3 kleine Zapfen gespalten, einen unpaaren dorsalen und ein Paar lateroventrale. Der erstere ist etwas zusammengedrückt, die letzteren tragen die Analstäbchen (Abb. 15 C); diese sind ziemlich klein, schlank und stark aufwärts gekrümmt. Sie sind gelbbraun, das innere Stück jedoch farblos, durch einen braunen Ring begrenzt, dessen Rand proximal scharf, distal unscharf ist. Beim Männchen fehlen Scheiden für Genitalfüsse und Penis. Auf der Ventralseite von X stehen distal feine, blasse, vorwärts gerichtete Spitzchen, $5{,}5 \times 1{,}2$ µ gross oder kleiner (dann in kurzen Querreihen zu 2—3 Stück). IX mit einer (dorso)lateralen Borste und (auf jeder Seite) einer analen Gruppe von 3—8 ziemlich kleinen, dorsalen Borsten; X auf der Ventralseite oral mit 3—4 ziemlich langen Borsten; Analstäbchen ohne Borsten.

Jahreszyklus.

Der Beginn der Flugzeit schwankt etwas von einem Jahr zum anderen, jedoch kann man normalerweise die Flugzeit von der zweiten Hälfte Juni bis zur zweiten Hälfte August rechnen. Esben-Petersen (2) gibt für andere jütländische Örtlichkeiten die Zeit von Juni bis September als Flugzeit an. Über die Lebensdauer des einzelnen Individuums lässt sich nichts sagen, jedoch kann diese in trockenen Perioden sicher lang sein, jedenfalls bei den Weibchen (vgl. S. 6).

Während der Flugperiode finden sich Imagines an den Ufern der Gewässer in ungeheuren Mengen. Bei Tage halten sie sich meist in der krautartigen Vegetation verborgen; wird diese geschüttelt, so fliegen sie in dichten Schwärmen über das Wasser, suchen sich aber rasch ein neues Versteck. Dennoch sieht man, selbst bei starkem Sonnenschein, eine nicht geringe Anzahl von Individuen herumlaufen oder ganz kurze Strecken fliegen; an schattigen Stellen kann man zuweilen kleine Schwärme über dem Wasser sehen. Trotz der grossen Menge von Tieren wurde eigentliche Schwarmbildung indessen nicht beobachtet.

Tabelle I. Wachstum der Larven.

	Zahl der untersuchten Larven	1. Stadium	2. Stadium	3. Stadium	4. Stadium	5. Stadium	Durchschnittslänge aller Larven in mm
Højris, $^{11}/_{8}$ 35	1352	82,5 %	17,5 %	..	..	..	1,11 ± 0,010
Højris, $^{10}/_{9}$ 40 (Pflanzen)	4814	22,5 %	77,4 %	0,1 %	..	..	1,34 ± 0,013
Højris, $^{10}/_{9}$ 40 (Steine)	2133	22,2 %	77,8 %	..	..	..	
Rold, $^{21}/_{10}$ 35	58	..	77,7 %	20,6 %	1,7 %	..	1,35 ± 0,025
Højris, $^{31}/_{12}$ 34	198	..	..	91,4 %	8,6 %	..	2,54 ± 0,031
Røde Mølle, $^{4}/_{1}$ 40	60	..	10,0 %	90,0 %	..	..	2,36 ± 0,039
Højris, $^{19}/_{4}$ 35	409	..	..	64,6 %	35,4 %	..	2,73 ± 0,037
Højris, $^{22}/_{5}$ 40	271	..	..	..	99,6 %	0,4 %	5,10 ± 0,039
Højris, $^{13}/_{6}$ 35	92	..	..	..	..	100 %	8,58 ± 0,011
Højris, $^{22}/_{6}$ 35	27	..	..	..	..	100 %	7,94 ± 0,145
Højris, $^{27}/_{6}$ 40	200	..	..	..	..	100 %	8,45 ± 0,085
Højris, $^{10}/_{7}$ 34	60	..	..	..	..	100 %	6,52 ± 0,100

Højris liegt an der eurythermen Sønderup Aa, Rold und Røde Mølle an der teilweise stenothermen Lindenborg Aa. Die Einsammlungen sind nach Jahreszeit geordnet. Sie rühren z. gr. T. aus verschiedenen Jahren her; dies bedeutet natürlich eine Schwäche des Materials, jedoch meine ich, dass die prinzipiellen Züge sich daraus erkennen lassen.

Am 11. August wurden — gleichzeitig mit Imagines — zahlreiche Larven im 1. Stadium und eine nicht geringe Anzahl im 2. Stadium gefunden. In Tabelle I ist die Veränderung des Bestandes im Laufe des Jahres angegeben und in Abb. 16 das Wachstum der Larven graphisch dargestellt. Wie man sieht, verläuft das Wachstum bis Neujahr recht gleichmässig und steht dann bis zum 19. April still; (die Stagnation kann vielleicht schon zu einem etwas früheren Zeitpunkt eingetreten sein). Der scheinbare Stillstand vom $^{10}/_{9}$—$^{21}/_{10}$ ist dadurch zu erklären, dass die letzte Probe aus der teilweise stenothermen Lindenborg Aa stammt. Hier herrscht im Spätsommer eine niedrigere Temperatur als in der eurythermen Sønderup Aa; dies veranlasst offenbar ein langsameres Wachstum. Später im Jahre ändert sich das Verhältnis; das Wasser der Lindenborg Aa ist nun wärmer als das der Sønderup Aa, und bis Neujahr hat, wie man sieht, die Lindenborg-Population auch die Sønderup-Population wieder eingeholt ($^{31}/_{12}$ und $^{4}/_{1}$). Dies zeigt, dass die Temperatur ein wesentlicher Faktor für die Winterstagnation ist. Im Frühjahr (nach dem 19. April) tritt eine gewaltige Steigerung der Wachstumsgeschwindigkeit ein, die nun bis zum Schluss der Entwicklung unverändert bleibt. Der scheinbare Rückgang gegen Ende der Kurve beruht auf dem Abgang als Puppe, durch den ja die grössten und best entwickelten Larven zuerst betroffen werden.

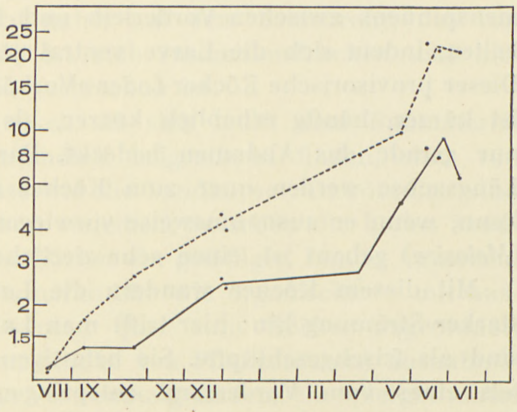

Abb. 16. Wachstumskurven der Larven (ausgezogene Linie) und der Köcher (unterbrochene Linie). Abszisse: Monate des Jahres, Ordinate (logarithmische Skala): Länge in mm. Vgl. Tabelle I, II und VI.

Schliesslich ersieht man aus der Tabelle, dass die Überwinterung auf den mittleren Larvenstadien (besonders dem 3. Stadium) stattfindet, und dass das 5. Stadium von kürzerer Dauer ist, als sonst gewöhnlich bei Köcherfliegen.

Biologie.

Die Larven schlüpfen aus den Eiern nach Verlauf von 1—2 Wochen; aus den feinen Detritusteilchen, die sich an den Stellen, wo die Eier abgelegt werden, in reichlicher Menge finden, baut sich die frischgeschlüpfte Larve einen zylindrischen Köcher mit weit offenem Hinterende (9, Abb. 6 A). Ich hatte Gelegenheit, den Beginn dieses Köcherbaues bei Larven in Gefangenschaft zu beobachten. Die ersten Partikel werden während des Zusammenspinnens zwischen Vorderleib und Hinterleibsspitze festgehalten, indem sich die Larve ventral stark zusammenkrümmt. Dieser provisorische Köcher (oder »Vorköcher« im Sinne Siltalas) ist kürzer, häufig erheblich kürzer, als die Larve, so dass er nur gerade das Abdomen bedeckt. Partikel mit ausgeprägter Längsachse werden quer zum Köcher angebracht, und dieser kann, wenn er ausnahmsweise vorwiegend aus Diatomeenfäden (*Melosira*) gebaut ist, einen sehr zierlichen Anblick bieten.

Mit diesem Köcher wandern die Larven nach Stellen mit starker Strömung hin; hier trifft man Larven, die kaum grösser sind als frischgeschlüpfte. Sie befestigen nun den Köcher mittels eines vom Vorderrand entspringenden Seidenbandes am Substrat. Ihr typischer Biotop sind die Steine am Grunde des Gewässers; die Larven vermeiden hier nicht einmal die Strecken mit stärkster Strömung (Strömungsgeschwindigkeit bis zu 150 cm/Sek., s. Tafel I, Abb. 1). Jedoch kann man auch an etwas ruhigeren Stellen, wo der Grund aus Sand besteht, grosse Mengen von Larven finden. Hier sind sie an die Vegetation gebunden, besonders an *Glyceria fluitans* oder ähnliche Formen mit bandförmigen Blättern; sie sitzen auf den äussersten Enden der Pflanzen, wo sie der Strömung des freien Wassers ausgesetzt sind. Bei Strömungsgeschwindigkeit von weniger als etwa 50 cm/Sek. trifft man kaum noch Larven an.

Der provisorische Köcher hat nur eine kurze Lebensdauer. Nachdem die Larve ihn befestigt hat, baut sie mit Sandkörnern weiter und streift schliesslich den provisorischen Köcher ab. Während der weiteren Entwicklung verwendet die Larve fast ausschliesslich feine Sandkörner als Baumaterial, deren Grösse sich mit dem Alter der Larve ändert; im 1. und 2. Stadium ist ihr Durchmesser durchschnittlich 33 μ, im 3. Stadium 42, im 4. 65

und im 5. Stadium 100 μ. Da aber die Grösse in jedem Stadium ziemlich schwankt, machen sich die Häutungen auf dem Köcher nicht deutlich bemerkbar, so wie es z. B. bei *Ecclisopteryx* (10, S. 565) der Fall ist. Auf den jüngeren Stadien sind die Sandkörner im Verhältnis zur Larve wesentlich grösser als später. Die grössten Sandkörner sind mehr oder weniger flach gedrückt und wenden eine der Breitseiten nach innen. Die einzelnen Sandkörner liegen recht dicht an einander, ohne sich jedoch gegenseitig zu berühren oder sich zu überdecken. Die Solidität des Köchers (Abb. 1) beruht daher auf dem Seidenrohr, das von sehr zäher Beschaffenheit ist, sich zusammendrücken und biegen lässt, aber kaum zu zerbrechen ist.

Die organischen Partikel, die sich meist dem Baumaterial als untergeordneter Bestandteil beigemischt finden, sind von gleicher Form und Grösse wie die Sandkörner. Gewöhnlich sind sie von dunkler Farbe und verleihen, zwischen die hellen Sandkörner eingesprengt, dem Köcher ein gefälliges, buntes Aussehen. Materialien — sowohl mineralische wie organische — mit ausgeprägter Längsachse werden quer zum Köcher angeordnet; dieser kann daher, wenn Stücke von Pflanzenfasern ausnahmsweise in grösserer Menge Verwendung finden, ein quergestreiftes Aussehen erhalten.

Will man das Wachstum des Köchers berechnen, so muss man die Länge der im Laufe der Entwicklung hinten abgebrochenen Stücke zur gegenwärtigen Länge des Köchers hinzurechnen. Die Länge (x) des zwischen zwei bestimmten Zeitpunkten abgebrochenen Stückes lässt sich nach der Formel $x = {}^1/_2 (b_2 - b_1)$ cot ${}^1/_2 v$ berechnen, wobei b_1 und b_2 die hintere Breite des Köchers zu den beiden Zeitpunkten und v den Scheitelwinkel des Köchers (Tabelle V) bedeuten, d. h. den Scheitelwinkel des Kegels, von dem der Köcher einen Stumpf bildet. (Die Herleitung dieser Formel wird aus der nebenstehenden Abb. A hervorgehen). Die beobachteten Werte für b und die daraus berechneten Werte für x sind in Tabelle VI aufgeführt. Bei

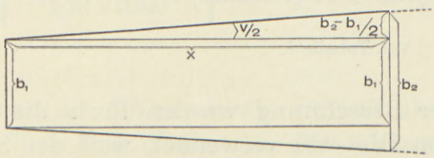

Abb. A. Schematische Darstellung des hinteren Abbruchs des Köchers. Man sieht, dass $\cot \frac{1}{2} v = \frac{x}{{}^1/_2 (b_2 - b_1)}$; hieraus ergibt sich $x = \frac{1}{2} (b_2 - b_1) \cot \frac{1}{2} v$.

Tabelle II. Variationsbreite und Durch-

	1. Stadium	2. Stadium
Højris, $^{11}/_{8}$ 35	0,87 — 1,13 0,965 ± 0,014	0,85 — 1,31 1,01 ± 0,007
Højris, $^{10}/_{9}$ 40 (Pflanzen)	(0,93) 1,06 — 1,34 1,165 ± 0,011	1,03 — 1,50 1,15 ± 0,008
Højris, $^{10}/_{9}$ 40 (Steine)	1,03 — 1,43 1,18 ± 0,012	(0,98) 1,03 — 1,79 1,225 ± 0,009
Rold, $^{21}/_{10}$ 35	...	1,33 — 2,05 1,66 ± 0,025
Højris, $^{31}/_{12}$ 34	...	...
Røde Mølle, $^{4}/_{1}$ 40	...	1,17 — 1,41 1,34 ± 0,04
Højris, $^{19}/_{4}$ 35	...	...
Højris, $^{22}/_{5}$ 40	...	...
Højris, $^{13}/_{6}$ 35	...	...
Højris, $^{22}/_{6}$ 35	...	...
Højris, $^{27}/_{6}$ 40	...	...
Højris, $^{10}/_{7}$ 34 (Pflanzen)	...	...

Vgl. Tabelle I.

der Berechnung wurden für v die Durchschnittswerte der Zeit von $^{11}/_{8}$—$^{19}/_{4}$ verwendet, weil der Scheitelwinkel am Ende der Entwicklung sich ändert, und der Abbruch ja im hinteren Teil des Köchers stattfindet.

Die so berechneten Wachstumszahlen (tatsächliche Länge $+\Sigma x$) sind in Abb. 16 graphisch dargestellt. Das Wachstum des

schnitt des Verhältnisses $\frac{\text{Köcherlänge}}{\text{Larvenlänge}}$.

3. Stadium	4. Stadium	5. Stadium	Alle
...	...	...	0,973 ± 0,013
1,08 – 1,38 1,20 ± 0,09	...	...	1,185 ± 0,006
...	...	...	
1,40 – 2,05 1,62 ± 0,06	2,04	...	1,65 ± 0,023
1,07 – 1,95 1,47 ± 0,016	1,21 – 1,72 1,41 ± 0,042	...	1,465 ± 0,015
1,11 – 1,96 1,40 ± 0,021	...	...	1,395 ± 0,02
1,15 – 2,73 1,96 ± 0,018	1,17 – 2,78 1,98 ± 0,032	...	1,96 ± 0,019
...	0,98 – 1,69 1,32 ± 0,011	1,25	1,32 ± 0,011
...	...	1,24 – 2,24 1,78 ± 0,032	1,78 ± 0,032
...	...	1,27 – 2,52 1,80 ± 0,058	1,80 ± 0,058
...	...	0,98 – 1,99 1,50 ± 0,012	1,50 ± 0,012
...	...	1,46 – 2,60 2,16 ± 0,045	2,16 ± 0,045

Köchers nimmt, wie man sieht, am $^{10}/_{9}$ etwas ab, verläuft aber dann gleichmässig bis zum $^{22}/_{5}$. Das Wachstum des Köchers, d. h. die Aktivität der Larve, wird also vom Winter nicht annähernd so stark beeinflusst wie das Wachstum der Larve. Zur Frühjahrszeit tritt eine gewaltige Steigerung der Wachstumsgeschwindigkeit des Köchers ein. Nach der Kurve scheint es,

als ob dies zu einem späteren Zeitpunkt ($^{22}/_{5}$) geschieht als die sprungweise Wachstumssteigerung der Larve ($^{19}/_{4}$). Indessen dürfte die Form der Kurve auf den ungewöhnlich strengen Winter 1940 zurückzuführen sein; es ist anzunehmen, dass in Wirklichkeit die Steigerung der Wachstumsgeschwindigkeit bei Larve und Köcher gleichzeitig einsetzt. An den Köchern vom 22. Mai 1940 sieht man nämlich eine scharfe Grenze zwischen dem hinteren, dunkleren, älteren Teil und dem vorderen, helleren, jüngeren; diese Grenze muss den Beginn einer Stagnationsperiode bezeichnen. Die vordere, hellere Partie ist $5{,}41 \pm 0{,}08$ mm lang; zieht man diese Länge von der Wachstumszahl des Köchers ab, so erhält man $4{,}45 \pm 0{,}13$ mm. Diese Zahl entspricht, wie die Kurve zeigt, der Wachstumszahl von Anfang Januar, und das war gerade der Zeitpunkt, an dem der strenge Frost einsetzte[1]. Hieraus ergibt sich, dass das Wachstum des Köchers zwar in normalen Wintern nicht aufhört, dass aber ein abnorm strenger Winter vermag, es zum Stillstand zu bringen (während der Frostperiode 1940 lag vermutlich die Temperatur des Wassers in der Sønderup Aa während der ganzen Zeit nahe dem Gefrierpunkt); dies lässt darauf schliessen, dass die abnorm strengen Winter der letzten Jahre sich der für die Art letalen Grenze nähern. Vielleicht lässt sich hierdurch die westliche Verbreitung der Art erklären.

Da normalerweise das Wachstum des Köchers während der Wintermonate nicht aufhört, während das Wachstum der Larve stark gehemmt wird, und da bei den meisten Köchern der rückwärtige Abbruch unbeträchtlich ist, so nimmt im Laufe des Jahres die Länge des Köchers im Verhältnis zur Larve erheblich zu (Tabelle II), bis er im Vorfrühling fast doppelt so lang ist wie die Larve; dieses Verhältnis erhält sich während der weiteren Entwicklung ziemlich unverändert. Wie die Tabelle zeigt, ist die relative Länge des Köchers eine Funktion der Jahreszeit, während das Larvenstadium dafür jedenfalls nur von sehr geringer Bedeutung ist. (Vgl. z. B. 1. und 3. Stadium am $^{10}/_{9}$ und 3. und 4. Stadium am $^{19}/_{4}$). Die Zahlen zeigen eine deutliche Tendenz, obwohl die Unregelmässigkeiten, wie zugegeben sei, im Verhältnis zum mittleren Fehler gross erscheinen. Vielleicht machen sich dabei kleine, lokale Milieuunterschiede (Beschaffung von

[1] Infolge dieser Stagnation sind die Köcher vom $^{22}/_{5}$ 1940 ungewöhnlich kurz, der entsprechende Punkt auf Abb. 16 somit »zu niedrig«.

Baumaterial u. dergl.) geltend, die nicht leicht zu überschauen sind. Hier sei nur erwähnt, dass der auffallend niedrige Wert vom 22. Mai 1940 der genannten Stagnationsperiode zuzuschreiben ist.

Tabelle III zeigt das Verhältnis zwischen Breite und Länge des Köchers. Wie man sieht, ist dieses Verhältnis zu ein und demselben Zeitpunkt bei den verschiedenen Larvenstadien sehr ungleich; es nimmt während der Entwicklung stark ab, so dass der Köcher der ausgewachsenen Larve ungewöhnlich schlank erscheint. Die senkrechten Kolonnen der Tabelle zeigen, dass das Verhältnis sich innerhalb eines Larvenstadiums im Laufe des Jahres nicht wesentlich ändert, jedenfalls kaum mehr, als sich durch Verschiebung im »durchschnittlichen Alter« des Larvenstadiums erklären lässt; (z. B. ist der Wert für das 2. Stadium am $^{4}/_{1}$ identisch mit dem für das 3. Stadium am $^{10}/_{9}$; am $^{10}/_{9}$ sind die meisten Larven des 3. Stadiums jung, am $^{4}/_{1}$ die meisten des 2. Stadiums alt). Da indessen die relative Länge des Köchers zunimmt, so bedeutet dies, dass er im Laufe des Winters geräumiger im Verhältnis zur Larve wird. Dies gilt innerhalb des einzelnen Larvenstadiums; bei den Häutungen dürfte die Geräumigkeit jedesmal wieder etwas abnehmen, da der Vorderleib der Larve an Breite zunimmt. Der innere Durchmesser des Köchers entspricht bei der ausgewachsenen Larve ziemlich genau ihrer Breite. Die verhältnismässig grosse Breite des Köchers auf den jüngeren Stadien ist besonders den im Verhältnis grösseren Baumaterialien zuzuschreiben.

Tabelle IV gibt das Verhältnis zwischen vorderer und hinterer Breite des Köchers an. Dieses Verhältnis ändert sich ähnlich wie das zwischen Länge und Breite. Es nimmt vom 1.—4. Stadium stark zu und bleibt dann bis zum Schluss der Entwicklung konstant. Das Verhältnis drückt die Konizität des Köchers aus, jedoch ist auch die relative Breite in diesem Ausdruck mitenthalten. Dagegen ist der Scheitelwinkel des Köchers (Tabelle V, trigonometrisch berechnet) ein Ausdruck für seine Konizität, bei dem jener Faktor ausgeschaltet ist. Wie man sieht, hält sich der Wert des Scheitelwinkels vom 1.—4. Stadium fast konstant, abgesehen vom Beginn des 1. Stadiums ($^{11}/_{8}$), wo er wesentlich geringer ist; dies beruht auf dem schnellen Wachstum des Köchers im Beginn dieses Stadiums (der Scheitelwinkel des provisorischen Köchers ist 0°). Die anscheinende Zunahme

Tabelle III. Variationsbreite und Durch-

	1. Stadium	2. Stadium
Højris, $^{11}/_{8}$ 35	0,29 — 0,54 0,375 ± 0,004	0,20 — 0,35 0,286 ± 0,003
Højris, $^{10}/_{9}$ 40 (Pflanzen)	0,27 — 0,40 0,314 ± 0,003	0,19 — 0,35 0,266 ± 0,003
Højris, $^{10}/_{9}$ 40 (Steine)	0,27 — 0,40 0,314 ± 0,004	0,21 — 0,33 0,259 ± 0,003
Rold, $^{21}/_{10}$ 35	...	0,19 — 0,28 0,232 ± 0,003
Højris, $^{31}/_{12}$ 34	...	...
Røde Mølle, $^{4}/_{1}$ 40	...	0,21 — 0,23 0,226 ± 0,005
Højris, $^{19}/_{4}$ 35	...	...
Højris, $^{22}/_{5}$ 40	...	...
Højris, $^{13}/_{6}$ 35	...	...
Højris, $^{22}/_{6}$ 35	...	...
Højris, $^{27}/_{6}$ 40	...	...
Højris, $^{10}/_{7}$ 34	...	...
Højris, undatiert	...	...
Alle	0,345 ± 0,003	0,268 ± 0,002

Vgl. Tabelle I.

schnitt des Verhältnisses $\frac{\text{Köcherbreite}}{\text{Köcherlänge}}$.

3. Stadium	4. Stadium	5. Stadium	Ruhelarven und Puppen
...	...	...	...
0,21 – 0,24 0,227 ± 0,01	...	...	...
...	...	...	...
0,17 – 0,28 0,212 ± 0,008	0,17	...	...
0,13 – 0,25 0,176 ± 0,002	0,13 – 0,20 0,161 ± 0,005	...	...
0,15 – 0,24 0,190 ± 0,003	...	...	...
0,12 – 0,24 0,161 ± 0,002	0,11 – 0,24 0,149 ± 0,003	...	...
...	0,12 – 0,21 0,154 ± 0,001	0,17	...
...	...	0,09 – 0,135 0,115 ± 0,002	...
...	...	0,08 – 0,145 0,120 ± 0,005	...
...	...	0,09 – 0,20 0,133 ± 0,002	...
...	...	0,085 – 0,165 0,110 ± 0,002	0,08 – 0,13 0,103 ± 0,002
...	...	...	0,085 – 0,14 0,106 ± 0,004
0,173 ± 0,001	0,155 ± 0,001	0,129 ± 0,001	0,104 ± 0,002

Tabelle IV. Variationsbreite und Durch-

	1. Stadium	2. Stadium
Højris, $^{11}/_{8}$ 35	1,00 – 1,43 1,13 ± 0,01	1,10 – 1,55 1,28 ± 0,01
Højris, $^{10}/_{9}$ 40 (Pflanzen)	1,13 – 1,45 1,27 ± 0,01	1,14 – 1,63 1,35 ± 0,01
Højris, $^{10}/_{9}$ 40 (Steine)	1,09 – 1,43 1,24 ± 0,01	1,14 – 1,52 1,33 ± 0,01
Rold, $^{21}/_{10}$ 35	...	1,24 – 1,72 1,44 ± 0,02
Højris, $^{31}/_{12}$ 34	...	...
Røde Mølle, $^{4}/_{1}$ 40	...	1,26 – 1,43 1,39 ± 0,03
Højris, $^{19}/_{4}$ 35	...	...
Højris, $^{22}/_{5}$ 40	...	...
Højris, $^{13}/_{6}$ 35	...	...
Højris, $^{22}/_{6}$ 35	...	...
Højris, $^{27}/_{6}$ 40	...	...
Højris, $^{10}/_{7}$ 34	...	...
Højris, undatiert	...	...
Alle	1,19 ± 0,007	1,325 ± 0,005

Vgl. Tabelle I.

schnitt des Verhältnisses $\frac{\text{Vordere}}{\text{Hintere}}$ Köcherbreite.

3. Stadium	4. Stadium	5. Stadium	Ruhelarven und Puppen
...	...	...	...
1,38 — 1,49 1,44 ± 0,03	...	...	...
...	...	...	...
1,38 — 2,04 1,63 ± 0,02	1,76	...	...
1,30 — 2,04 1,67 ± 0,01	1,61 — 2,19 1,76 ± 0,03	...	...
1,41 — 1,88 1,62 ± 0,02	...	...	...
1,34 — 2,49 1,66 ± 0,01	1,36 — 2,52 1,95 ± 0,03	...	...
...	1,36 — 2,52 2,04 ± 0,02	1,92	...
...	...	1,54 — 2,68 2,09 ± 0,07	...
...	...	1,67 — 2,55 2,13 ± 0,08	...
...	...	1,33 — 2,86 1,87 ± 0,025	...
...	...	1,50 — 2,60 2,04 ± 0,04	1,58 — 2,32 1,99 ± 0,03
...	...	...	1,43 — 2,70 1,81 ± 0,07
1,66 ± 0,009	1,99 ± 0,015	1,95 ± 0,022	1,94 ± 0,031

Tabelle V. Variationsbreite und Durch-

	1. Stadium	2. Stadium	3. Stadium
Højris, $^{11}/_{8}$ 35	0° — 7°,09 2°,33 ± 0,11	1°,95 — 5°,73 3°,60 ± 0,08	...
Højris, $^{10}/_{9}$ 40 (Pflanzen)	1°,49 — 5°,15 3°,73 ± 0,12	2°,07 — 6°,07 3°,85 ± 0,08	3°,20 — 4°,35 3°,89 ± 0,37
Højris, $^{10}/_{9}$ 40 (Steine)	1°,49 — 5°,62 3°,40 ± 0,12	2°,29 — 6°,07 3°,61 ± 0,07	...
Rold, $^{21}/_{10}$ 35	...	2°,17 — 5°,84 3°,92 ± 0,17	3°,32 — 6°,75 4°,58 ± 0,27
Højris, $^{31}/_{12}$ 34	...	...	2°,52 — 5°,26 3°,82 ± 0,05
Røde Mølle, $^{4}/_{1}$ 40	...	2°,63 — 3°,89 3°,54 ± 0,21	3°,20 — 5°,15 4°,10 ± 0,08
Højris, $^{19}/_{4}$ 35	...	...	2°,52 — 5°,95 3°,84 ± 0,04
Højris, $^{22}/_{5}$ 40	...	...	...
Højris, $^{13}/_{6}$ 35	...	...	...
Højris, $^{22}/_{6}$ 35	...	...	...
Højris, $^{27}/_{6}$ 40	...	...	...
Højris, $^{10}/_{7}$ 34	...	...	...
Højris, undatiert	...	...	...
Alle	2°,93 ± 0,08	3°,80 ± 0,04	3°,87 ± 0,03

Vgl. Tabelle I.

schnitt des Scheitelwinkels des Köchers.

4. Stadium	5. Stadium	Ruhelarven und Puppen	Alle
...	...	...	2,92 ± 0,09
...	...	...	3°,66 ± 0,07
...	...	...	
4°.01	...	...	4°,08 ± 0,20
3°,43 — 4°,58 3°,94 ± 0,09	...	...	3°,83 ± 0,05
...	...	...	4°,04 ± 0,10
2°,74 — 5°,61 3°,86 ± 0,06	...	...	3°,84 ± 0,05
2°,74 — 5°,62 4°,36 ± 0,03	4°,69	...	4°,36 ± 0,03
...	2°,18 — 4°,47 3°,26 ± 0,08	...	3°,26 ± 0,08
...	2°,40 — 4°,12 3°,23 ± 0,37	...	3°,23 ± 0,37
...	1°,72 — 5°,38 3°,25 ± 0,06	...	3°,25 ± 0,06
...	2°,29 — 4°,01 3°,09 ± 0,06	2°,06 — 3°,55 2°,87 ± 0,07	3°,08 ± 0,06
...	...	2°,06 — 3°,66 2°,62 ± 0,11	2°,62 ± 0,11
4°,20 ± 0,03	3°,22 ± 0,03	2°,80 ± 0,06	...

im 4. Stadium ($^{22}/_{5}$) ist auf die oben erwähnte unnormale Stagnation zurückzuführen, durch die die Larve gezwungen wurde, während der Steigerung der Wachstumsgeschwindigkeit im Frühling den Köcher verhältnismässig breit zu bauen. Während des 5. Stadiums nimmt der Wert deutlich und recht erheblich ab.

Tabelle VI. Durchschnittswerte von *b* und *x* (siehe Text).

	b	*x*
Højris, $^{11}/_{8}$ 35	0,31 mm ± 0,008	0,15 mm ± 0,003
Højris, $^{10}/_{9}$ 40	0,32 » ± 0,0013	0.23 » ± 0,006
Rold, $^{21}/_{10}$ 35	0,335 » ± 0,0048	0,54 » ± 0,014
Højris, $^{31}/_{12}$ 34	0,37 » ± 0,0033	0,31 » ± 0,008
Røde Mølle, $^{4}/_{1}$ 40	0,355 » ± 0,0029	1,83 » ± 0,042
Højris, $^{19}/_{4}$ 35	0,49 » ± 0,0046	0,38 » ± 0,009
Højris, $^{22}/_{5}$ 40	0,515 » ± 0,0063	4,36 » ± 0,135
Højris, $^{13}/_{6}$ 35	0,80 » ± 0,0165	3,82 » ± 0,222
Højris, $^{22}/_{6}$ 35	0,765 » ± 0,0404	6,12 » ± 0,165
Højris, $^{27}/_{6}$ 40	0,915 » ± 0,0124	3,67 » ± 0,114
Højris, $^{10}/_{7}$ 34	0,755 » ± 0,0167	
Ruhelarven und Puppen	0,845 » ± 0,0255	...

x ist sowohl für $^{31}/_{12}$ wie für $^{4}/_{1}$ im Verhältnis zum $^{21}/_{10}$, für $^{13}/_{6}$—$^{10}/_{7}$ im Verhältnis zum $^{22}/_{5}$ berechnet. Vgl. übrigens Tabelle I.

Die Seidengrundlage des Köchers besteht aus zwei Schichten, die sich besonders bei alten, leeren Puppenköchern leicht, bei neueren nur schwer von einander trennen lassen. In der äusseren Schicht kreuzen sich die Seidenfäden unregelmässig. Diese Schicht ist zwischen den Sandkörnern stark verdickt, so dass sie nach Entfernung des Sandes aus flachen Zellen besteht, die durch schmale, gelbbraune Scheidewände getrennt sind (Tafel II, Abb. 5). Auch in der inneren Schicht, die die äussere wie eine dünne Tapete auskleidet, haben die Fäden einen etwas unregelmässigen Verlauf, ziehen jedoch vorwiegend in longitudinaler und transversaler Richtung. Die einzelnen Fäden sind im 5. Stadium etwa 14 μ dick.

Das Hinterende wird durch eine gelbbraune, ziemlich dicke, flach kegelförmige Membran (Abb. 17, C, D) verschlossen; sie besteht vorwiegend aus zirkulären Fäden, denen jedoch in den

inneren Schichten auch radiäre beigemischt sind. Die Membran wird in der Mitte durch eine kreisrunde oder schwach ovale, häufig etwas unregelmässige Öffnung durchbohrt; ihr Durchmesser beträgt im 1. Stadium 0,1—0,18 mm, im 2. bis zu 0,23, im 3. bis 0,26, im 4. und 5. Stadium bis 0,4—0,5 mm. Die Breite der Membran richtet sich nach der sehr schwankenden hinteren Breite des Köchers. Sie kann bei breiten Köchern im 5. Stadium bis zu 0,3 mm betragen, während die Membran an den schmalsten Köchern nur als Verdickung des Randes erscheint oder bei den allerschmalsten und längsten häufig sogar ganz fehlt. An der Aussenseite der Membran können ganz feine Sandkörnchen befestigt sein; dies scheint am häufigsten bei jüngeren Stadien der Fall zu sein.

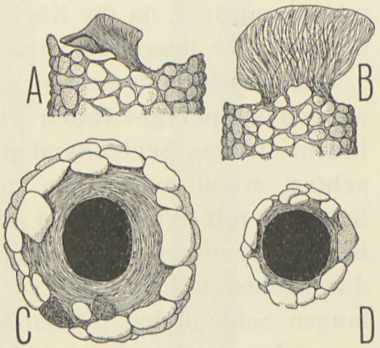

Abb. 17. Larvenköcher, 5. Stadium. A. und B. Vorderenden zweier Köcher, die zwei verschiedene Typen des Seidenbandes zeigen. $^{14}/_{1}$. A. war an einer Wasserpflanze befestigt; die Schiefheit des Stieles ist vermutlich als Resultante des Zuges der Strömung und der Schwerkraft entstanden. C. Hinterende eines verhältnismässig kurzen Köchers. D. dasselbe von einem verhältnismässig langen. $^{21}/_{1}$.

Es bleibt noch eine sehr charakteristische Eigenschaft des Köchers zu besprechen, nämlich seine Befestigung am Substrat während der gesamten Entwicklung durch ein vom Vorderende des Seidenrohrs ausgehendes Seidenband (Abb. 17, A, B). Dieses kann schmal und drehrund oder breit und flach sein; in den jüngeren Stadien scheint ausschliesslich die erste Form vorzukommen, während die letztere gegen Ende des Larvenlebens vorherrscht. In beiden Formen verbreitert sich das Band distal zu einem grossen »Tellerchen«. Die Strömung bewirkt unmittelbar, dass sich das Vorderende des Köchers gegen sie kehrt; (die Köcher können durch die Strömung ein wenig hin und her bewegt werden).

Die Larven befestigen ihre Köcher an den Partien der Steine (oder Pflanzen), die am meisten der Strömung ausgesetzt sind; auf Steinen sind sie meist reihenweise quer zur Strömung angeordnet. Infolge ihrer grossen Zahl machen die Larven sich stark bemerkbar; sie befestigen ausserordentlich häufig ihren Köcher an dem einer anderen Larve, ein wenig hinter dem

Vorderrand desselben. Auf diese Weise können ganze Ketten von Larvenköchern entstehen. Das starke Wachstum gegen Ende der Larvenentwicklung führt zu heftigem Platzmangel, durch den die Larven gezwungen werden, buchstäblich in mehreren Stockwerken über einander zu wohnen; hierdurch entstehen Klumpen von sehr charakteristischer Form (Tafel II, Abb. 6). Alle Köcher eines Klumpens liegen selbstverständlich parallel; seine gegen die Strömung gekehrte Vorderseite ist treppenförmig angeordnet, da die Köcher in jedem Stockwerk etwas gegen die darunter liegenden zurückspringen.

All dies ist indessen nicht so zu verstehen, als sei die Larve an den einmal gewählten Platz gefesselt. Sie kann wegziehen, indem sie das Seidenband durchnagt; das kann man oft beobachten, wenn man einen mit Larven besetzten Stein in einer Schüssel mit Wasser oder an einer Stelle des Wasserlaufes anbringt, die ihnen nicht zusagt. Nachdem die Larve das Band durchgebissen hat, kann sie sich an einem mindestens 20 cm langen Seidenfaden treiben lassen, an dem sie sich dann langsam, aber stetig wieder gegen die Strömung aufwärts arbeitet.

Besonders scheinen die jüngeren Larven dazu zu neigen, ihren Platz zu wechseln; später nimmt diese Neigung erheblich ab und hört gegen Ende des Larvendaseins völlig auf. Der Köcher von älteren Larven des 5. Stadiums ist stets solider befestigt als der von jüngeren; häufig lässt auch die Art und Weise, wie das bis 10 mm lange, vordere Stück gebaut ist, erkennen, dass die Larve während seines Baues den Platz nicht gewechselt hat. (Dies wäre ja auch für Larven in den unteren Schichten der erwähnten Klumpen unmöglich). Oft findet man nämlich nicht nur ein einziges, vom Vorderende des Köchers ausgehendes Band, sondern eine ganze Reihe von Bändern hinter einander an der Ventralseite des Köchers (Abb. 18 B); besonders sieht man dies bei Köchern, die an anderen befestigt sind. Die Bänder sind, wie Abb. 18 A zeigt, nicht in einer regelmässigen Reihe, sondern je nach der Form der Unterlage angeordnet[1]. Noch häufiger sieht man, dass bei direkt am Stein befestigten Köchern ein mittlerer Längsstreifen an der Ventralseite des Seidenrohres nicht von Sandkörnern bedeckt, sondern am Substrat befestigt ist. (An einem solchen Köcher fand ich eine sehr merkwürdige Abnor-

[1] Jedes Band entspricht zweifellos einem ehemaligen Vorderende.

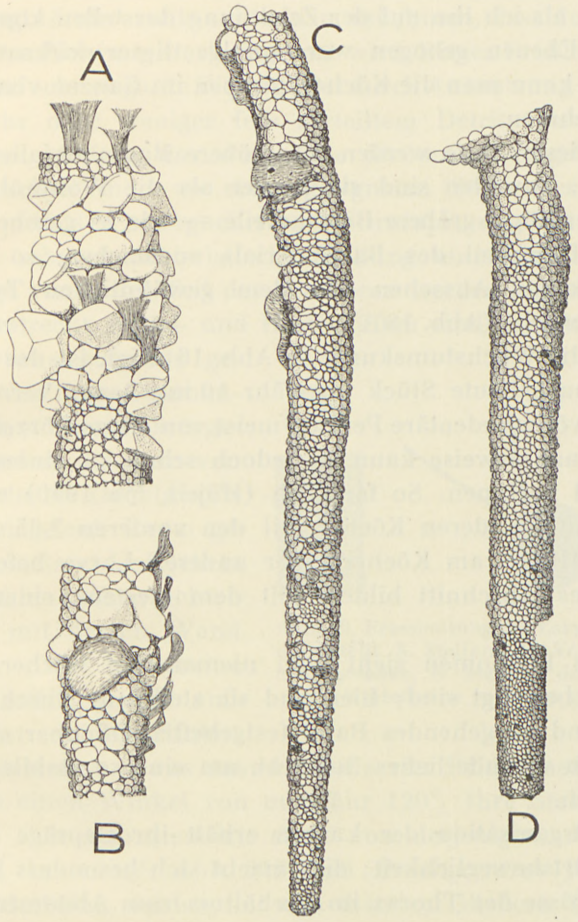

Abb. 18. A.—C. Köcher ausgewachsener Larven, D. Puppenköcher. $^{7}/_{1}$. A. und B. zeigen den vorderen Teil von 2 Köchern, A. von der Ventralseite, B. von rechts. C. Köcher von links, D. von rechts. Näheres siehe Text.

mität; innerhalb der am Substrat befestigten Seidenschicht fand ich eines zweite, die von feinen Sandkörnchen bedeckt war). Vom Rand des »nackten« Streifens aus kann sich ein »Tellerchen« auf dem Stein ausbreiten. Solche Köcher weichen häufig von der regelmässigen, geraden Form ab und formen sich nach der Unterlage; namentlich findet sich recht allgemein am Übergang vom »losen« zum »festen« Teil ein stumpfwinkliger Knick (Abb. 18 D), oder der Köcher ist ganz unregelmässig gekrümmt (Abb. 18 C). Der abgebildete Köcher war sogar noch unregel-

mässiger, als ich ihn auf der Zeichnung darstellen konnte, da er in zwei Ebenen gebogen war. Infolge dieser wirksameren Befestigung kann man die Köcherklumpen im Ganzen von den Steinen abnehmen.

Auf dieser Stufe werden oft gröbere Baumaterialien benutzt als sonst; zuweilen sind sie breiter als die Wohnröhre selbst. Wenn derartige, gröbere Bestandteile — wie es geschehen kann — den Hauptteil des Baumaterials ausmachen, so entstehen Köcher, deren Aussehen von dem gewöhnlichen Typus sehr stark abweicht (Abb. 18 A).

Wie die Wachstumskurve in Abb. 16 zeigt, ist das ganze im 5. Stadium gebaute Stück ungefähr 10 mm lang. Vermutlich ist aber die völlig sedentäre Periode meist von etwas kürzerer Dauer; ganz ausnahmsweise kann sie jedoch schon zu einem früheren Zeitpunkt beginnen. So fand ich (Højris, $^{22}/_{5}$ 1940) eine Larve im 4. Stadium, deren Köcher mit den vorderen 3,65 mm, d. h. fast zur Hälfte, am Köcher einer anderen Larve befestigt war. Der »lose« Abschnitt bildete mit dem »festen« einem Winkel von 155°.

Dieses Phänomen sieht man niemals bei Köchern, die an Pflanzen befestigt sind; hier sind sie stets nur durch ein vom Vorderrand ausgehendes Band festgeheftet. Offenbar sind Pflanzen ein zu veränderliches Substrat, um eine so stabile Bauweise zuzulassen.

Die Organisation der Larven erhält ihr Gepräge durch die geringe Ortsbeweglichkeit; dies macht sich besonders in der geringen Grösse des Thorax im Verhältnis zum Abdomen bemerkbar. Die ausserordentliche Schlankheit der Larve wird dadurch erreicht, dass ihr Wachstum im Wesentlichen als Verlängerung des Abdomens stattfindet; sie ermöglicht ihrerseits die Schlankheit des Köchers und bewirkt daher, dass der Strömung eine möglichst kleine Angriffsfläche geboten wird. Ebenso sind die Beine nicht als typische Gangbeine ausgebildet, sondern einer anderen Funktion angepasst, über die unten Weiteres berichtet wird.

Die Befestigung des schlanken, glatten, nach hinten stark verschmälerten Köchers mit dem Vorderende gegen die Strömung ist eine besonders wirksame Methode zur Beseitigung der Schwierigkeiten, die der Aufenthalt in stark fliessendem Wasser mit

sich bringt. Andererseits sollte man annehmen, dass durch sie der Zugang zur Nahrung stark begrenzt wird. Dieses Problem wird indessen auf sehr elegante Weise gelöst durch Ausnutzung der Mengen von mehr oder weniger fein zerteiltem Detritus und anderen Nahrungsmaterials, das durch die Strömung mitgeführt wird.

Beobachtet man an einer geeigneten Stelle des Ufers die Larven mittels Wassergucker und Stirnlupe, so sieht man, dass sie eine sehr charakteristische Stellung einnehmen (Abb. 19). Kopf und vorderster Teil des Prothorax werden aus dem Köcher herausgestreckt. Mittel- und Hinterhüften sind vorwärts und zugleich etwas aufwärts gestreckt, das Coxa-Trochantergelenk stark auswärts gebeugt, sodass das Bein von Trochanter bis Klaue (das Femur-Tibiagelenk kaum gebeugt) vom Rand des Köchers »ausstrahlen«, einen rechten Winkel mit dessen Wand bildend. Die Mittelbeine sind ungefähr gerade aufwärts gestreckt, die Hinterbeine ausserdem etwas auswärts; die vier Beine »bedecken« zusammen einen Winkel von ungefähr 120°. Ihre Stellung unterscheidet sich also ziemlich stark von derjenigen, die Murphy (8) und Lloyd (5, Abb. 217) bei *Brachycentrus* beschreiben. Die Vorderbeine werden mit den distalen Gliedern (Trochanter — Klaue) unter dem Kopf gehalten, und zwar gerade vorwärts gestreckt und ein wenig gespreizt, so dass ihr Abstand von einander etwa gleich der anderthalbfachen Kopfbreite ist (Trochanter-Femur divergieren ein wenig, das Femur-Tibiagelenk ist schwach gebeugt, so dass Tibia-Klaue wieder etwas konvergieren).

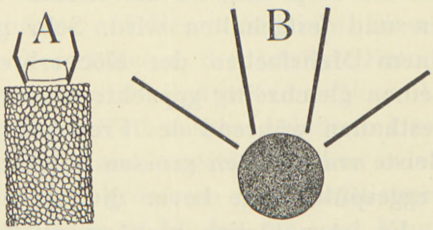

Abb. 19. Fresshaltung der Larve schematisch dargestellt. A. Stellung der Vorderbeine von oben gesehen, B. Stellung der Mittel- und Hinterbeine von vorn gesehen.

Da die Innenkanten von Mittel- und Hinterbein gerade gegen die Strömung gerichtet und die Beine, wie oben erwähnt, stark zusammengedrückt sind, bieten sie der Strömung nur geringen Widerstand. Durch ihren Dornenbesatz (S. 34) sind sie vorzüglich zum (passiven) Ergreifen und Festhalten feiner Detrituspartikel geeignet; man sieht daher auch stets einen feinen »Schleier« von Detritus auf der Innenseite der Beine. Von Zeit zu Zeit wird

abwechselnd eins der Beine gegen den Kopf hin gebeugt und gleichzeitig zusammengefaltet (d. h. Tibia-Klaue werden vollständig gegen die Innenkante des Femur gebeugt, wobei sich die Klaue über die Vorderseite des Femur legt. Dieser Vorgang wird sehr schnell ausgeführt, fast wie ein Schlag). Dann werden die Detrituspartikelchen durch die Dornen und Haardorne des Vorderbeines abgefegt. Die Vorderbeine formen nun aus den Partikeln einen Futterklumpen und halten ihn fest, während die Larve ihn verzehrt. Wird einmal ein grösseres Stückchen gefangen, so beugt sich das betreffende Bein augenblicklich gegen den Kopf hin, wo die »Beute« durch die Vorderbeine ergriffen und festgehalten wird. Sehr grosse Stücke (Länge bis zu einem Mehrfachen der Köcherbreite) werden von allen vier Beinen gleichzeitig gepackt; diese helfen dann auch mit beim Festhalten während des Fressens. Gewöhnlich wird jedoch das Meiste von solchen grossen Stücken durch die Strömung wieder weggespült, lange bevor die Larve mit der Mahlzeit fertig ist.

Es ist natürlich nicht möglich, die Nahrungsaufnahme bei den jüngeren Larvenstadien im Einzelnen zu studieren; da aber die Larven schon im 1. Stadium die beschriebene, charakteristische Haltung haben, und da bereits in diesem Stadium der Besatz von Dornen und Haardornen auf den Innenkanten der Beine entwickelt ist, darf man es als sicher ansehen, dass die Larve während ihrer ganzen Entwicklung dieselbe Technik anwendet.

Nicht nur die Beine der Larve, sondern — wie oben angedeutet — ihre ganze Morphologie wird durch ihre Lebensweise durchgreifend beeinflusst. Die Doppeldorne des Abdomens (s. S. 43) sind zweifellos als Retentionsorgane aufzufassen. Bei den eruciformen Larven kommen Retentionsorgane an den Seiten des Abdomens ganz allgemein vor; jedoch sind sie gewöhnlich nach vorwärts gerichtet und tragen so dazu bei, das Herausgleiten der Larve aus dem Köcher zu verhindern. Bei *Oligoplectrum* sind sie indessen rückwärts gerichtet und dienen dazu zu verhindern, dass die Larve in den Köcher zurückgepresst wird, während sie ihre Freßstellung mit in der Strömung ausgestreckten Mittel- und Hinterbeinen einnimmt. Ein interessantes Beispiel dafür, wie selbst kleine morphologische Züge durch eine spezielle Biologie ihr besonderes Gepräge erhalten. — Die Borsten

des Metepimerons (S. 30) bilden ein Gitter, das den Eingang zur analen Partie des Köchers auf der Ventralseite absperrt. Ähnliche Gebilde finden sich auch bei anderen Larven, jedoch nicht so vollkommen ausgebildet. Vermutlich ist eine derartige Absperrung bei *Oligoplectrum* infolge der fast konstant vorwärts gestreckten Mittel- und Hinterhüften besonders erforderlich.

Über die Art der Nahrung gibt der Darminhalt Aufschluss; er besteht im Spätsommer ($^{11}/_{8}$ und $^{10}/_{9}$) vorwiegend aus Diatomeen, unter denen eine breite, ovale Form vorherrscht. Sie kann bis 35 µ lang sein, also im Verhältnis zu den Mundteilen der Larve im 1. Stadium von beträchtlicher Grösse; dies erklärt vielleicht das Fehlen der Innenbürste auf diesem Stadium. Diese wäre sozusagen im Wege beim Verschlucken der harten Diatomeen, die im ganzen verschlungen werden. Später (vom $^{21}/_{10}$ ab) besteht der Darminhalt zum grössten Teil aus bräunlichem Detritus, und zwar teils aus ganz fein zerkleinerten Stücken von unbestimmbarer Herkunft, teils aus grösseren mit deutlicher Phanerogamen-Struktur. Letztere können bis zu 0,8 mm lang sein. Auch Stücke von Algenfäden oder Pilzhyphen können vorkommen, ebenso sind Bruchstücke von tierischer Herkunft (Insektenlarven) nicht selten. Vermutlich stammen sie von toten Tieren, die von der Strömung mitgeführt wurden.

Hinsichtlich der Ernährung verhalten sich am $^{10}/_{9}$ die ältesten Larven (3. Stadium) ebenso wie die jüngsten (1. Stadium), am $^{21}/_{10}$ die jüngsten (2. Stadium) ebenso wie die ältesten (4. Stadium). Es ist daher anzunehmen, dass der Übergang von vorwiegender Diatomeennahrung zu vorwiegender Detritusnahrung nicht mit dem Alter der Larven zusammenhängt, sondern mit der Jahreszeit und durch die geringere Diatomeenproduktion im Winterhalbjahr infolge schlechterer Licht- und Temperaturverhältnisse bedingt ist. Man könnte deshalb erwarten, dass im Frühling und Frühsommer die Diatomeen wieder den Ehrenplatz auf der Speisekarte einnehmen; das ist aber nicht der Fall. Die Diatomeen sind zwar zahlreich, bilden aber nur einen kleinen Bruchteil des Darminhaltes. Hierbei spricht vermutlich die Grösse der Larven mit.

Ich konnte feststellen, dass frischgeschlüpfte Larven in Gefangenschaft, bevor sie mit dem Bau des provisorischen Köchers beginnen, eine Mahlzeit von Diatomeen einnehmen, die sich an

den Stellen, wo die Eier abgelegt werden, in grosser Menge finden. Ich vermag nicht zu sagen, ob das auch normalerweise im Freien geschieht, halte es aber für sehr wahrscheinlich.

Die Ernährungsbiologie von *Brachycentrus*, wie sie von MURPHY (8) und LLOYD (5, S. 367; 6, S. 82) dargestellt wird, weicht ziemlich von der oben geschilderten bei *Oligoplectrum* ab. In Anbetracht des fast völlig gleichen Baues der Beine bei beiden Gattungen halte ich eine wesentlich grössere Ähnlichkeit der Ernährungsweise für wahrscheinlich; ich möchte daher annehmen, dass die Angaben der beiden Autoren auf unzureichenden Untersuchungen beruhen, oder dass sie vielleicht die Larven unter nicht ganz natürlichen Verhältnissen beobachtet haben. Da *Brachycentrus* in meinem Untersuchungsgebiet nicht vorkommt, konnte ich leider keine Vergleiche anstellen.

Auf dieselbe Weise, wie die Larve sich mit Nahrung versorgt, verschafft sie sich auch Baumaterial. Mehrmals sah ich Larven ein Sandkorn ergreifen, das sie mit Mandibeln und Vorderbeinen festhalten, während sie sich im Köcher um ihre Achse drehen, um eine geeignete Stelle am Vorderrand zum Festspinnen zu finden; dieser Prozess erfordert geraume Zeit.

Zuweilen kann man sehen, dass Larven pumpende Bewegungen (etwa 1 mm vor und zurück) im vorderen Teil des Köchers ausführen und sich gleichzeitig um ihre Längsachse drehen. Wahrscheinlich findet hierbei die innere Seidenaustapezierung statt. Eine Larve unterbrach diesen Prozess mehrmals, streckte den Thorax aus dem Köcher heraus und »untersuchte« die Aussenseite des Köchers, indem sie mit Mittel- und Hinterklauen darauf schlug.

Recht oft sieht man Larven gleichsam im Köcher etwas vorwärts »springen« und ihre Mittel- und Hinterklauen in die Unterlage einschlagen; es war mir indessen nicht möglich festzustellen, wodurch diese Bewegung verursacht oder was mit ihr bezweckt wird.

Der Puppenköcher findet sich an denselben Stellen wie der Larvenköcher. Vor der Verpuppung versieht die Larve den Vorderrand des Köchers mit einem trompetenförmigen Kragen (Abb. 18 D und 20—21), der die direkte Fortsetzung der Köcherwandung bildet und meistens 2—3 mal so breit ist wie die vordere Köcheröffnung. Dieser Kragen wird gewöhnlich aus Sandkörnern gebaut (die ebenso wie im vorderen Teil des Köchers ziemlich

grob sein können), jedoch bildet die Verwendung organischen Materials keineswegs einen Ausnahmefall. Besitzt dieses Material eine ausgesprochene Längsachse, so wird es tangential angeordnet; ist es breit und flach, so wird es in die Ebene der Kragenwandung eingebaut (Abb. 21 C). Ab und zu werden dünne Stücke verwendet, die viel länger als der Durchmesser des Kragens, ja zuweilen fast so lang sind wie der Köcher. Von solchem Material wird nur ein kleines Stück festgesponnen; seine Enden werden von der Strömung rückwärts gebogen und flottieren frei im Wasser. Schliesslich können auch Kopfkapseln verschiedener grosser Insektenlarven, Deckflügel von Käfern u. dergl. Verwendung finden, wodurch der Kragen ein ganz groteskes Aussehen erhalten kann. Kragen, zu deren Bau grössere organische Bestandteile benutzt wurden, sind am breitesten, bisweilen fast fünf mal so breit wie der Köcher. Das Baumaterial wird auf der Hinterseite der Seidengrundlage befestigt; dies fällt jedoch nicht unmittelbar in die Augen, da die Seide am Kragen

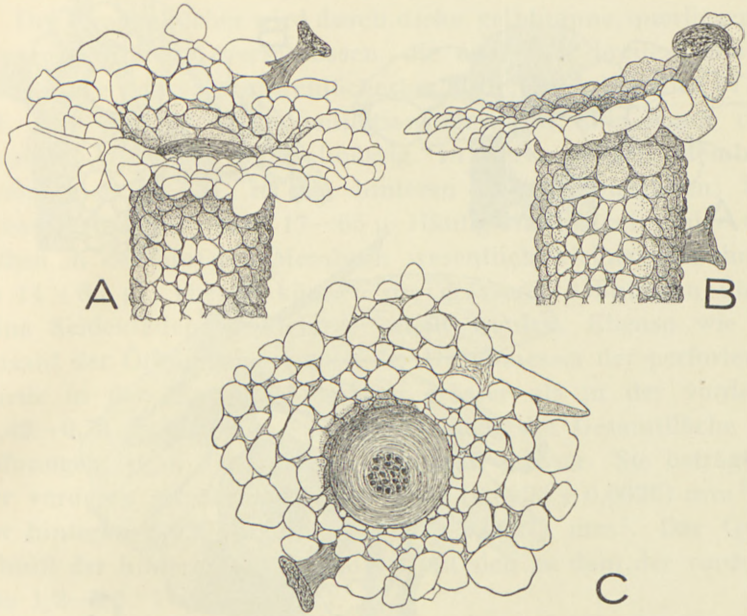

Abb. 20. Kragen des Puppenköchers, A. von oben, B. von rechts, C. von vorn. $^{11,5}/_{1}$. Der Köcher war durch die beiden sehr soliden Bänder an zwei anderen Puppenköchern befestigt.

weit dünner und durchsichtiger ist als am Köcher. Die innere »Tapete« des Köchers setzt sich nicht auf den Kragen fort.

Die ventrale Partie des Kragens bildet meistens mit der Aussenwand des Köchers einen sehr stumpfen Winkel; dorsal-

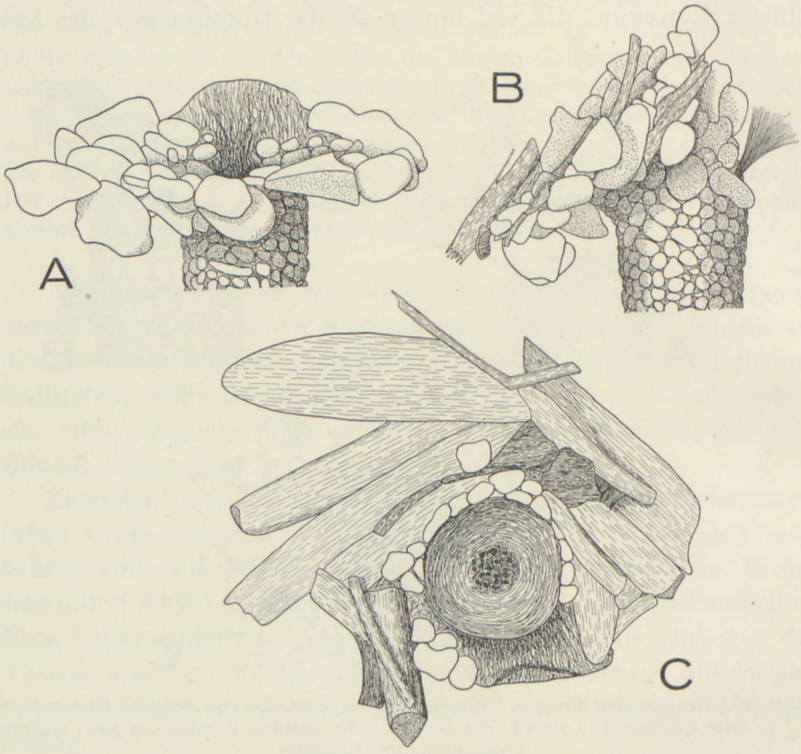

Abb. 21. Kragen von drei verschiedenen Puppenköchern, A. von oben, B. von rechts, C. von vorn. $^{11,5}/_{1}$. Die äusserst unregelmässige Form von B. ist durch ungünstige Lage des Köchervorderendes verursacht.

wärts verkleinert sich dieser, so dass die dorsale Partie des Kragens nahezu senkrecht auf der Köcherwand steht (Abb. 20B). Hierdurch erhält der Kragen eine Länge, die vom 0,52—1,15 fachen der Köcherbreite schwankt; durchschnittlich beträgt sie das $0{,}81 \pm 0{,}04$ fache derselben. Diese Schiefheit wird durch die Unterlage, auf der der Köcher befestigt ist, bedingt. Wenn nämlich das Vorderende des Köchers ausnahmsweise einmal frei liegt, so wird der Kragen ganz radiärsymmetrisch und steht überall senkrecht auf der Köcherwandung.

Die ventrale Partie des Kragens ist gewöhnlich an der Befestigung des Köchers auf der Unterlage mitbeteiligt; zuweilen ist er auch nur durch diese Partie auf der Unterlage befestigt. In diesen Fällen fehlt die Bedeckung mit Sandkörnern entweder auf einem peripheren, parabolischen Ausschnitt oder auf der ganzen ventralen Partie des Kragens (Abb. 21 A, C). In anderen Fällen (Abb. 20) ist der ganze Kragen von Sandkörnern bedeckt.

Der Puppenköcher wird durch dicke, gelbbraune, querliegende, ebene Membranen verschlossen, die aus einer breiten, soliden Randpartie und einer durchlöcherten Mittelpartie bestehen (Tafel III, Abb. 7_{5-6}, III). Die Ventilationsöffnungen sind rund oder länglich, oft etwas unregelmässig. In der vorderen Membran finden sich 10—21, in der hinteren 14—30 von ihnen; ihre Grösse beträgt 31—97 × 17—65 μ. Häufig sind jedoch einige derselben in der hinteren Membran wesentlich kleiner (bis herab zu 14 × 8,5 μ); ebenso können die grösseren Öffnungen durch feine Seidenfäden in kleinere geteilt werden. Ebenso wie die Anzahl der Öffnungen ist auch der Durchmesser der perforierten Partie in der hinteren Membran grösser als in der vorderen (0,49—0,74 bezw. 0,34—0,57 mm); auch die Gesamtfläche der Öffnungen ist in der hinteren Membran grösser. Sie beträgt in der vorderen Membran 0,0175—0,052 (0,0298 ± 0,0026) mm^2, in der hinteren 0,035—0,0725 (0,0538 ± 0,0027) mm^2. Der Querschnitt der hinteren Öffnungen verhält sich zu dem der vorderen wie 1,2—3,2 : 1 (1,94 ± 0,11).

Die vordere Membran liegt unmittelbar hinter dem Kragen, die hintere ziemlich weit vom Hinterende des Köchers, da die Puppenkammer nicht viel länger ist als die Puppe. Etwas (etwa 2—5 mm) hinter der hinteren Membran (d. h. 1,6—7,1 mm vom Hinterende des Köchers) findet sich in der Köcherwand eine grössere Öffnung (Abb. 18 D), meist auf der Ventralseite, häufig aber auch an der rechten oder linken Seite; dagegen sah ich sie niemals dorsal.

Durch die Form der vorderen Membran wird die Stellung der grossen Borsten auf Clypeus und Labrum der Puppe verständlich; sie müssen, um als Putzwerkzeuge dienen zu können, etwas aufwärts gestreckt stehen.

Es liegt in der Natur der Sache, dass ich niemals an einem und demselben Köcher die Schliessung des Puppenköchers beob-

Tabelle VII. Schliessung des Puppenköchers.

Nr.	Kopf d. Larve	Seitenloch	Vordere Membran	Hintere Membran
1	vorwärts	÷	Feines Netz	÷
2	»	÷	» »	÷
3	»	÷	» »	÷
4	rückwärts	÷	» »	÷
5	»	÷	» »	÷
6	»	÷	» »	÷
7	»	÷	» »	÷
8	»	÷	» »	÷
9	»	÷	» »	÷
10	»	÷	» »	÷
11	»	÷	» »	÷
12	»	+	» »	÷
13	»	+	» »	÷
14	»	+	» »	÷
15	»	+	» »	÷
16	»	+	» »	÷
17	»	+	» »	÷
18	»	+	» »	÷
19	»	+	» »	÷
20	»	+	» »	÷
21	»	+	» »	÷
22	»	+	» »	Randpartie(ganz schmal)
23	»	+	» »	— und sehr weitmaschige Mitte
24	vorwärts	+	÷	— (schmal)
25	»	+	÷	—
26	»	+	÷	— und wenige Fäden in der Mitte
27	»	+	÷ (am Rande deutliche Reste des feinen Netzes)	— und weitmaschiges Netz in der einen Seite
28	»	+	÷	— und einige Fäden in der einen Seite
29	»	+	÷	— und weitmaschige Mitte
30	»	+	÷	— und sehr weitmaschiges Netz
31	»	+	÷	fast vollendet
32	»	+	Randpartie (äussert schmal)	Randpartie und weitmaschige Mitte

(Fortsetzung nächste Seite).

Tabelle VII (Fortgesetzt).

Nr,	Kopf d. Larve	Seiten-loch	Vordere Membran	Hintere Membran
33	vorwärts	+	Randpartie (ganz schmal)	Randpartie und einzelne Fäden über d. Mitte
34	»	+	— (schmal)	—
35	»	+	— —	—
36	»	+	— —	—
37	»	+	— —	— und weitmaschige Mitte
38	»	+	— —	— und sehr weitmaschige Mitte
39	»	+	— (ziemlich schmal)	— und weitmaschige Mitte (solide Stränge)
40	»	+	—	—
41	»	+	—	—
42	»	+	—	— und einzelne Fäden
43	»	+	—	— und weitmaschige Mitte (solide Stränge)
44	»	+	—	— und sehr weitmaschige Mitte (feine Stränge)
45	»	+	—	— und sehr weitmaschige Mitte
46	»	+	— und ein paar Fäden	vollendet
47	»	+	— und weitmaschiges Netz	Randpartie und einzelne Fäden in der Mitte
48	»	+	— —	— und weitmaschiges Netz
49	»	+	— — (sehr solide Stränge)	fast (?) vollendet
50	»	+	vollendet	Randpartie (recht schmal)
51	»	+	—	—
52	»	+	—	—
53	»	+	—	— und einige Fäden in der einen Seite
54	rückwärts	+	—	—
55	»	+	—	—
56	»	+	—	— und weitmaschiges Netz m. feinen Fäd.

(Fortsetzung nächste Seite).

Tabelle VII (Fortgesetzt).

Nr.	Kopf d. Larve	Seitenloch	Vordere Membran	Hintere Membran
57	rückwärts	+	vollendet	Randpartie und sehr weitmaschige Mitte
58	»	+	—	fast vollendet
59	»	÷	Feines Netz	Randpartie
60	vorwärts	+	Randpartie	÷
61	»	÷	— und weitmaschiges Netz	÷
62	»	+	Weitmaschiges Netz, am Rande dichtliegende, konzentrische Fäden	÷
63*	rückwärts	+	vollendet	vollendet
64*	»	÷	Feines Netz	÷

* NB: Ruhelarven!

achten konnte; jedoch habe ich so viele Stadien des Prozesses im Freien beobachtet (Tabelle VII), dass ich seinen Verlauf mit grosser Sicherheit zu rekonstruieren vermag.

Wenn die Larve den Kragen fertig gebaut hat, verschliesst sie das Vorderende durch ein feinmaschiges Netz aus dünnen Seidenfäden (Tabelle VII, Nr. 1—3; Tafel III, Abb. 7_I). Dann wendet sie sich im Köcher (Nr. 4—11), beisst eine Öffnung in seine Wandung (Nr. 12—21) und beginnt hierauf mit dem Bau der hinteren Membran (Nr. 22—23). Zuerst baut sie aus konzentrischen Fäden die unperforierte Randpartie; dann spinnt sie kreuz und quer Fäden über das »Fenster« in seiner Mitte (Tafel III, Abb. 7_I). Durch beständige Verengerung der so gebildeten Maschen durch Zufügung neuer und Verdickung der vorhandenen Fäden (Tafel III, Abb. 7_{II}) entsteht die Siebplatte der Membran. Jedoch macht die Larve nur selten diese Membran gleich auf einmal ganz oder fast fertig (Tabelle VII, Nr. 31, 46 und 49); gewöhnlich begnügt sie sich damit, die feste Randpartie zu spinnen und das »Fenster« durch ein weitmaschiges Netz aus feineren oder gröberen Fäden (Nr. 29, 30, 32, 37—39, 43—45 und 48), oder zuweilen nur durch wenige, über das Fenster gespannte Fäden (Nr. 26—28, 33, 42, 47 und 53) zu schliessen. Manchmal baut sie auch nur die Randpartie (Nr. 25, 34—36, 40,

41, 51 und 52), die sie in einzelnen Fällen nicht einmal fertig macht (Nr. 24, 50). Dann wendet sie sich abermals und beseitigt das zarte Netz am Vorderende (Nr. 24—31. Dies ist also ein provisorisches Gebilde, das nur dazu dient, Angriffe zu verhindern, während die Larve an der hinteren Membran arbeitet). Hierauf beginnt die Larve den Bau der vorderen Membran auf dieselbe Weise wie die hintere (Tabelle VII, Nr. 32—53, Tafel III, Abb. 7_{2-4}). Diesmal führt sie indessen die Arbeit zu Ende, bevor sie sich wieder umdreht und die hintere Membran fertig macht (Nr. 54—58). Schliesslich wendet sie sich zum letzten Mal und ist nun bereit, in das Stadium der Ruhelarve einzugehen.

Die Larve geht jedoch nicht immer nach diesem Schema vor; die vier in Nr. 59—62 gezeigten Fälle lassen sich nicht darin einordnen. 59 zeigt indessen nur, dass die Larve zuweilen unterlässt, ein Seitenloch zu beissen, was ich auch recht häufig bei fertigen Puppenköchern sah (dasselbe zeigt 61); dagegen zeigen 60—62, dass die Larve zuweilen die definitive vordere Membran früher beginnt als die hintere. Zugleich ist bei 61 und noch mehr bei 62 die Anlage jener überhaupt abnorm.

Der Bau des Puppenköchers und das wiederholte Umwenden ist sicher für die Larve sehr ermüdend; in einzelnen Fällen vermag sie auch nicht, ihn zu vollenden, sondern geht, wie Nr. 63 und 64 zeigen, schon vorher ins Ruhestadium über. Im ersteren Fall war die Larve offenbar nicht mehr imstande, die letzte Wendung auszuführen, im letzteren kam sie sogar nur so weit, das provisorische feine Netz im Eingang zu bauen.

Diesen zwei Fällen von »abnormen Puppenköchern« lassen sich indessen hunderte von normalen gegenüberstellen. Dagegen bilden 3 Fälle (Nr. 60—62, die sich obendrein noch durch 2 zweifelhafte Fälle ergänzen lassen) von »abnormem Verfahren« beim Bau des Puppenköchers von insgesamt 62 Fällen einen recht hohen Prozentsatz.

Es ist leicht verständlich, warum die Fläche der hinteren Ventilationsöffnungen grösser ist als die der vorderen. Da das Vorderende des Köchers gegen die Strömung gerichtet ist, so entsteht aussen vor der vorderen Membran ein Stauungsdruck (durch das Vorhandensein des Kragens noch gesteigert), der dazu beiträgt, Wasser durch die Öffnungen zu pressen. Das »Sei-

tenloch« stellt zweifellos eine Sicherheitsvorrichtung dar für den Fall, dass der hintere Teil des Köchers verstopft wird; dies kann z. B. durch kleine Fadenalgen geschehen, die ausserordentlich häufig im hinteren Teil des Köchers wachsen. Es könnte einfacher scheinen, das hintere Stück des Köchers ganz zu beseitigen; das »Seitenloch-System« gewährt aber sicher besseren Schutz gegen das Eindringen von Feinden. Das komplizierte Verfahren bei Schliessung des Köchers wird vom gleichen Gesichtspunkt aus verständlich. Es könnte am natürlichsten scheinen, wenn die Larve zuerst die vordere Membran fertig spinnen und dann die hintere bauen würde. In diesem Fall wäre sie aber während des Spinnens der vorderen Membran rückwärts ungeschützt; da diese Arbeit sicher alle ihre Kräfte mit Beschlag belegt, ist sie leichter angreifbar. Deshalb legt die Larve zuerst die schnell gebaute, provisorische Membran an; ebenso nimmt sie sich deshalb nicht die Zeit, die hintere Membran ganz fertig zu machen, sondern wendet sich wieder und ersetzt die gebrechlichere, provisorische Membran durch die solide, definitive, vordere Membran.

Die Beinstellung der Ruhelarve ist anders als sonst bei Köcherfliegen üblich, und zwar werden alle drei Beinpaare vorwärts gestreckt. Die Femora der Vorder- und Mittelbeine werden vertikal, die der Hinterbeine horizontal oder ein wenig aufwärts gehalten. Die Vorderbeine werden im Femur-Tibiagelenk schwach, Mittel- und Hinterbeine sehr stark gebeugt.

Es gelang mir nicht zu sehen, wie eine Puppe den Puppenköcher verlässt; sicher bietet das Auskriechen gegen die starke Strömung gewisse Schwierigkeiten. Die Strömung muss jedoch an der Mündung des Köchers infolge des vom Kragen verursachten Stauungsdruckes sehr abgeschwächt werden; dies ist vermutlich die (wichtigste) Funktion des Kragens. Die leeren Köcher zeigen, dass die vordere Membran nicht zerbissen, sondern einfach losgestossen wird.

Verbreitung.

Da *Oligoplectrum maculatum* an stark fliessendes Wasser gebunden ist, ist die Form in Dänemark keineswegs gemein und, wie zu erwarten, nur aus Jütland bekannt. In Himmerland (der Landschaft zwischen Limfjord und Mariagerfjord) ist die Art

Tabelle VIII. Die Verbreitung von *Oligoplectrum maculatum* in Himmerland.

es bedeutet teilweise stenotherm, e eurytherm bzw. ausgesprochen eurytherm (vgl. 10, S. 328). k. B. bedeutet kleiner Bach, m. B. mittelgrosser Bach, g. B. grosser Bach, Fü. Flüsschen, Fu. Fluss. n. L. bedeutet natürlicher Lauf, l. r. leicht reguliert, s. r. stark reguliert (schnurgerader Kanal). +++ bedeutet sehr grosser *O.*-Bestand (an günstigen Stellen bis 10 000 pro m²), ++ grosser Bestand (bis 5 000 pro m²), + kleiner Bestand (selbst an günstigen Stellen < 100 pro m²); schliesslich bedeutet (+), dass die Art zwar gefunden wurde, jedoch in so kleiner Anzahl, dass ihr Vorkommen als zufällig zu bezeichnen ist. (Siehe auch S. 3).

1a	2	Lindenborg Aa, Oberlauf	1512	es	g. B.	n. L.	+++
1b	3	» » , Unterl. .	1313	e	Fü.	n. L.	(+)
2	28	Østeraa	1312	es	g. B.	s. r.	(+)
3	29b	Gulbæk	1212	e	m. B.	s. r.	+
4	32	Hasseris Aa	1212, 1312	e	g. B.	l. r.	+
5	33	Binderup Aa	1211, 1311	e	Fü.	n. L.	+++
6	35	Sønderup Aa	1410, 1411 1511	e	Fü.	n. L.	+++
7	..	Dybvad Aa	1309	e	g. B.	l. r.	++
8	..	Bjørnsholm Aa, Herredsbæk	1309	e	g. B.	s. r.	++
9	..	Bjørnsholm Aa, Faldbæk	1409	e	m. B.	s. r.	+
10	..	Trend Aa	1408, 1409 1508, 1509	e	Fü.	n. L. —l. r.	+++
11	..	Tværbæk	1409, 1509	e	g. B.	n. L.	+++
12	..	Lerkenfeld Aa	1510 1609, 1610	e	Fü.	n. L.	+++
13	..	Lilleaa	1609	e	m. B.	n. L.	+
14	..	Simested Aa	1709, 1710	e	Fü.	n. L.	+++
15a	..	Skals Aa, Vasehus Bro.	1811	e	Fü.	n.L.-l.r.	+
15b	..	» » , Løvel Bro ...	1810	e	Fu.	l. r.	++
16	..	Skravad Bæk	1810	e	g. B.	l. r.	+++
17	..	Hørup Møllebæk	1810	e	k. B.	l. r.	(+)
18	..	Hodal Bæk	1712	e	m. B.	s. r.	+
19	..	Karls Møllebæk	1713	e	m. B.	l. r.	++
20	..	Villestrup Aa	1613	e	Fü.	n. L.	+++
21a	39a	Lundgaards Bæk, Rostrup	1613	es	g. B.	n. L.	(+)
21b	39b	Lundgaards Bæk, Blegdø	1613	es	g. B.	n. L.	+++

jedoch allgemein verbreitet und gehört hier zu den Leitformen der grösseren Wasserläufe. In Tabelle VIII sind sämtliche Fundstellen aus Himmerland verzeichnet. Einige der erwähnten Gewässer habe ich früher (10, S. 327—337) kurz beschrieben. Die

Zahlen der zweiten Kolonne geben die Nummern dieses Verzeichnisses an, die der vierten Kolonne die Nummern der Messtischblätter (Dänisches Geodätisches Institut), auf denen die betreffenden Örtlichkeiten zu finden sind.

Wie man zunächst sieht, fehlt die Art in stenothermen Gewässern vollständig. Nur Nr. 1a, 2 und 21 gehören zur Gruppe teilweise stenothermer Gewässer; bei Nr. 2 handelt es sich um einen äusserst kleinen Bestand, und in Nr. 21 kommt *O. maculatum* (in nennenswerter Menge) nur im untersten Teil des Baches vor, kurz vor seiner Mündung in Nr. 20; es ist anzunehmen, dass die Aufrechterhaltung des Bestandes hier von ständigem Zuflug von Imagines von Nr. 20 her abhängig ist. Das Verhalten von Nr. 1a ist sehr eigentümlich; während der letzten zwei km des Wasserlaufes durch den Wald nimmt die Anzahl der Individuen so stark ab, dass die Art zuletzt fast ganz verschwindet; das beruht vielleicht darauf, dass der stenotherme Charakter des Gewässers durch den Zufluss zahlreicher kleiner Quellen noch verstärkt wird. Die Zahl meiner Temperaturmessungen ist jedoch nicht ausreichend, um dieses Verhältnis richtig zu beurteilen.

Demnächst zeigt sich, dass im Schema kleine und mittelgrosse Bäche nur schwach vertreten sind; unter den Gewässern mit grösserem *Oligoplectrum*-Bestand findet sich nur 1 mittelgrosser Bach (19), und dieser ist obendrein ziemlich gross, so dass ich ihn eigentlich nur vorsichtshalber in der Gruppe der mittelgrossen Bäche untergebracht habe. Dagegen ist der Bestand an *Oligoplectrum* in allen Flüsschen von Himmerland sehr gross (oder jedenfalls gross), ausgenommen Nr. 1b; das fast vollständige Fehlen der Art in diesem Wasserlauf beruht vielleicht darauf, dass die Lindenborg Aa in ihrem Unterlauf nur ein geringes Gefälle hat und daher der Art nur an wenigen Stellen geeignete Lebensbedingungen bietet.

Wie aus dem vorigen Kapitel hervorgeht, ist starke und schnelle Schwankung der physikalischen Verhältnisse des Gewässers von Ort zu Ort für die Art eine Lebensbedingung (oder jedenfalls günstig). So werden die Eier an ruhigen Stellen abgelegt, während die Larven an Stellen mit rascher Strömung leben; die Hauptnahrung der grösseren Larven (pflanzlicher Detritus) wird dagegen wohl vorzugsweise an den ruhigeren Stellen

des Gewässers hervorgebracht. Derartige abwechselnde Verhältnisse finden sich in natürlich fliessenden Gewässern, während die Strömungsgeschwindigkeit eines Wasserlaufs nach Regulierung gewöhnlich über lange Strecken hin konstant wird. Binderup Aa (5) und Sønderup Aa (6) sind in ihrem Oberlauf stark reguliert. In der Sønderup Aa fällt die obere Grenze für die Verbreitung von *Oligoplectrum* ziemlich genau zusammen mit dem Beginn des natürlichen Laufes; in der Binderup Aa liegt sie ein paar km weiter abwärts. In den oberen, stark regulierten Strecken der Simested Aa (Nr. 14) fehlt die Art gleichfalls. Dagegen findet sich ein grosser Bestand in dem stark regulierten Herredsbæk (Nr. 8), und zwar auf einer kürzeren Strecke mit Steingrund und starker Strömung in dem sonst recht langsam fliessenden Bach. Das Fehlen der Art in kleineren Bächen liegt vielleicht daran, dass diese nicht genügende Abwechslung in bezug auf die physikalischen Verhältnisse bieten. In diesem Zusammenhang sei hervorgehoben, dass kleine eurytherme Bäche mit ganz natürlichem Lauf recht selten sind.

Dies ist jedoch kaum ausreichend, um die Verbreitung der Art zu erklären; es scheint mir naheliegend, an Unterschiede in der Produktivität der Gewässer zu denken, die, wie früher (11) kurz von mir skizziert, durch verschiedenartigen geologischen Aufbau der Niederschlagsgebiete verursacht sein könnten. Die jüngeren Larven leben ja vorzugsweise von Diatomeen, die von der Strömung mitgeführt werden, und zwar wohlgemerkt nicht von Planktondiatomeen, sondern von kriechenden Formen, also von Elementen der Mikroflora, die von der Strömung losgerissen wurden. Zweifellos gehört eine sehr grosse Diatomeenproduktion dazu, um Nahrung für die Myriaden junger Larven zu beschaffen; die Annahme, dass die Diatomeenproduktion einen begrenzenden Faktor bildet, hat daher viel für sich. Die Tatsache, dass die Wachstumsgeschwindigkeit im Frühjahr und Frühsommer weit grösser ist als im Spätsommer (Abb. 16), wo die Larven von Diatomeen leben, weist entschieden in dieselbe Richtung. Ebenso kann man mit gutem Grund annehmen, dass die Grösse der Diatomeenproduktion vom Gehalt des Wassers an Nährsalzen, besonders an Nitraten und Phosphaten, abhängt. In dem genannten Aufsatz habe ich es zum mindesten wahrscheinlich gemacht, dass die hochliegende Kreide von Himmerland auf den

Gehalt des einsickernden Wassers an diesen Salzen konservierend wirkt. — Ich will ein paar Beispiele nennen, die keinesfalls gegen diese Erklärung für die Verbreitung von *Oligoplectrum* sprechen. Die Skals Aa (Nr. 15) entspringt im Kreis Viborg und bildet mit ihrem Unterlauf einen Teil der Südgrenze von Himmerland. Im Beginn dieser Strecke (15a) ist ihr Bestand an *Oligoplectrum* spärlich; 13 km weiter abwärts (8 km von der Mündung), nachdem der Fluss mehrfach Zufluss von der Himmerlandseite erhalten hat (die Wassermenge nimmt auf dieser Strecke sehr stark zu), wird der Bestand gross, und noch grösser ist er in dem himmerländischen Nebenfluss, dem Skravad Bæk. Der Karls Møllebæk (19) mit grossem *Oligoplectrum*-Bestand mündet in den Mariagerfjord etwa 10 km östlich von Hobro; 6 km weiter westlich mündet der Valsgaard Bæk, in dem sich kein *Oligoplectrum* findet. Beide Gewässer gleichen einander ausserordentlich und führen, jedenfalls im Sommer, ungefähr die gleiche Wassermenge; jedoch gehört das Niederschlagsgebiet des Valsgaard Bæk zu den wenigen Strecken in Himmerland, wo der hochliegende Untergrund aus Tertiär besteht. — Die ganze Frage ist indes noch nicht ausreichend bearbeitet. Ich hoffe, später in einer zusammenfassenden Übersicht über die Fauna der Gewässer von Himmerland darauf zurückkommen zu können.

Aus dem Süsswasserbiologischen Laboratorium
der Universität Kopenhagen.

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Abb. 1. Binderup Aa bei der Pannum Brücke. Typischer Larvenbiotop. Hochsommer. Tiefe etwa 0,5 m.

Abb. 2. Sønderup Aa bei Hyldal, Kirchspiel Suldrup. Im Vordergrund typischer Laichplatz, im Hintergrund typischer Larvenbiotop. Hochsommer.

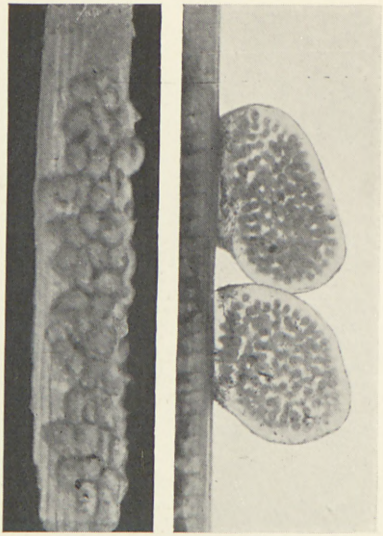


Abb. 3. Links Stück eines *Sparganium*-Blattes mit vielen Laichmassen ($^{4}/_{5}$), rechts einzelne Eiklumpen von der Breitseite gesehen ($^{3,5}/_{1}$).

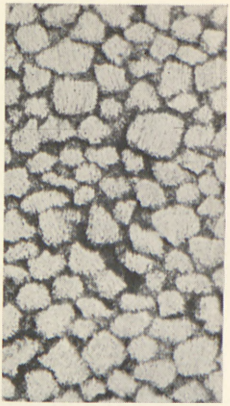


Abb. 5. Seidengrundlage des Köchers von aussen gesehen, nach Entfernung der Sandkörner. $^{20}/_{1}$.

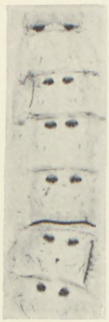


Abb. 4. Haft- und Bewegungsapparat der Puppe. $^{13}/_{1}$.

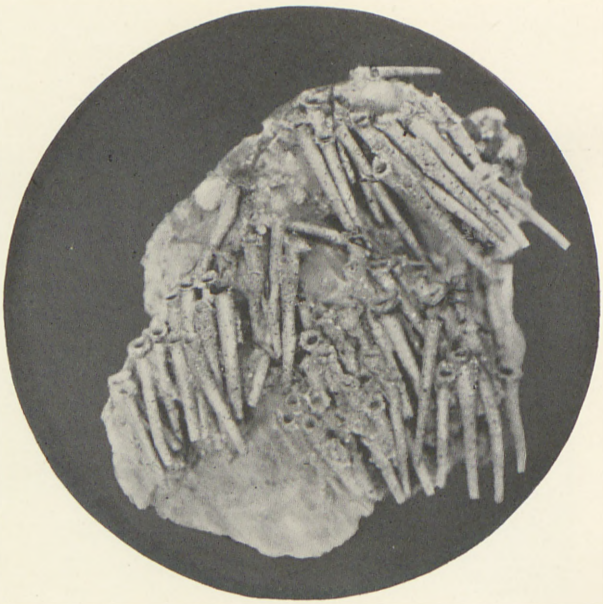


Abb. 6. Kleiner Stein aus der Sønderup Aa mit Larven- und Puppenköchern. $^{1}/_{1}$.

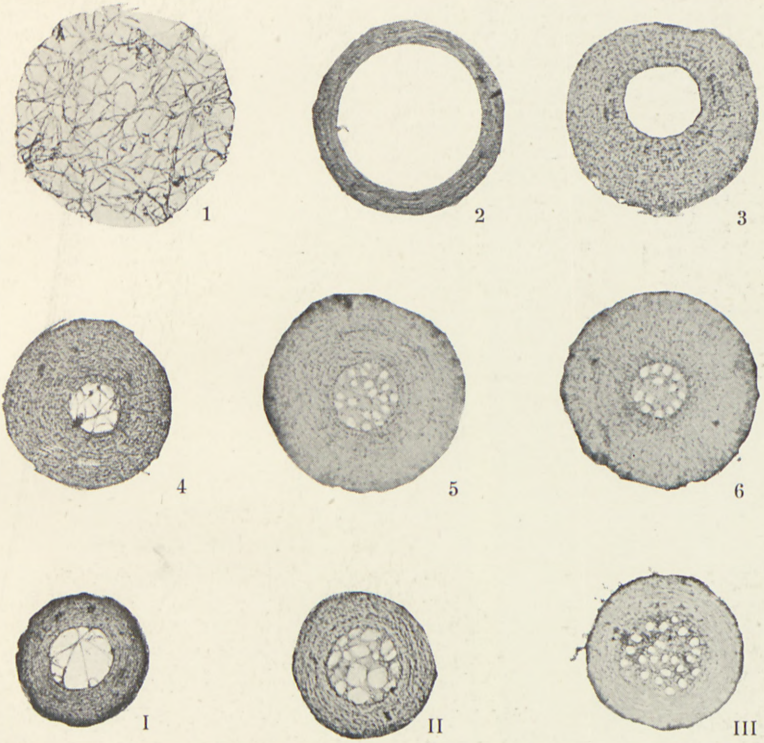


Abb. 7. Entwicklung der Puppenmembranen. $^{20}/_{1}$. 1—6 vordere, I—III hintere Membran. 5, 6 und III fertige Membranen.

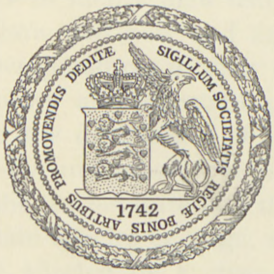
DET KGL. DANSKE VIDENSKABERNES SELSKAB
BIOLOGISKE MEDDELELSER, BIND XIX, NR. 3

PROBLEMS OF HEAT DEATH AND HEAT INJURY

EXPERIMENTS ON SOME SPECIES OF *DIPTERA*

BY

ELLINOR BRO LARSEN



KØBENHAVN
I KOMMISSION HOS EJNAR MUNKSGAARD
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CONTENTS

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I. Introduction.

The following work is published as a part of the investigations conducted by Professor MATHIAS THOMSEN on the biology of various species of flies, especially those associated with houses and domestic animals.

In a previous paper (E. BRO LARSEN & M. THOMSEN, 1940) the main subject was the duration of development of the species concerned at various constant temperatures within a temperature zone which permits the continuation of development. Those experiments showed that up to a certain point temperature has an accelerating effect on development, but if the temperature rises further the effect is injurious, development is retarded, various irregularities occur, and if still higher temperatures are applied, development is no longer completed and the insects die after a shorter or longer period.

For several reasons it was desirable, however, to study the reactions of the species to fatally high temperatures, partly because measurements show that now and again these insects are exposed to such fatal temperatures in their natural environment, partly because heat plays an important part in some of the methods advocated for the destruction of the eggs, larvae, and "pupae" of the house-fly and other species. So it is of interest to know the height of the temperature and the length of exposure necessary to kill a certain species or stage, and how great a percentage is likely to be affected by a given exposure.

Finally these investigations might perhaps claim some theoretical interest, as reports on the influence of fatal temperatures on insects are somewhat scanty and insufficient. In some of these previous experiments there is no statement of the duration

of the exposure; in others it was not possible to make sure if the insects actually were exposed to the temperature registered; moreover the exact determination of the age of the insects used was often difficult.

Most closely related to the subsequent investigations is OOSTHUIZEN's work 1935 on the influence of fatal temperatures on the confused flour beetle *Tribolium confusum*, in which eggs larvae, pupae and adults were exposed to high temperatures at varying intervals and at varying degrees of humidity; fertility under these influences was also examined, the results being discussed. In DARBY and KOPP's work (1933) on *Anastrepha ludens* (*Diptera, Trypetidae*) the age of the insects used in the experiments was considered, as well as the question whether they actually may be expected to have the temperature registered. MELLANBY (1932) examined the influence of the humidity of the air on the determination of the thermal death point, and like BUXTON (1931) found that at temperatures so high that a short exposure is fatal, humidity has no effect, provided that the test insects are so small that their temperature cannot be lowered essentially by evaporation, whereas with long periods of exposure low humidity is unfavourable owing to evaporation. However, most of the investigations on insects are confined to a bare determination of the thermal death point and often, employing the method given by BODENHEIMER (1929), to a determination of the phases of activity of insects under rising temperatures, a method which excludes the time factor in determining the fatal temperature. This factor, however, is of great importance, theoretically as well as practically; for instance it is often necessary, to use the lowest possible fatal temperature in order to avoid damage to the medium inhabited by the insects, e. g. flour, dung, bulbs, etc. For investigations dealing with heat injury as seen mainly from a theoretical point of view, organisms other than insects were generally employed, e. g. bacteria, spores of fungi, seeds and cells of plants, blood corpuscles etc., organisms readily supplying an abundance of uniform material. However, as there is a lack of such comprehensive investigations on insects, I have, in spite of the rather primitive experimental technique, attempted to procure a material as copious as possible, for the elucidation of the influence of the fatal temperatures.

My special thanks are due to Professor MATHIAS THOMSEN for his interest and suggestions during the years in which the investigations were carried out. I would also express my thanks to the Carlsberg Foundation which has given a grant for the work. Further, I am indebted to Dr. OLE HAMMER for collecting wild flies from farms and fields and to Mrs. RACHEL BAGER and Professor K. A. C. BONDORFF for valuable help in the statistical treatment of the results.

II. Technique and test animals.

The experiments were started during the winter of 1934 and continued till the winter of 1936 and thereafter at intervals, when suitable material was at hand, until 1938.

The following species were employed: *Musca domestica, Lyperosia irritans, Stomoxys calcitrans, Haematobia stimulans and Scatophaga stercoraria.*

Musca domestica is the species most thoroughly investigated, eggs, larvae and puparia at different stages of development have been examined. As to the four other species puparia and larvae have been examined.

If the purpose is to experiment with fatally high temperatures the technique must be another than that of the experiments, mentioned in the introduction, on the influence of temperature on the duration of development, since a very slight change of temperature causes a marked difference in the injurious effect to be examined, and it is difficult to keep a constant temperature in dung. Hence the experiments have been carried out by immersing the objects in a water-bath of the required temperature; here it is rather easy to keep the temperature very constant for a tolerably short experimental period.

The experiments have been carried out in two ways, adapted to the particular problems to be examined: I) What is the influence of a given exposure on the further growth of the individual? II) How long can an individual live at a certain temperature?

I. In the first experimental series the technique was very simple; at the beginning the objects were placed in small very thin-walled glass tubes which were immersed in a water-bath of the required temperature, later, however, they were immersed directly into the water in small gauze bags. Controls showed no difference between the results of the two methods, but the latter was by far the more convenient and provided the most uniform heating.

The experimental period having been expired, the bags were taken out, puparia and eggs were placed on moist sand at 25° C. to emerge larvae being confined in dishes containing dung. When emerging time arrived the culture glasses were watched and notes were made of the time of emergence and the final number of emerged insects; malformations during the pupation of the larvae and in the emerged insects were recorded, all puparia not broken were opened, and the moment when death had occurred was determined as exactly as possible by observing the stage of the pupa or larva.

All experiments were made at a relative humidity of 100 per cent which is very near to the optimum and corresponds to the normal humidity of the nutritive medium—dung—of the insects.

In all experiments the larvae and puparia used were taken from cultures kept at 25° C.

The experimental temperature has ranged from 40—56° C. adjusted according to previous experience of the temperature susceptibility of the individual species and stages; the experiments were made with intervals of 1° C. The experimental period generally was $^1/_4$, $^1/_2$, 1, 2, 4, 8, 16 and 32 minutes, so that the time of exposure for the same temperature increases on a logarithmic scale. In order to stabilise the established values, however, periods of exposure were often inserted between those given above.

It is to be noticed that no experiment lasted more than 32 minutes, partly because it was difficult to keep a constant temperature for a longer period, the heating of the water-bath not being automatically regulated, partly because it was to be feared that if longer experimental periods were used the effects of irrelevant factors might be felt, for instance hunger, deficiency of oxygen etc.

MELLANBY (1932) states that the death point of lice and fleas is influenced by starvation, but since both animals feed on highly watery food, it is probable that it is the large amount of liquid which makes the well-nourished insects more resistant.

II. As to the second experimental group, for which larvae only were used, a wide glass tube (fig. 1) was placed in the water-bath, one end of the tube being closed, while the other had a pierced rubber stopper, from which a short thermometer projected into the tube. Fused into the tube near its closed end

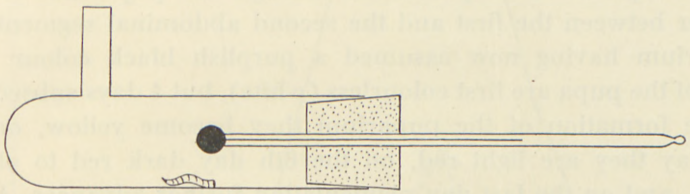


Fig. 1. Glass tube for temperature resistance experiments (see text).

was a branch which emerged from the surface of the water. When the air of the glass tube had reached the required temperature a larva was put through the branch tube down into the experimental tube, whereafter the reaction of the animal could be watched through the water from above. The time of occurrence of the various recognisable phases was noted, as also how long a time passed before death occurred (see later).

III. Experiments on *Musca domestica*.

1. Experiments of type I.

a. Experiments on puparia.

An accurate knowledge of the various easily recognisable phases of the development in the puparium, the time of their occurrence and their duration, is necessary, in order to know at which stage the insect is affected and how long after the exposure death occurred in the unbroken puparia.

At 25° C. the development of *Musca domestica* from the hardening of the larval cuticle—the formation of the puparium—until

the emergence of the fly, takes about 6 days. Hardening lasts from 2—3 hours, during which the colour changes from white to purplish red, this being followed by a final larval stage accompanied by a partial ecdysis (FRAENKEL 1938). This fourth larval stage lasts about 18 hours. Afterwards another and complete ecdysis takes place, whereby the larva becomes a pupa; at the beginning the head of the pupa is still invaginated (cryptocephalic stadium), but during the following day the head is everted (phanerocephalic stadium). After this the two minute tubular spiracles are protruded through the puparium on the border between the first and the second abdominal segment, the puparium having now assumed a purplish black colour. The eyes of the pupa are first colourless (white), but 4 days subsequent to the formation of the puparium they become yellow, on the 5th day they are light red, on the 6th day dark red to almost black, and on the last day pigmentation further advances. A few hours before emergence the movements of the frontal sac commence (E. BRO LARSEN and M. THOMSEN 1940).

For a preliminary examination of the susceptibility of the various stages experiments have been made on puparia at 8 different stages at identical temperatures. In fig. 2 they are named according to the time elapsing, counted by days, after the puparium has formed, as follows: 0: quite young, white puparіae; $^3/_4$: puparіae about 18 hours old containing larvae of the 4th larval stage; 1: puparіae 1 day old, containing pupae; 2: puparіae 2 days old etc., up to 6: puparіae 6 days old immediately before eclosion. The figure shows the result, the percentage of emergence of the 8 stages having been plotted, partly when the time of exposure lasted 1 minute (the full curve), partly after an exposure of 2 minutes (the stippled curve). It will be seen that the emergence percentage, i. e. the heat resistance, is the highest for 3 days old puparіae and the lowest for the stages: 0, $^3/_4$ and 6. The stage of the white puparium (0), however, does not last more than half an hour; hence it is difficult to procure sufficient material of this stage for a long experimental series. Stage 6 shows much irregularity and is difficult to determine with accuracy, since it is to symbolize the condition immediately prior to emergence. Thus there remains the "$^3/_4$ stage", lasting 18 hours; this stage has been chosen as a representative

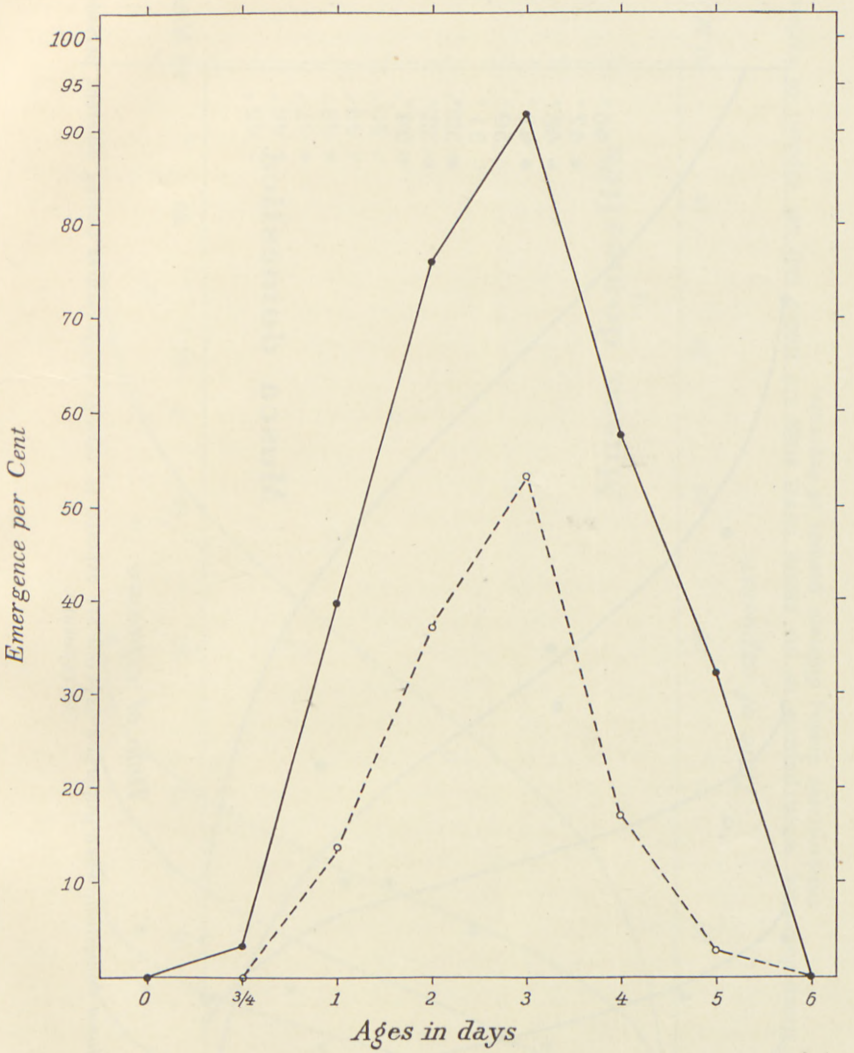


Fig. 2. Temperature resistance curves for eight age classes of puparia at the same temperature, ——— for 1 minute's exposure, ------ for two minute's exposure.

of the most sensitive stage or a very sensitive one at least, while 3 days old pupariae were chosen for experiments as the least sensitive.

The experiments were carried out according to the following principle: In each series at identical temperatures, experiments

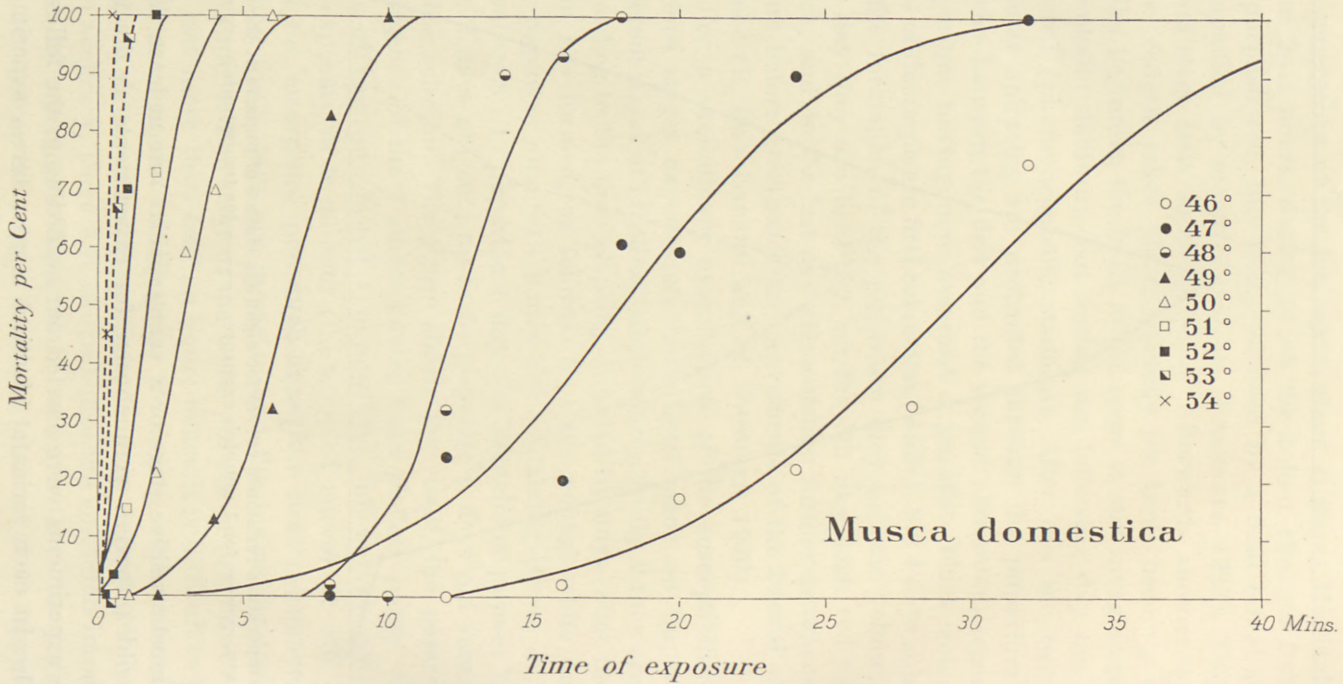


Fig. 3. The mortality in *Musca domestica*, when puparia 3 days old are exposed to various temperatures during different periods of exposure.

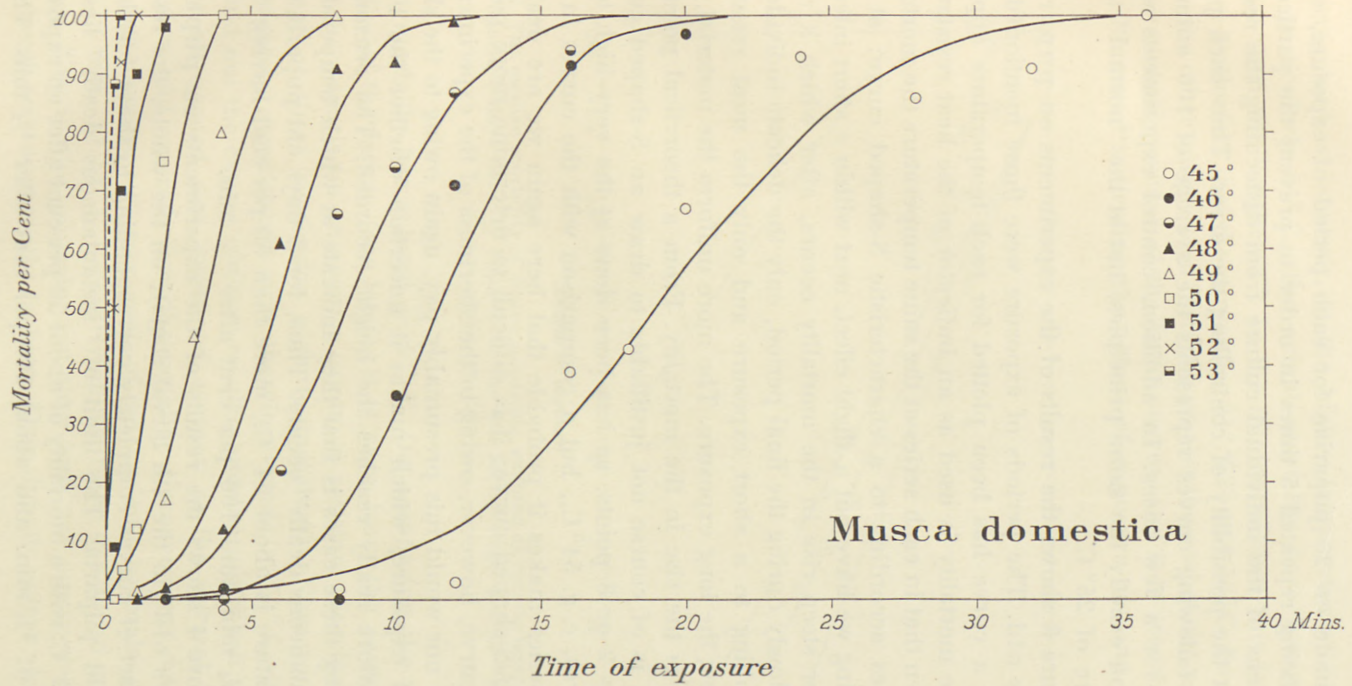


Fig. 4. The mortality in *Musca domestica*, when puparia in the fourth larval stage (18 hours old) are exposed to various temperatures during different periods of exposure.

were made on 20 puparіae for each period of exposure, each series being repeated 5 times in order to prevent the particular conditions of the individual culture from dislocating the results without the possibility of controlling the error. Thus each point of the following curves represents the average of 100 animals, except for a few cases. In addition, control experiments were made on exactly the same principles, but at the "normal" temperature of 25° C.

Figure 3 shows the results of the experiments on puparіae 3 days old. The periods of exposure were those mentioned on pag. 6, a curve has been plotted for each temperature applied and the mortality is used as an indicator of the heat resistance. It is seen that in each series at the same temperature the mortality increases according to a characteristic S-shaped curve: at the beginning we have but a slight effect, next within a short interval a rather steep rise in the mortality occurs, after which it rises more slowly during the final period, only the delicate individuals succumbing to a short exposure and only the most resistant surviving the long exposure. The more uniform the material, the steeper is the rise in the mortality. From a theoretical point of view it is of course not justifiable to draw an S-shaped curve through 2 or 3 points, as has been done at the very high temperatures, e. g. 54° C., but a comparison with the curves in the other series makes it probable that here again we are dealing with an S-shaped curve; it is difficult to determine more points of the curve, however, owing to the shortness of the experimental period, nor would this presumably pay, again owing to the short time of exposure, which makes it uncertain whether the body temperature really reaches the height recorded. The reason of including these cases is that they indicate at which temperature "instantaneous death" occurs. Thus, for 3 days old puparia this temperature is about 54° C., more than 50 per cent having died after $^1/_4$ min. and 100 per cent after $^1/_2$ min.

Figure 4 shows the results of the experiments on puparia $^3/_4$ days old (in the 4th larval stage); on the whole, the results give a set of curves completely corresponding to those for the 3 days old puparia. The limit of "instantaneous death" lies at 53°—52° C. with a mortality of about 50 per cent after an exposure period of $^1/_4$ min. and about 100 per cent after $^1/_2$ min. There

are irregularities, however, at the higher temperatures: 53°, 52° and particularly 51° C., the actual mortality here being much higher than the "expected". In the numerical data this is manifested by the fact that the curves for 51°, 52° and 53° C. lie remote from the other curves by a distance greater than what corresponds to the mutual position of the latter curves, and it is seen in fig. 5 that the curve indicating the extent of exposure causing 50 per

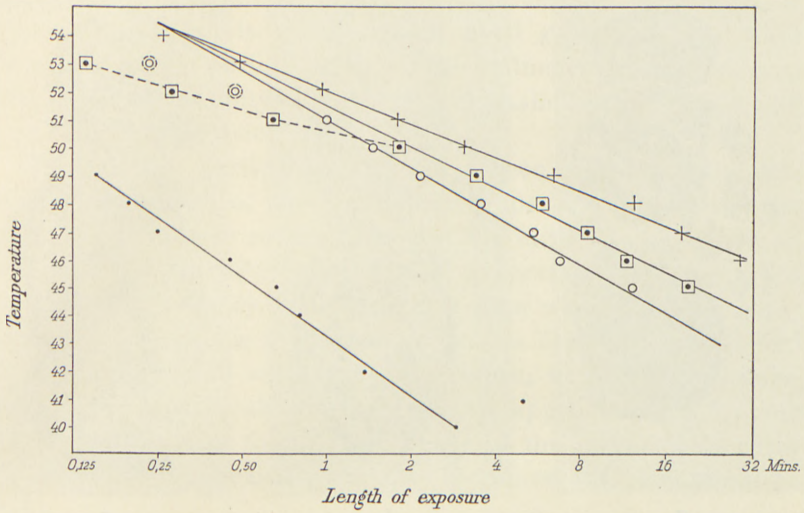
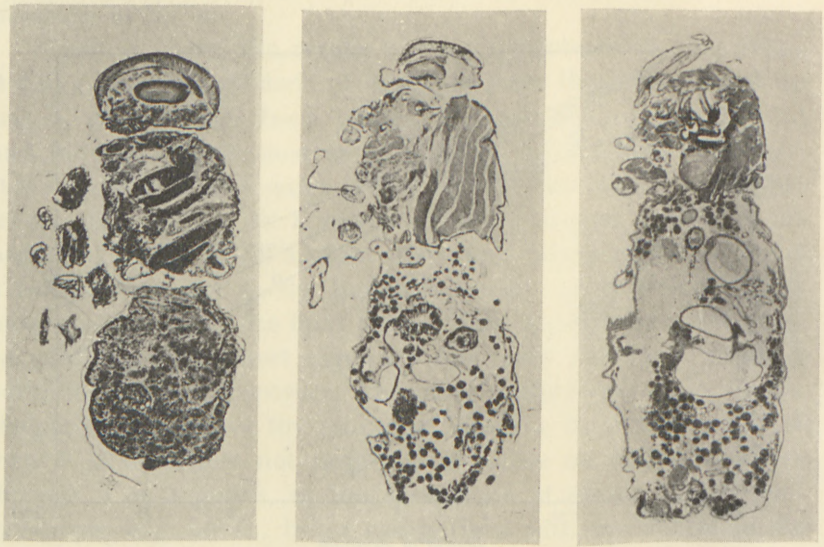


Fig. 5. The median mortality of *Musca domestica*, exposed at various temperatures. + Puparia 3 days old. ⊡ Puparia in the 4th larval stage. ○, ◎ Full-grown larvae. • Eggs.

cent mortality has a bend at 51° C. On comparing the susceptibility of the larvae (see later), it is seen from the same figure that the puparia on an average are more resistant than the larvae except at these particular high temperatures. Numerous supplementary experiments were therefore made at 51° C. in order to find out the causes of these results, and the unbroken puparia were all opened and examined as soon as the normal time of eclosion had expired. Now the peculiar discovery was made that a large number—47 per cent of all the test animals exposed for $^1/_2$ min. at 51° C.—were lying as fully developed and coloured flies in the puparia, but they had not emerged because the head was apparently lacking. Occasionally wings, legs and

setae were fully pigmented, the pupal skin ruptured etc., but the flies had not been able to break the puparium, because, among other reasons, the frontal sac was not capable of functioning. It is not correct, however, to say that there was no head, for various series of sections of "headless flies" (fig. 6 *c*) showed the head lying invaginated in the thorax of the fly. There were all



a *b* *c*

Fig. 6. Sections of *Musca* pupae *a:* Normal pupa, just before emergence. *b:* Heat-treated pupa with small head, just before emergence. *c:* Heat-treated "headless" pupa, just before emergence.

transitional stages from the completely undeveloped head to a fully developed head with pigmented eyes. A partly developed head may be everted; we then have flies with very small heads, fully pigmented but with undeveloped mouth-parts and small eyes (6 *b*). Thus the development of the head as well as the eversion itself may be disturbed or inhibited by heat effect, the development in the puparium continuing almost normally in other respects. Hence, several of the aforesaid 47 per cent would have emerged, since they were found alive and mobile in the puparia, if the lack of head had not prevented emergence, in which case the 51° C. curve would lie as do the other curves. In the tables these animals have been entered as dead in the stage "with red

eyes" or "with black eyes", judged according to the pigmentation of the body, since I thought it incorrect to place them under the stage "head absent", a stage characterising the transition of the normal puparium from the 1st to the 2nd day.

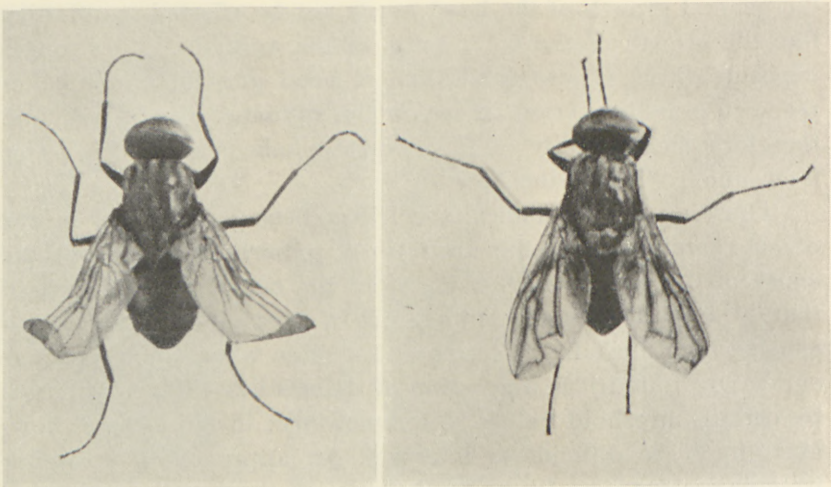
The explanation of this peculiar deformity is probably that special substances inducing the eversion of the head are destroyed by the influence of the heat, so that eversion is hindered, while the general growth of the head needs not be affected, continuing like the growth of the other parts of the animal. With stronger exposure there is also inhibition of head growth, which takes place just in the period in which the influence is applied, and therefore the mortality of the cryptocephalic stage is high (see later under "discussion").

Thus in this particular case it has been possible by means of an examination of the unruptured puparia to determine the cause of the change in the course of the curve, for it appeared that at a certain high temperature a special defect occurred causing high mortality. I suppose that often where such irregular curves are found it is a question of defects like this, conditioned by certain threshold values of temperature; in most cases, however, they are difficult to interpret. An approach to a similar irregular curve was found in the case of the larvae, and cases are mentioned repeatedly in the literature (cf. Bělehrádek 1935).

Apart from these instances deformities in the emerged flies are met with in the series of experiments on 3 days old puparia as well as in those aged 18 hours. Thus vesiculous wings filled with liquid are frequently found, and incomplete extension of the wings, which appear short, bent upwards (fig. 7a). A considerable deposit of pigment along the veins forming broad, brown or brownish black bars, is often seen (fig. 7 *b*). This very characteristic deformity appears mainly when third day puparia are exposed for a short time only at the highest temperatures: 54°, 53° and 52° C.

Günther Bodenstein (1940), in *Musca domestica*, by stimulating puparia at 42° C. at various intervals brought about a great number of various modifications of a kind similar to those mentioned above, e. g. "vesiculous wings", "drooping wings", "wings expanded", "wings crumpled"; in addition, however, his

material contains a number of other interesting modifications and mutations as to the form of the wing and the arrangement of the veins, modifications which resemble specific and racial characters of a number of other *Diptera*. As my examinations were brought to an end as early as 1938, I had not at that time become aware of modifications like the latter, for one thing



a *b*

Fig. 7. *a*: Heat-treated imago of *Musca domestica* with curled and expanded wings, *b*: *Musca domestica*, imago with pigment along the veins as a consequence of heat exposure during pupal life.

because the examinations in themselves were made for quite another purpose; hence only the most noticeable of them have been recorded. This is most unfortunate, for in a material so large and varying, numerous instances of the kind of modifications detected by BODENSTEIN were certainly present.

In order to compare three days old puparia with puparia of other stages, I have employed another method of representing the injury at high temperature: the exposure giving 50 per cent emergence or 50 per cent mortality being plotted in the coordinate system. This procedure is often used in medicine, f. inst. in analyses of the effect of poisons or in the standardisation of medicaments; it is stated what dose is necessary for a certain result: death, cramp, cure etc. in 50 per cent of the individuals used in the experiment.

This method of representation has likewise been used when comparing the resistance to heat of various species and various stages within the same species. If in the experiments on the puparia the temperature is plotted on the ordinate and the logarithm of the time on the abscissa, the result, as will be seen in fig. 5, will be nearly a straight line; the relation between the temperature and the logarithm of the time of exposure is consequently a linear function. (The temperature interval being so small, the picture of the curve will be almost the same if the logarithm of temperature is also used, as when calculating the temperature coefficient *b* (see p. 41)). In other words, the linear function means that the injury shows a linear proportionality to the percentage of increase of the exposure, not to the linear increase.

If on fig. 5 we compare the exposure necessary to bring about a mortality of 50 per cent, we see that everywhere it is less for puparia in 4th larval stage ("$^3/_4$") than for the 3 days old puparia. *A priori* it seems likely that this is due to the fact that the older pupariae have the shorter period left before emergence so that the active period of an advancing destruction caused by the heat will be shorter than in young puparia not due for emergence until several days later, however, the experiments mentioned on p. 8 on still older stages show that the whole explanation is not to be found here. In my opinion the essential point is that the influence on the young puparia takes place at a time when the latter are specially sensitive, because the metamorphosis is extremely active, numerous changes are in progress, for instance ecdysis, presumably hormonally conditioned, of the 4th larval stage to the pupa, the above mentioned eversion of the head and the formation of the limbs, the decomposition of the larval fat-body and the building up of the imaginal one, in short a period of profuse secretion of hormones and extensive formation of mitoses. It has been shown (see Bělehrádek 1935) that dividing cells are particularly susceptible to the exposure to heat compared with resting cells, so that the cause of the high mortality may be due to the large number of cells in mitosis.

The greater resistance of the 3 days old puparia suggests a quiet period in the pupal life and other circumstances point in the same direction. As is well known, measurement of the meta-

bolism in the course of the pupal life gives a U-shaped curve, the metabolism falling considerably during the middle part of the pupal life, to rise afresh towards the end of it. The phenomenon is known in a large number of insects, e. g. *Calliphora vomitoria* (WEINLAND 1906), *Tenebrio molitor* (KROGH 1914), *Phormia, Lucilia, Scatophaga* (TAYLOR 1927), *Lucilia sericata* (COUSIN 1932), and *Drosophila melanogaster* (POULSON 1935). KROGH advances the hypothesis that the metabolism is an expression of the quantity of organized tissue present, so that the drop in the beginning of the pupal life corresponds to histolysis, the rise to histogenesis. However, the explanation of metabolism as expressing the advance of the histolysis and the histogenesis is uncertain, since both processes in some cases may be completed before the drop in the metabolism occurs (WIGGLESWORTH 1939).

My experience seems to indicate that what we have to deal with is nothing but a resting period in the metamorphosis, the great resistance to heat and likewise to cold and to desiccation (E. Bro Larsen 1943) suggests that a quieter period of the pupal life has set in. The same explanation is suggested by the fact that hibernation in some closely allied species examined (there is no diapause in *Musca domestica*), e. g. *Lyperosia irritans, Haematobia stimulans* and *Scatophaga stercoraria*, takes place during this period. It is much more probable that hibernation takes place during a resting period than during a period with numerous katabolic and anabolic processes.

The hypothesis outlined above may also explain the fact that puparia 5 and 6 days old are more sensitive than those 3 days old; in the former the pigmentation of eyes, hairs, legs and wings as well as emergence coincides with or immediately follows exposure to heat, i. e. exposure is applied during a very active and critical period of the pupal life. It is difficult to make any definite pronouncement on the problem, of course, since the presence of essential processes evading observation may very well be imagined, including such as do not require an increased metabolism, e. g. possibly the differentiation of the brain or the like; nevertheless the great power of resistance to external influences coinciding with the period of low metabolism indicates a resting period.

b. Experiments on larvae.

For these experiments use was made of fully grown larvae with the formation of fat beginning, but still having food in the intestine; and as in the case of the puparia 5 groups of 20 larvae each were used for each temperature and each period of exposure. The result is as in the case of the puparia a series of S-shaped curves. One of the five series is pictured in Plate I. The values of the exposure causing 50 per cent mortality are seen in fig. 5; it is a straight line, when the logarithm of the period of exposure is used.

Larvae seem to be less resistant than puparia to high temperatures, this is shown by the position of the line of 50 per cent mortality below that for the puparia. This is apparently inconsistent with experiences made in nature, where puparia are always found in cooler places, just as laboratory experiments show that larvae prefer a higher temperature than that prevailing in the places in which the puparia are found (E. og M. Thomsen 1937); but in actual fact it is not possible to compare the circumstances. In the experiments with fatally high temperatures the question is not one of temperatures at which it is possible for the insects to live, but of an injury done by transitory exposure to a temperature otherwise fatal, hence it is comprehensible that, in a series of interdependent processes, a displacement will have greater consequences on the result of emergence for larvae than for pupae. In the first place larvae have to live for a longer time than pupae before emerging as flies, and in the second place the exposure is immediately followed by the formation of the puparium and its hardening, which is a very critical period. It has been demonstrated that these processes are induced by hormones secreted by the "ring gland" and if the activity of this hormone is inhibited the formation of the puparium is prevented (Fraenkel 1935, Hadorn 1937).

The greater sensibility of the larvae is also demonstrated in fig. 8, which shows the mortality rate at 49° C., the extraordinarily great susceptibility of the eggs being particularly noticeable. At 51° C., however, it is seen, as mentioned on pag. 13, that the young puparia are more susceptible than the larvae. In this connection it

may be mentioned that DARBY and KAPP (1933) in heat experiments on *Anastrepha ludens* find that the larvae are the less susceptible. But quite apart from it being a question of another species, and that quite different conditions may possibly play a part, it is to be noticed that the larvae are recorded as "living" if only they move after the heat application, while the pupae

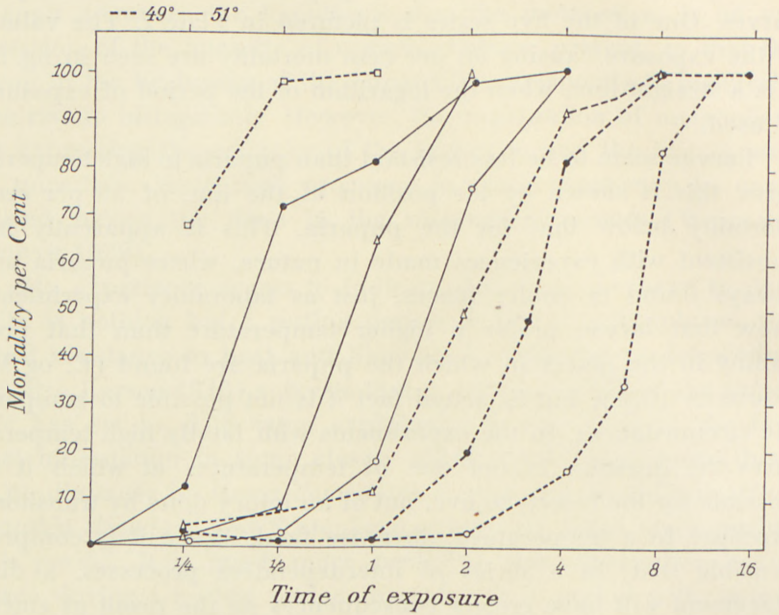


Fig. 8. Mortality of *Musca domestica* exposed to 49° ----- and 51° ———: 3 days old puparia ○, 18 hours old puparia ●, larvae △ and eggs □.

are not recorded as "living" till the moment of emergence, whereas larvae as well as puparia in my experiments are not entered as "living" until they have succeeded in carrying development through to emergence. On attempting a valuation similar to that for *Anastrepha ludens* we find that the larvae at any rate are less susceptible than the young puparia.

In the earlier experiments on the dependence of development on temperature (E. BRO LARSEN and M. THOMSEN 1940, pag. 22) larvae were kept at temperatures above the optimum, e. g. 39°, 40°, 41° C. In the behaviour of these larvae the effect of the high temperatures manifested itself in the form of increasing restless-

ness and loss of weight. The same effect is observed from exposure to fatally high temperatures if the exposure is slight only. The larvae move along quickly and restlessly as soon as the gauze bag is opened; but if the exposure is increased either by raising

Table 1.

	0 m.[1]	5 m.	10 m.	20 m.	30 m.	40 m.	24 hours
53° C.							
1/4 m.	5	50	45	70	..	85	90
1/2 m.	0	2	3	3	..	15	15
52° C.							
1/4 m.	5	40	55	70	..	95	95
1/2 m.	0	5	5	10	..	10	45
1 m.	0	0	0	0	..	0	30
51° C.							
1/4 m.	40	80	95	100	..	100	100
1/2 m.	15	55	90	100	..	100	100
1 m.	0	5	20	20	..	20	85
2 m.	0	0	0	0	..	0	5
50° C.							
1/4 m.	45	80	100	100	100	100	100
1/2 m.	20	95	65	100	100	100	100
1 m.	1	3	30	65	75	90	95
2 m.	0	0	0	0	0	0	40
4 m.	0	0	0	0	0	0	0

In the first column is given the time of exposure for each temperature. In the following columns is given the number of larvae capable of penetration into the dung at the given moment.

the temperature or by applying it for a longer period, the movements of the insects are paralysed. When the experiment is finished the paralysed animals lie motionless on the medium for a shorter or a longer time, and it is possible to obtain a measure of the heat injury in the first hand by recording the time of the return to mobility (table 1). If the paralysis ceases in the course of an hour, the insects are often able to penetrate into the dung and complete their development; but if they are completely insusceptible to excitation for more than about two hours, only

[1] m = time in minutes after end of exposure.

incomplete recovery takes place, and even if the insects after the course of 24 hours start moving a little, the injury sustained during the 24 hours will be so great that pupation and further development are inhibited.

In addition to paralysis other characteristic changes are often noticed in larvae affected by heat, for instance rhytmical, pulsating muscular movement, lack of capacity for orientation to the light, a capacity normally very well developed in *Musca* larvae. Similarly, miscolourings occur, brown and dark spots, particularly in the alimentary canal; and even if mobility is restored, the animals being able to penetrate into the dung, digestion seems to be difficult, possibly because certain enzymes have been destroyed by the heat or because the internal organs are still paralysed. The former view is held by Oosthuizen (1936) who has observed similar phenomena in larvae of *Tribolium confusum*.

Finally it may be mentioned that the ability of the full grown larvae to tolerate desiccation (see E. Bro Larsen 1943) is lost to a great extent; the skin becomes dull and flabby, and if the culture dishes are not covered, the larvae will dry up before the cessation of the paralysis. As has been stated, the formation of puparia is rendered difficult, this manifesting itself partly by the inhibition of the normal contraction, so that tapering, larva-like puparia appear (see Plate 1); if this abnormality is not too great, apparently normal flies may emerge. Further the hardening may be incomplete, which generally results in the loss of these puparia, because *e. g.*, like quite young puparia, they are very susceptible to desiccation.

In addition it is characteristic that the formation of the puparium is retarded, partly, as a matter of course, owing to the general paralysis, but partly also because dislocations in the interaction of the various processes seem to delay the formation of the puparia, and it has been noticed that the latter may be delayed up to 48 hours compared with control animals at the same temperature, a phenomenon also found by Oosthuizen in the various stages of *Tribolium confusum*.

A characteristic phenomenon, even if occurring rather sporadically, which likewise I consider related to abnormally high temperature, and which may therefore be mentioned in this

connection, is the occurrence of larvae that are unable to form a puparium. They are extraordinarily big, transparent-yellowish larvae, very glossy and distended; the normal white fat-body, seen towards the end of larval life through the cuticle, is not observable in these insects. These abnormal larvae wander restlessly about for several days after the time when pupation should normally have occurred, after which they either die or harden into a very incompletely developed puparium, characterized by its larval shape and incomplete pigmentation and hardening, and no flies emerge from these puparia. I have found these larvae in mass-cultures which have been exposed to too much heat, as well as in my experiments at fatally high temperatures; my conjecture is that the raised temperature has damaged the hormones which condition the physiological changes taking place as a preliminary to pupation.

In this connection it may be stated that Mellanby (1938) presumes that the puparium-forming hormones in *Lucilia sericata* are destroyed at 25°—37° C., because larvae at these temperatures do not develop further than the stage of prepupal diapause and fail to form puparia; if hereafter the temperature is lowered, puparia are formed after some time. Hadorn (1937) has described similar retardations and defects in the formation of puparia in a mutant of *Drosophila melanogaster* ("lethal giant"); it seems that these larvae secrete too small a quantity of the hormone of the ring gland, for it is known that the implantation of ring gland from normal larvae causes a formation of puparia. Thus it is also possible that it is a question of a spontaneous mutation without any relation to the influence of high temperature.

c. Experiments on eggs.

The incubation period of the eggs of *Musca domestica* is very short; as susceptible and non-susceptible periods alternate, it is difficult to procure material as uniform as desirable; hence a larger material has been used for these experiments than for those on the puparia and the larvae; the results nevertheless were not satisfactory.

Eggs quite newly laid were used for the experiments, and the temperatures employed were 41° (1061 eggs), 42° (1623 eggs), 44° (1276 eggs), 45° (1587 eggs), 46° (611 eggs), 47° (422 eggs),

48° (531 eggs) and 49° (339 eggs). For the control experiments 900 eggs were used, these control eggs on an average showing a mortality of 2.8 per cent.

A straight line through the exposures causing 50 per cent mortality is below the curves of the puparia and larvae (fig. 5). Consequently, eggs seem to be much more susceptible than larvae and puparia; however, a direct comparison between the physiological resistance of the different stages is actually not possible; thus it must be remembered that the units of time employed for exposure are comparatively much greater in the case of the eggs, the developing period of which is so very short (14 hours at 25° C.), while the puparia are exposed for a much smaller fraction of their total developing period.

Nothing can actually be concluded from these experiments but the fact is that, if the various stages mentioned above are given an exposure numerically identical, the mortality will be highest in the eggs, much lower in the larvae and young puparia and lowest in 3 days old puparia.

With the eggs the influence of the heat manifests itself also in a characteristic way apart from the rise of mortality. Hatching is retarded, as compared with the control eggs, and even if the development of the embryo proceeds so far that the larvae are seen within the eggs, with belts of spines, their movements are so slight that it is hard for the larvae to pierce the egg shell, whereby hatching is retarded or prevented for this reason alone. If hatching is successful the larvae often are so delicate that they die immediately after.

Here again characteristic brown spots appear during embryonic development after strong exposure.

2. Experiments of type II.

In this type of experiments the larvae were exposed to a definite temperature (see under technique) and a record was taken of the moment of death; still it must be observed that there is no guarantee that what is recorded is the actual death, since this cannot superficially be distinguished from heat rigidity. At intervals, however, a quivering of the skin is to be seen, even when movement has otherwise ceased, as long as the insect is exposed

to the heat. After the cessation of this reaction, I have not succeeded in restoring the insect to life by bringing it back to normal temperature; hence I have recorded this moment as the moment of death.

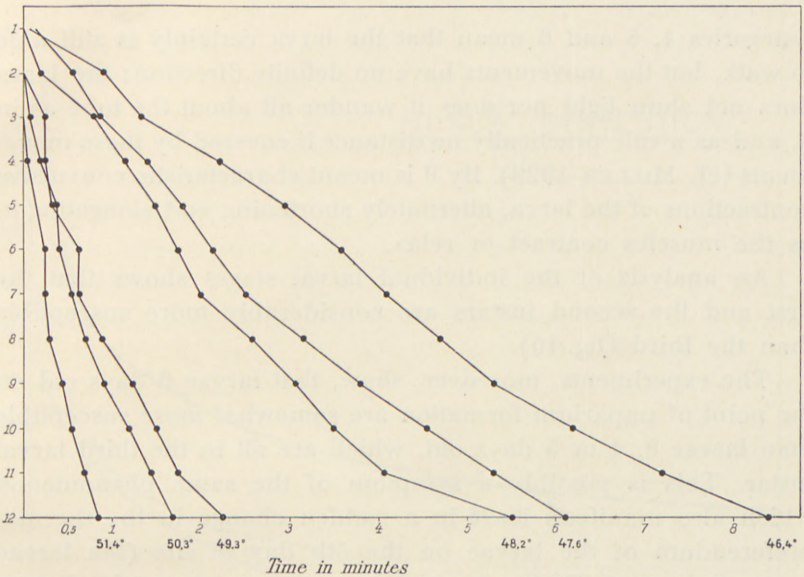


Fig. 9. The succession of the injury of larvae of *Musca domestica* when exposed to different temperatures (see text p. 25-26).

Gradually, as the changes caused by the heat exposure become greater, the individual insect undergoes a series of rather characteristic phases which can be watched through the wall of the glass tube. With rising temperature the stages are passed in quicker and quicker succession, as is seen from fig. 9 which shows the condition in full grown larvae.

A distinction is made between:

1. Normal movements.
2. Lively movements.
3. Violent movements.
4. Uncontrolled movements of gait.
5. Uncontrolled movements. a.
6. — — b.
7. Movements of front part and hind part.

8. Slight movements of front part and hind part.
9. Pulsating movements.
10. Slight pulsating movements.
11. Skin quivering and heat rigidity.
12. Heat rigidity and death.

Categories 4, 5 and 6 mean that the larva certainly is still able to walk, but the movements have no definite direction; the larva does not shun light nor does it wander all about the tube as in 3, and as a rule practically no distance is covered by these movements (cf. MILLER 1929). By 9 is meant characteristic convulsive contractions of the larva, alternately shortening and elongating it, as the muscles contract or relax.

An analysis of the individual larval stages shows that the first and the second instars are considerably more susceptible than the third (fig. 10).

The experiments, moreover, show, that larvae 6 days old on the point of puparium formation are somewhat more susceptible than larvae 3, 4 or 5 days old, which are all in the third larval instar. This is possibly a symptom of the same phenomenon which also manifests itself in a sudden change in the thermopreferendum of the larvae on the 5th day of life (the larvae were all kept at 25° C. at which temperature they take 6 days for their development), so that it is much lower during the last two days than on the previous days (E. THOMSEN and M. THOMSEN 1937). On the other hand the curve of heat resistance is highest for the big larvae 4 and 5 days old, and the drop on the 6th day is not so marked as might be expected, considering the evident lowering of the thermopreferendum of the larvae; so that in all probability the question is merely of a somewhat greater sensitivity owing to the changes which take place as a preliminary to the formation of the puparium.

If the temperatures used are plotted on the ordinate, and the logarithm of the time from, the start of the exposure until the occurrence of death, on the abscissa, the result will be little short of a straight line, expressing that the logarithm of the longevity—which here is an expression of the reversed dose of the exposure—is in inverse ratio to the temperature, or to get equal effect when the temperature de-

creases arithmetically the time must increase geometrically. Fig. 12 represents this phenomenon regarding *Musca domestica, Lyperosia irritans, Stomoxys calcitrans, Haematobia stimulans* and *Scatophaga stercoraria*. It should be noticed that at the very

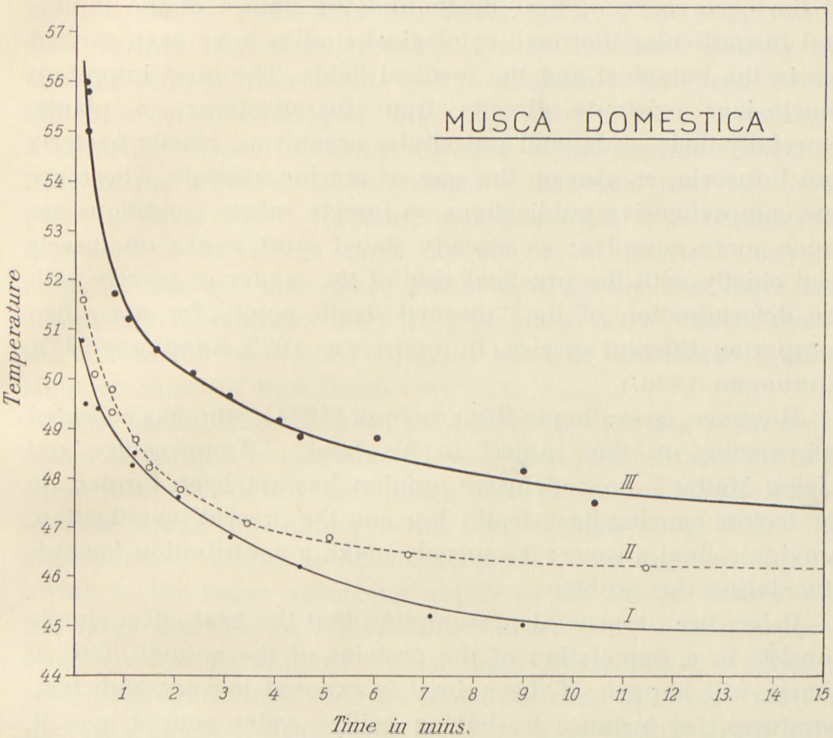


Fig. 10. Resistance curve for larvae of *Musca domestica* for the three instars.

high temperatures the points are above the straight line, i. e. a longevity is recorded greater than should be expected; such is the case with big larvae when the temperature of "instantaneous death" is approached. The explanation is presumably that during the short exposure the big larvae are not heated up to the temperature recorded; but there is also the possibility that, if the space is not completely saturated with vapour, they may, for a short time only, lower their temperature by evaporation (see Mellanby 1932).

3. Discussion.

On the causes of heat death and heat injury.

Numerous writers have worked on the problem of the causes of the occurrence of heat death and the nature of the injury, and in particular thorough cytological studies have been carried out in the botanical and the medical fields. The most important conclusions originate directly from investigations on plants, especially their seeds, and unicellular organisms, chiefly bacteria and infusoria, as also on the eggs of marine animals. There are few comprehensive publications on insects, where conditions are much more complex; as already stated most works on insects deal chiefly with the practical side of the matter or merely with the determination of the "thermal death point" for use when comparing different species (Bodenheimer 1925, Krogerus 1932, Nordberg 1936.)

However, according to Bělehrádek (1935), who has collected information on this subject in his book: "Temperature and Living Matter", no conclusive opinion has yet been formed on the factors causing heat death. Nor can the present investigation, provide a final answer; it can only make a contribution towards elucidating the problem.

It has been a general presumption that the heat effect simply consists in a coagulation of the proteins of the animal. This of course will happen, if the animal is exposed to very high temperatures, for instance by having boiling water poured over it, and it is easily understood that the heat coma, in which the animals lie extended, stiff and tense, was a temptation to biologists to assume a coagulation or fixation of a nature similar to that mentioned above. However, the fact that the heat coma may be transient, the animal once more becoming active, has been difficult to explain, since coagulation of protein only to a slight degree is a reversible process; and when moreover it was maintained that heat coma may occur and vanish an indefinite number of times, this being a normal link of the diurnal activity of the animal, there was considerable disagrement between the observations of chemists as to protein coagulation and the observations made of what actually takes place in nature. Similarly, it is

remarkable that heat injury occurs at a temperature much lower than that causing a coagulation of the proteins in question.

Among the other theories advanced to explain heat death is that of the destruction of the enzymes of the organism; this theory is supported by the fact that enzymes have an optimum temperature, at which their effect is greatest; if the temperature rises further the effect of the enzymes decreases quickly on account of their destruction, and in addition it has been shown that destruction is delayed, when the water content is reduced, a reduction eauses the same lowering of the injurious effect of heat on the animals. However, active enzymes may be found even in tissue killed by heat; and moreover, it is remarkable that even if in certain cases the activity of enzymes may be restored, this will take place much more slowly and more imcompletely in enzymes than in protoplasm as a whole; hence the destruction of enzymes may at most be considered a contributory cause of heat death.

The resemblance between the paralysis occurring when organisms are subject to excessively low oxygen pressure and the heat coma, has occasioned the theory of asphyxiation as the cause of heat death, the notion being that at high temperature the absorption of oxygen could not keep pace with the consumption owing to the lesser absorbing ability of the heated tissues and the large increase in metabolism. In the case of temperatures relatively low in animals with a high consumption of oxygen, several circumstances indicate that deficiency of oxygen plays a part as a cause of heat death, but it is objected that death often occurs so early that oxygen deficiency cannot possibly have set in.

The same is true of an accumulation of toxins in the organism as a cause of heat death. As a result of increased metabolism owing to the heat, poisonous metabolites have been supposed to accumulate in the organisms; in certain cases this, no doubt, is so, but it may be stated here, as with the theory mentioned above, that the injury often sets in so rapidly that there can be no question of an accumulation of metabolites.

Finally a theory much favoured is that the influence of heat causes a change in the fats of the protoplasm, the lipoids, so that at the critically high temperatures the injury consisted of the melting of the cell fats. It is taken for granted that the melting

points of the lipoids of animals have a relation to the temperature at which they are formed so that heat-adapted animals have fats with a high melting-point, whereas the lipoid melting-point of cold-adapted animals would be lower. This theory of the melting of the lipoids as the cause of heat death is supported by the observation that animals adapted to heat often have a greater resistance to fatally high temperatures than those adapted to cold. Various investigations of the chemical and physical changes of the cells point in the same direction, whereas others weaken the theory (Bělehrádek 1935, p. 214).

All in all it may be said that none of the above theories alone covers the cause of heat death and heat injury.

If we ask what contribution the present investigation makes to the elucidation of the problem, it must be said at once that it does not support the theory of the general coagulation of proteins; heat death does not occur at a definite temperature, there must always be a combination of temperature and time so that the "thermal death point" as expressing the coagulation point of the proteins of the species concerned must be abandoned.

However, in the present investigations special stress is laid upon the determination of the time at which the heat death occurs, and by opening unbroken puparia and by the analysis of dead eggs we find that the characteristic feature is that development continues for a shorter or a longer time after the heat exposure. Under severe exposure the effect of the injury advances rapidly, death occurring early; under less severe exposure there is considerable development before death occurs. An attempt is made to illustrate this in table 2, where the results of all the experiments on 3 days old puparia are grouped, arranged to temperature and duration of exposure. It will be seen that with the weak stimulations, for instance with short periods of exposure, development continues for a long time after the cessation of the exposure, death occurring only late; with stronger stimulations death interrupts development at an earlier stage.

In addition, we see from table 3 that the mortality rate is not evenly distributed over all stages of development but is more frequent at certain stages. The table gives the course of the experiments on $^3/_4$ day old, and 3 days old puparia.

All experiments with periods of exposure of $^1/_4$, $^1/_2$, 1, 2, 4,

Table 2. Puparia 3 days old. Mortality and Emergence of Flies.

Temperature	Time of exposure	Stages of development					
		0	1 Yellow eyes	2 Red eyes	3 Black eyes	4 Half emerged	5 Emerged
54° C.	1/4 m.	6	..	6	21	14	92
	1/2 m.	40	17	45	32	..	..
53° C.	1/4 m.	..	..	2	1	2	97
	1/2 m.	..	..	23	29	8	43
	1 m.	45	14	16	21	1	4
52° C.	1/4 m.	..	..	..	..	..	101
	1/2 m.	..	..	3	2	1	96
	1 m.	15	..	19	28	9	29
	2 m.	34	22	26	18	..	..
	4 m.	95	5	..	..	..	..
51° C.	1/4 m.	1	..	..	..	..	99
	1/2 m.	..	..	..	3	..	97
	1 m.	..	..	9	6	3	82
	2 m.	..	..	27	43	5	25
	4 m.	2	41	51	6	..	..
	8 m.	50	44	6	..	..	..
50° C.	1/4 m.	..	..	..	..	..	40
	1/2 m.	2	..	..	..	..	38
	1 m.	1	..	..	..	..	99
	2 m.	..	..	9	10	4	77
	4 m.	11	..	20	34	13	24
	8 m.	2	21	52	5	..	..
	16 m.	19	14	3	..	..	..
	32 m.	100	..	..	..	..	..

Column 0: Number of individuals died during or immediately after exposure.

Column 1: Number of individuals died in pupal stage with yellow eyes (4 days old).

Column 2: Number of individuals died in pupal stage with red eyes (5 days old).

Column 3 and 4: Number of individuals died in pupal stage with black eyes or half emerged (6 days old).

Column 5: Number of individuals emerged.

8, 16 and 32 minutes are here summarized, including the supplementary experiments with other periods of exposure, than those originally planned. For 3/4 day old puparia there are the

following phases: 0: death occurs during exposure or immediately afterwards, viz. in the 4th larval stage; 1 and 2: death occurs after the formation of the pupa, but prior to the eversion of the head (1), or during eversion, so that a very small head results (2); 3: death occurs during the period of white eyes; 4: during the period of yellow eyes; 5: during the period of red eyes; 6: during the period of black eyes; 7: indicates that emergence has taken place. For 3 day old puparia the following phases are distinguished: 0: death occurs during exposure or immediately afterwards, viz. during the stage of white eyes; 1: death occurs during the stage of yellow eyes; 2: death occurs during the stage of red eyes; 3: death occurs during the stage of black eyes; 4: death occurs during emergence so that the puparium is broken, but the fly too much weakened to complete the process; 5: emergence apparently normal.

It is seen from tables 2 and 3 that when the exposure is increased, either by raising the temperature or prolonging the period, the mortality rises; but there is a remarkable difference between the two groups in table 3; where young puparia in the 4th larval stage are exposed, death chiefly occurs during or immediately after exposure, but when older puparia are subjected to the same doses, death occurs more frequently later, i. e. when development has passed the resting stage and reached the sensitive stages in which the formation of the imago is completed.

Table 3. Percentage of Mortality and Emergence of Flies.

Stage of development reached	Puparia 3/4 days old	Puparia 3 days old	Stage of development reached
0.* 4th larval stage ...	32 per cent		
1. headless	6 —		
2. small head			
3. eyes white	1 —	8 per cent	0.* eyes white
4. — yellow	1 —	6 —	1. — yellow
5. — red	4 —	12 —	2. — red
6. — black	4 —	12 —	3. — black 4. head everted
7. emergence.....	53 —	61 —	5. emergence
	101 per cent (4668 Ind.)	99 per cent (5126 Ind.)	

* 0 = stage at exposure.

It is also seen from this table that no matter what the exposure the mortality is the lowest during the stages of white and yellow eyes, whereafter it rises. Similarly it is seen that mortality during the exposure or immediately after is remarkably high, if the exposure takes place in the critical 4th larval stage, whereas it is but one fourth if the exposure takes place during the stage with white eyes.

The same is true of the experiments on the full grown larvae. If the results of all experiments are summarized, it is seen that the mortality within the period from exposed larva to emerged fly is not evenly distributed over the different stages, a calculation of the mortality showing the following figures:

Table 4. Percentage of Mortality and Emergence of Flies.

Died during or immediately after exposure	Died during formation of puparium	Died during the rest of pupal life	Emerged from larva-like puparia	Emerged from normal puparia
	a b c			
39 per cent	3—8—1 per cent	3 per cent	1 per cent	45 per cent

The columns *a*, *b* and *c* are stages of abortive formation of puparium, so-called "Larva-like puparia" or larval puparia (see Plate I) being formed. If the formation of the puparium is successful, no more than 3 per cent die during the whole pupal life, while 45 per cent develops to flies. Of the "Larva-like puparia" only 1 per cent develops, and here the formation of puparia proved to be a critical stage with high mortality (see also Plate I).

These observations, which show the successive advance of injury after exposure, and the higher frequency of death in some stages than in others, first and foremost are inconsistent with the theory of a general coagulation of proteins as the cause of heat death; nor do these observations support the theory of death owing to the melting effect of the temperature on the fats of the protoplasm.

The experience that with an exposure at one time the result appears at a later stage and that some stages are more suscep-

tible than others suggests a relation to the phenomenon that in development there is an alternation between labile and stable periods—active periods and resting periods. During the labile periods are induced the processes which later on will make their mark upon developments. We know only incompletely how this induction is carried out, but very likely certain determinator or induction substances are secreted during the labile periods, their task being to predestine, to start and regulate processes during embryonic as well as the post-embryonic development. Having the above phenomena in view I think it most likely that the injurious effect of temperature consists in a destruction or weakening of such determinator substances; for the consequence involved will be that the various processes are relatively displaced, i. e. that the equilibrium of development is disturbed.

If the destruction is only slight, the displacements will be so small that development can be completed, with or without defects as the result. If the displacements of the processes necessary to development are too great, development comes to a standstill and the animal will die, not so often in the resting periods as in the active periods, which require very intimate interaction between the various processes. If exposure takes place immediately prior to or during an active period, death or the defects will occur in this; if it takes place during a resting period, the consequences will mainly be seen when the effect of the determinator substances acting during the resting period is on the point of manifesting itself. This conforms with experiments made for instance on *Lepidoptera* (Prochnow 1914) in which the heat influence takes place in a critical period shortly after pupation, whereas the result does not appear until the pigmentation of the wings takes place during the last days of pupal life.

I am not of the opinion that there is any special cause of heat death, as sought by several investigators; there is no fundamental distinction between the injurious effect of heat from heat defect to heat death.

The nature of thermal injury to the living organism is, however, so very complex (cp. Bělehrádek 1935) that one cannot imagine it to be due to one single cause. The very characteristic general paralysis due to the influence of fatally high temperatures suggests, first and foremost, that the nervous system is

attacked; this is not to be wondered at, since in numerous other fields it has been found that the cells of the nervous system are more sensitive than the other cells of the organism. Here, as little as in the case of a destruction of determinator substances, the direct cause of the injury need be the high temperature itself. I think it most likely, that the injury in itself is due to the formation of toxic metabolites arising in consequence of the high temperature, perhaps poisonous substances of a kind similar to the histamines appearing if mammalian tissue is heated. The linear relation of the injurious effect to the logarithm of the dose recalls phenomena known in medical science in case of poisoning of the organism.

An accumulation of toxic metabolites in the cells is due probably to a defective oxygenation during cellular metabolism, the absorption of oxygen being deficient in heated tissues, i. e. a kind of asphyxiation within the individual cells. On the basis of my experiments I consider that the objection made that the heat exposure is often so short that there can be no question of oxygen deficiency or of accumulation of metabolites, is not well supported; the injury seems to be of the same nature with the shortest periods of exposure—$^1/_4$ min.—as well as with the longest. Moreover, it is known in medicine that toxic metabolites (histamines) may appear almost instantaneously. It is likewise known that a very brief reduction (a few seconds) of the supply of oxygen may paralyse, wholly or in part, the activity of brain cells. For instance, transitory pressure on the carotid arteries, about 15—30 seconds, will cause disturbances of vision and uncontrolled movements of the musculature of the limbs and the face; additional pressure causes paralysis (cp. the uncontrolled movements of *Musca* larvae prior to the occurrence of the paralysis). Thus it may very well be imagined that a brief oxygen deficiency may cause the formation of toxins, which take a long time to wash out of the organism (cp. the duration of paralysis in fly larvae) and which may destroy substances essential to development, so that the processes of development will be displaced and the animal at last will die.

Presumably it is incorrect to resort to such phenomena as the reduced capacity of absorption of heated tissues, etc. for there is no specific effect of fatal temperatures; exposing the

insects to lower temperatures results in similar symptoms of a poisoning of the organism, paralyses, defects etc. The more general explanation no doubt is that the high as well as the low temperatures are outside the temperature range to which the species is adapted and inside which the processes of the animal proceed harmoniously, without poisoning the animal.

IV. Comparison between various species of flies.

In addition to *Musca domestica* four other species have been examined, viz. *Stomoxys calcitrans*, *Lyperosia irritans*, *Haematobia stimulans* and *Scatophaga stercoraria*. These five species are characterized by being associated with the domestic animals, the nutrition of their larvae is almost identical, they all live in dung, and they are all capable of existing in cow dung, though *Musca domestica* prefers pig or horse dung. Moreover, it is common to them that they produce several generations throughout a year, and that the duration of development from egg to imago is short—shortest for *Musca domestica:* 11 days at 25° C. However, there are characteristic differences in their reactions to temperature. In a previous publication the duration of development of these species in relation to temperature has been studied (E. Bro Larsen and M. Thomsen 1940), and in another paper a comparison of the thermopreferenda of the larvae has been carried out on the basis of laboratory experiment, the results having been placed in relation to existing experience of the behaviour of the species in nature (E. Thomsen and M. Thomsen 1937).

The biology of the species examined according to observations in this country may briefly be characterized as follows: *Musca domestica* and *Stomoxys calcitrans* are both indoor forms. *Musca domestica* is particularly thermophilous, the larvae live in the strongly fermenting pig dung, and the species has a marked summer maximum. *Stomoxys calcitrans* is less thermophilous, the larvae live in the less fermentive cow and calf dung; the occurrence of the maximum depends on the temperature of the cow byre and the available quantity of nourishment (blood),

i. e. the number of cattle present, generally it occurs in the autumn when the cows are taken to the byre.

The other three species breed in the open. *Lyperosia irritans* is a marked summer form with a maximum in July-August, *Haematobia stimulans* is a spring and autumn form with a pronounced summer depression, and the same is true to a still

Table 5. Ecological data of species studied.

	Thermal constant	Threshold of development	Optimum temperature	Shortest duration	Upper temp. limit	Phenology	
						Summer	Winter
Musca domestica	146	12.2	33.2	6.92 d.	40	Several generations throughout the summer.	Slow development indoors.
Stomoxys calcitrans	193	12.3	31.4	10.75 d.	35	do.	do.
Lyperosia irritans	141	12.9	32.3	7.54 d.	36	Midsummer maximum.	pupal diapause.
Haematobia stimulans	189	10.7	28.3	10.96 d.	31	Spring and autumn maxima; summer depression.	do.
Scatophaga stercoraria	372	2.1	25.3	15.58 d.	27	do.	do.

greater extent of *Scatophaga stercoraria*, for it appears earlier in the spring and disappears later in the autumn, so that the summer depression becomes more pronounced (O. Hammer 1941). The table above gives information of the data obtained so far regarding the ecological constants of the species and their behaviour in nature. (For the explanation of the termini see E. Bro Larsen and M. Thomsen 1940, p. 16).

1. Experiments on puparia.

Owing to the difficulty of procuring uniform material the experiments have been carried out only on puparia of *Stomoxys* and *Scatophaga* of an age corresponding to the most resistent stage in *Musca domestica* and only for every second degree: 40°,

42°, 44°, 46° C. etc. The results are series of S-shaped curves as an expression of the mortality at the different degrees of exposure, and a curve through the points of 50 per cent of mortality is little short of a straight line (see fig. 11). If the curves of 50 per cent mortality for *Musca domestica* and the two other species are compared, it is seen that the puparia of *Scatophaga* are the least resistant, those of *S. calcitrans* somewhat more resistant, while

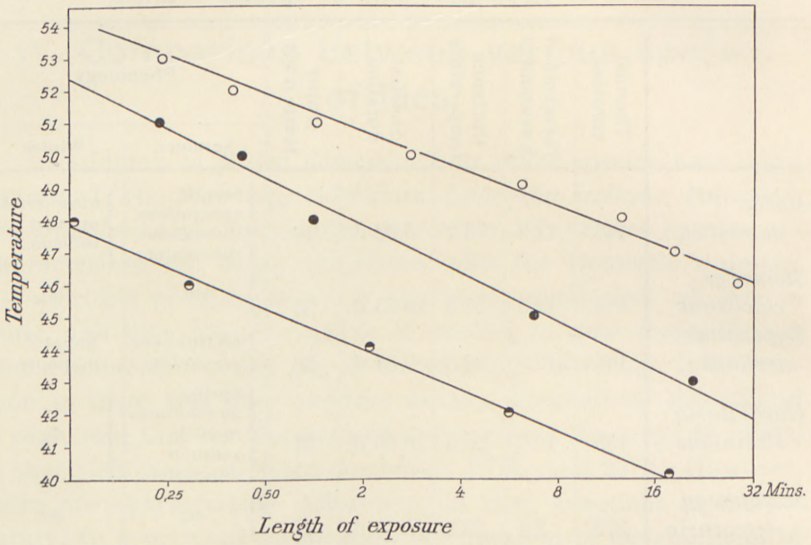


Fig. 11. The median mortality of *Musca domestica*, *Stomoxys calcitrans*, and *Scatophaga stercoraria*, exposed as puparia with white eyes to different temperatures and for various periods of exposure. ○ *Musca domestica*, ● *Stomoxys calcitrans*, ◓ *Scatophaga stercoraria*.

the resistance to high temperature is greatest in *Musca domestica*, which corresponds exactly to the information provided by table 5. On opening dead puparia we find the same phenomena as those seen in the puparia of *M. domestica:* that the pupae exposed to the smallest dose of heat have been able to continue development for several days after the exposure, while those exposed to larger doses die shortly after or during the exposure.

2. Experiments on larvae.

All experiments on larvae were carried out according to experimental type II in 1935.

The results are pictured in fig. 12 indicating the time elapsing from the beginning of the fatal exposure until the occurrence of death. The points represent mean values of individual experiments summed up for each half degree; generally each point is the average of about ten individual experiments, only in the case of the lower temperatures the average is of less than ten. For the points representing very high temperatures the same holds good

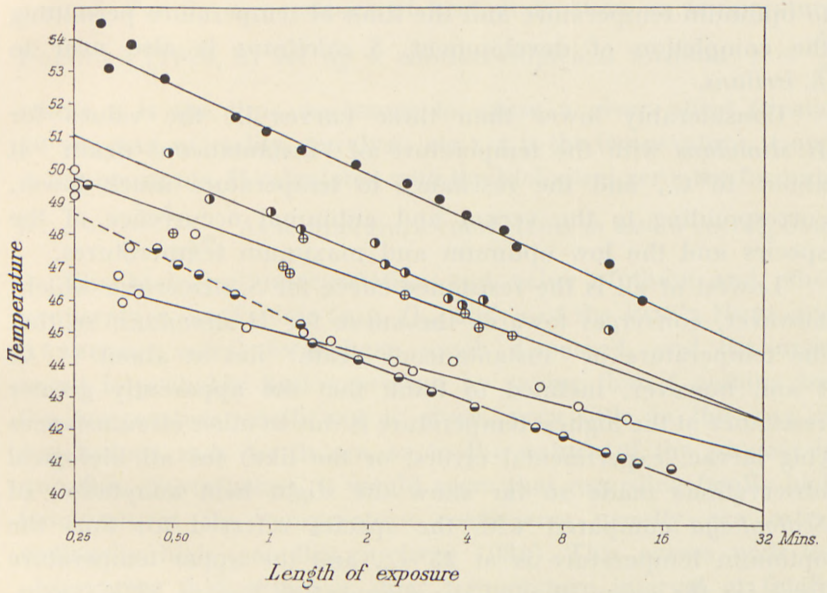


Fig. 12. Temperature resistance curves for larvae of ● *Musca domestica*, ◑ *Lyperosia irritans*, ⊕ *Stomoxys calcitrans*, ○ *Haematobia stimulans*, ◒ *Scatophaga stercoraria*.

as in the case of *M. domestica:* the larvae have a greater viability than should be expected in view of the temperature recorded; this is true particularly of *Scatophaga stercoraria* having the biggest larvae.

The results are seen to be in close accordance with the ecological facts given in table 5 on p. 37. At the top is *M. domestica*, the species on the whole showing the greatest adaptation to high temperatures, with the temperature for "instantaneous death" lying at about 53° C. (death occurring within the first half minute).

Next below is *L. irritans* with the temperature for "instanta-

neous death" at 50—51° C.; evidently it has a somewhat lower resistance to temperature corresponding to its outdoor life in the only slightly fermentative cow dung, but with a marked summer maximum revealing adaptation to high temperature. Table 5 shows that even regarding "optimum temperature" and "upper temperature limit of development" it comes next to *M. domestica*.

The curve of *S. calcitrans* is very near to this curve, with the temperature of "instantaneous death" at 48—49° C. In respect to optimum temperature and the limit of temperature permitting the completion of development, *S. calcitrans* is also near to *L. irritans*.

Considerably lower than these curves lie the values for *H. stimulans* with the temperature of "instantaneous death" at about 46° C., and the resistance to temperature much lower, corresponding to the vernal and autumnal occurrence of the species and the low optimum and maximum temperatures.

Lowest of all is the resistance curve for *S. stercoraria* which, however, converges towards the curve for *H. stimulans*, so that the temperature of "instantaneous death" lies at about 47° C. I am, however, inclined to think that the apparently greater resistance at the highest temperature is due to other circumstances (big larvae, experimental errors, or the like) for all biological observations made so far show the slight heat adaptation of *Scatophaga* compared with the species referred to; thus the optimum temperature is at 25° C., and the upper temperature limit for the completion of development as low as 27° C.

Hence it is seen that the results obtained from the application of fatally high temperatures show conformity with the habits of the animals in nature and their relation to temperature. Nieschulz (1933) on the basis of another experimental principle (see Bodenheimer 1925) found a similar agreement when determining the fatal heat maximum for imagines of *Musca domestica* (46.5°), *Stomoxys calcitrans* (43.8°), and *Fannia canicularis* (40.9°) corresponding to the thermopreferendum of the three same species, determined as 33.1°, 27° and 20.5°.

For the dependence of biological processes on temperature Bělehrádek (1926) drew up an empirical formula: $y = \frac{a}{t^b}$, in which y is the time, t the temperature and a and b are constants.

If the temperature is calculated from the biological zero (comp. E. Bro Larsen and M. Thomsen 1940, p. 16), the formula will be: $y = \frac{a}{(t-\alpha)^b}$ in which α is the biological zero, and if we write $b = 1$ the formula is: $y\ (t-\alpha) = a$. This is the formula of thermal summation which consequently is a particular case of Bělehrádek's formula. b is called the temperature coefficient and is considered a characteristic of the course of the process concerned. For the dependence of heat destruction on temperature Porodko (1926, b) set up a similar empirical formula: $z = \frac{A}{t^m}$ where z is the time necessary to cause a given effect (death, 50 per cent mortality, paralysis etc.), t is the temperature, A and m are constants. If calculated with the biological zero, the formula is: $z = \frac{A}{(t-\alpha)^m}$. As temperature coefficients m and b correspond.

The biological interpretation and value of these and other temperature coefficients (e. g. Q_{10} and μ of the van 't Hoff and Arrhenius rules) have been much discussed, and the main result is probably best expressed by saying that if nothing but the temperature coefficient is given, very little in the way of conclusions may be drawn as to the nature of the process in question. Nevertheless, it would seem that regarding fatally high temperatures the temperature coefficients usually are extraordinarily high (see Bělehrádek 1931). This agrees with the observation that within a short temperature interval at fatally high temperatures the effect of the injurious processes is doubled, while the velocity of the process is doubled within a much longer interval with moderately high or low temperatures. Thus the duration of development of *Musca domestica* at 21.5° C. is 15.67 days, at 30.3° C. 8.04 days, the velocity is doubled within an interval of 8.8° C. On the other hand at 48.6° C. *Musca* larvae will be killed in 4 minutes and at 49.2° C. in 1.8 minutes, a doubling of the intensity within less than 2° C. In accordance with this we find the temperature coefficient of the first mentioned process to be: $b = 1.002$, if $\alpha = 12°$ C., while in the latter case b is found to be 7.8 for $\alpha = 33.2°$ C. (the optimum temperature). This simply means that the supraoptimal temperature interval up to instantaneous destruction is only small.

An attempt of characterising the five species examined by means of *b* will give:

	α	*b*	α	*b*
Musca domestica	0	24.8	33.2	7.8
Lyperosia irritans	0	26.2	32.3	8.4
Stomoxys calcitrans	0	35.5	31.4	11.5
Haematobia stimulans	0	38.7	28.3	13.8
Scatophaga stercoraria	0	26.1	25.3	10.7

For the biological zero the optimum temperature was chosen from the argument that at the point at which mortality is the lowest and the completion of development most rapid, the heat injury will be least. This point therefore may be regarded as the biological zero for the special injurious processes of temperature.

It is seen from the table that for the four related *Muscidae b* rises with the increasing susceptibility to temperature; however, for *Scatophaga b* is 26.1 and 10.7 respectively and the course of the curve is another (fig. 12), so that *b* in no simple manner expresses the relation between the susceptibility of the five species to heat influence. Hence, we must be content to give a numerical expression of the empirical facts, as for instance:

The longevity is 4 min. for:

M. domestica at	48.5° C.
L. irritans -	46.0° C.
S. calcitrans -	45.5° C.
H. stimulans -	43.5° C.
S. stercoraria -	42.9° C.

In these comparative experiments the experimental animals are stimulated until they die; hence there is a question of an instantaneous destruction of vital substances and not, as in the time-limited exposure, of a chock resulting in a displacement of the equilibrium of processes and leading to the death of the animal.

If then the cause of death is to be sought in a destruction or melting of the lipoids of the cells (see p. 29) the succession of the melting points of the various species should be as given on p. 37: *Musca domestica, L. irritans, L. calcitrans, H. stimulans, S. stercoraria.* I have not had the opportunity to examine whether

this is the case, but even if it were, one must be cautious of drawing conclusions that are too general and believing in a simple relation between the melting point of the lipoids and the resistance to fatally high temperatures. If we take FRAENKEL's experiments (1938, b) on the thermal adaptation of *Calliphora* larvae, we find that if the larvae are kept at 12° C. and 31° C., the power of resistance of the latter to fatally high temperatures is about twice the resistance of the former, and equivalent injury at 39° being reached in 5 hours in the latter, but in 3 hours in the former, and a rise in the melting point of the lipoids is actually demonstrated. But if we take the two species *Musca domestica* and *Haematobia stimulans*, the resistance of *Musca* at 46° C. is 28 times greater than that of *Haematobia*, an equivalent effect being obtained in half a minute in *Haematobia* and in 14 minutes in *Musca;* and even if my experiments have been merely preliminary, I have found that if *Musca* larvae are kept at about 18°—20° C. their thermal resistance nevertheless is much greater than that of *Haematobia* larvae kept at 25° C., just as the difference of the resistance of *Musca* larvae kept at 25° C., and of those kept at 33° C. is so small that I am not even sure that I have demonstrated it. The very great difference found between the resistance of different species is of an extent much wider than what can be reached through experimental adaptation, and it cannot be directly due to differences in the melting points of the lipoids.

Statistical Treatment.

It appears from the foregoing that there is an individual variation in reaction, even if the exposure is the same, for we get a certain percentage of mortality and a certain percentage of emergence, not an all or none reaction, which result is due to the fact that all individuals were not equally resistant to the heat. Among the dead unbroken puparia is seen a marked variation of the degree of the fatal injury—some have died immediately after the exposure, others not until immediately before emergence (compare table 2 as also the individual experiments of the figs. 3 and 4). I have attempted to illustrate the results diagrammatic-

ally in fig. 13. The distance between the two parallel lines x and y indicates the variation of the test animals, the weakest specimens being found along the line x, those of medium resistance in the middle, and the most resistant along the line y. Along the horizontal axis the intensity of the exposure is plotted, the lowest

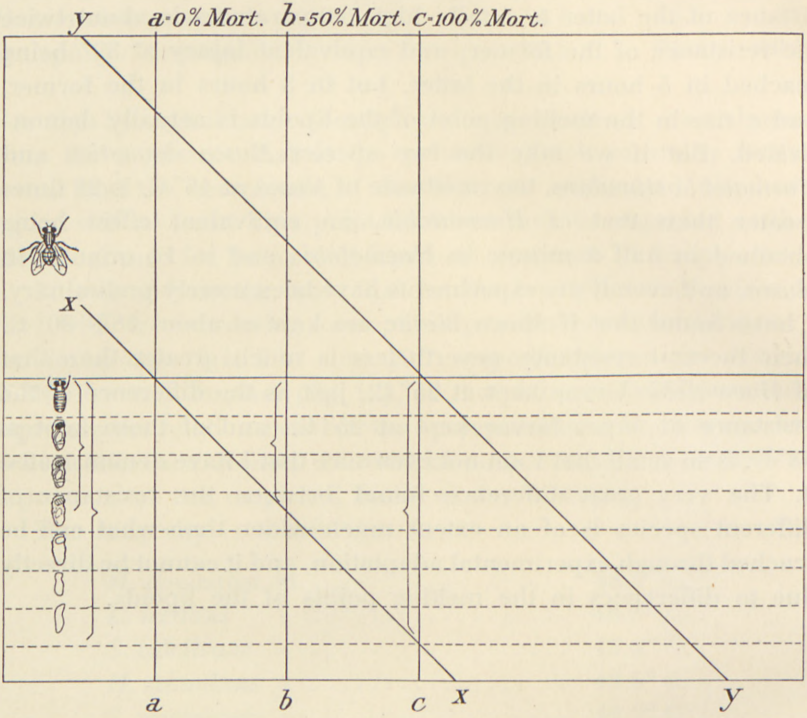


Fig. 13. The influence of variability in a given population of puparia exposed to heat (see text).

exposures being found on the left, the most severe on the right. Along the vertical axis diagrammatical drawings show the stage of development at which death occurred (cp. the classification on p. 32). The upper horisontal line represents the boundary between emergence and death, beyond this are the emerged flies[1].

If a definite heat dose b is given, both weak and resistant individuals are damaged, as the vertical line from b is meant

[1] In the diagrams no regard has been taken to the varying sensibility of the individual stages.

to illustrate, the consequence being that a percentage of 50 will emerge and a percentage of 50 will die, viz. the 50 per cent nearest to the x-line. Within the latter, however, some died in the stage with yellow eyes, some with red eyes and some with black eyes, and still others died immediately before emergence should have taken place. In the same way a continuity of the injury must be imagined among the emerged animals, only it evades analysis to a greater extent; an examination of fertility and viability in the survivors, would, however, probably bring it to light.

If we take another exposure, a, a percentage of 100 flies will emerge, while the exposure c results in a mortality of 100 per cent, and these puparia are more severely injured ("more dead") than in case of exposure b, so that some will be dead immediately after the exposure, some in the 4th larval stage etc.

In order to obtain a truer picture of the extent of injury, these circumstances ought to be taken into consideration and corrections made in the curves of mortality for the extent of the injury to the dead puparia. This was done, the result being that the course of the S-shaped mortality curves become more clearly and regularly S-shaped. This suggests that the S-shaped curve is an expression of properties in the experimental material.

As mentioned it is characteristic of the curves that at the beginning mortality increases at an ever accelerating rate, afterwards increasing at an ever falling rate; the turning point is at about 50 per cent mortality, at which figure the rate is the highest, the curve being approximately symmetrical about this point. Moreover all curves are identical, the mortality rate alone being different. The most likely explanation of this shape of curve is that it is an expression of the individual variation among the experimental animals regarding the quality: resistance to heat. Thus the S-shaped curve is the summation curve deduced from the probability curve concerned, expressing for instance for the curve fig. 3 at 49° C. that 25 per cent of the specimens are just incapable of surviving an exposure of 1.4 minutes, that 50 per cent of the specimens are just incapable of surviving an exposure of 2.1 minutes and so on.

In order to test whether the distribution in question is a normal distribution, corrections were first made in respect of the

natural mortality, i. e. the mortality always found within the experimental cultures, independent of the intensity of the exposure. This was determined by control experiments at 25° C. Afterwards a summation curve of a normal distribution was plotted to fit as close as possible the points of the experimental curve, and the deviation of the points from the normal curve was tested by their standard error, and the standard error of the whole curve was determined. Thus the criterion of whether or not the experimental distribution is normal, is the size of these deviations measured by the standard error of the adjustment curve. If the deviations are distributed according to the probability curve the adjustment has succeeded. In table 6 is shown the figures according to the normal distribution and their actual positions.

Table 6.

3-days old puparia			¾-days old puparia			Full-grown larvae		
Within	Was observed	Normal	Within	Was observed	Normal	Within	Was observed	Normal
0.10 μ	0	3	0.10 μ	5	3	0.10 μ	2	3
0.20 μ	1	6	0.20 μ	11	7	0.20 μ	6	7
0.30 μ	5	8	0.30 μ	12	10	0.30 μ	9	10
0.40 μ	8	11	0.40 μ	14	13	0.40 μ	12	13
0.50 μ	10	13	0.50 μ	15	16	0.50 μ	16	16
0.70 μ	15	18	0.70 μ	19	22	0.70 μ	20	22
0.90 μ	20	22	0.90 μ	22	27	0.90 μ	25	27
1.10 μ	27	26	1.10 μ	30	31	1.10 μ	32	31
1.30 μ	31	28	1.30 μ	30	34	1.30 μ	36	35
1.50 μ	33	30	1.50 μ	40	36	1.50 μ	39	37
2.00 μ	35	33	2.00 μ	42	40	2.00 μ	43	41
2.50 μ	..	35	3.00 μ	42	42	3.00 μ	43	43

The differences are not greater than that they may be due to chance, and it is to be noticed that in all cases the curve seems to be a little more acute than the normal curve, the low values being slightly lower, the high values slightly higher than those of the distribution curve, a feature that is known from other fields of biology.

Thus it has been made probable that the S-shaped curves are an expression of a normal distribution with respect to the

property of resistance to heat. Bělehrádek in "Temperature and Living Matter" writes that the S-shaped curves frequently met with in biology, often quite simply may be transposed into curves of distribution; an attempt at this was indeed made by e. g. Henderson Smith (1923) with the mortality curves of the fungus *Botrytis cinerea*, in experiments with killing by means of phenol and heat, and in the latter case he finds that the experiments are covered by Student's distribution curve. Among insects, the material used for the experiments is often so difficult to deal with that the results only rarely can be treated statistically, the experimental data are too scarce and heterogeneous, whereas in experiments on bacteria and spores of fungi an almost unlimited abundance of data may be obtained, and the simplified conditions of life mean that the material is more homogeneous.

The statistical material has been deposed in the library of the Royal Veterinary and Agricultural College, Bülowsvej, Copenhagen, from where it may be had on loan on application.

Summary.

1. The influence of fatally high temperatures on various stages of development has been examined in the following species of *Diptera*: *Musca domestica*, *Lyperosia irritans*, *Stomoxys calcitrans*, *Haematobia stimulans*, *Scatophaga stercoraria*. The animals used for the experiments have been exposed to the effects of high temperatures during a definite time interval ($^1/_4$, $^1/_2$, 1, 2, 4, 8, 16 and 32 min.) and the extent of the injury has been examined. Full-grown larvae were exposed to high temperatures, and a record was taken of the time elapsing before death occurred.

2. *Musca domestica*. Puparia, 3 days old, $^3/_4$ day old, full-grown larvae in the 3rd larval stage, and eggs, were tested. A linear relation was found between the logarithm of the period of exposure and the temperature, when 50 per cent of mortality was taken as a measure of the injury.

3. It is shown that susceptibility to fatally high temperatures increases in succession as follows: puparia 3 days old, puparia $^3/_4$ day old, larvae, eggs.

4. Larvae of *Musca domestica, Lyperosia irritans, Stomoxys calcitrans, Haematobia stimulans* and *Scatophaga stercoraria* were exposed to fatally high temperatures, and it was found that if the temperature rises in arithmetical progression the effect increases geometrically, the relation between the temperature and the logarithm of the time taken to kill the insect at the temperature in question being a straight line.

5. It is shown that the susceptibility to heat in the five species of *Musca domestica, Lyperosia irritans, Stomoxys calcitrans, Haematobia stimulans, Scatophaga stercoraria* increases in the succession given above, whereby conformity is created between that succession and the other biological constants of the species, as well as previous experience on their behaviour in nature.

6. It is shown: a) that death from heat often does not occur during or immediately after exposure, but later in the development; b) that mortality mainly occurs soon after the close of the exposure if the latter is strong, but that it occurs only later in life if it is slight; c) that mortality, regardless of the stage in which exposure takes place, is not evenly distributed in the period after the exposure, but is found in those developmental periods in which activity is greatest, and in which the most intimate interaction between the various processes is demanded (hatching, development and pigmentation of the organs); d) the hypothesis is advanced that the injury partly consists in a total or partial destruction of induction substances in the labile periods, so that the consequences of the heat exposure do not appear until the processes normally induced by the induction substances were about to co-operate. If then the equilibrium is seriously disturbed, development will stop and the animal will die; if the character of the disturbance is less serious, development continues in a more or less defective way.

7. If the exposure takes place in periods with an intense activity (formation of mitoses), mortality during or immediately after the exposure is greater than if the exposure takes place during a resting period.

8. It is made probable that the S-shaped curves of mortality are simply an expression of the variation in the experimental material, and the curves are compared with the curve of normal distribution.

9. At determination of the temperature coefficient *b* (or *m*) (according to BĚLEHRÁDEK's formula) for the various species named under 5, the following figures are obtained: 24.8, 26.2, 35.5, 38.7, 26.1 respectively, i. e. very high temperature coefficients, which agrees with previous experience of the great acceleration of the processes exposed to fatally high temperatures.

(From the Zoological Laboratory of the Royal Veterinary and Agricultural College, Copenhagen.)

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PLATE I.

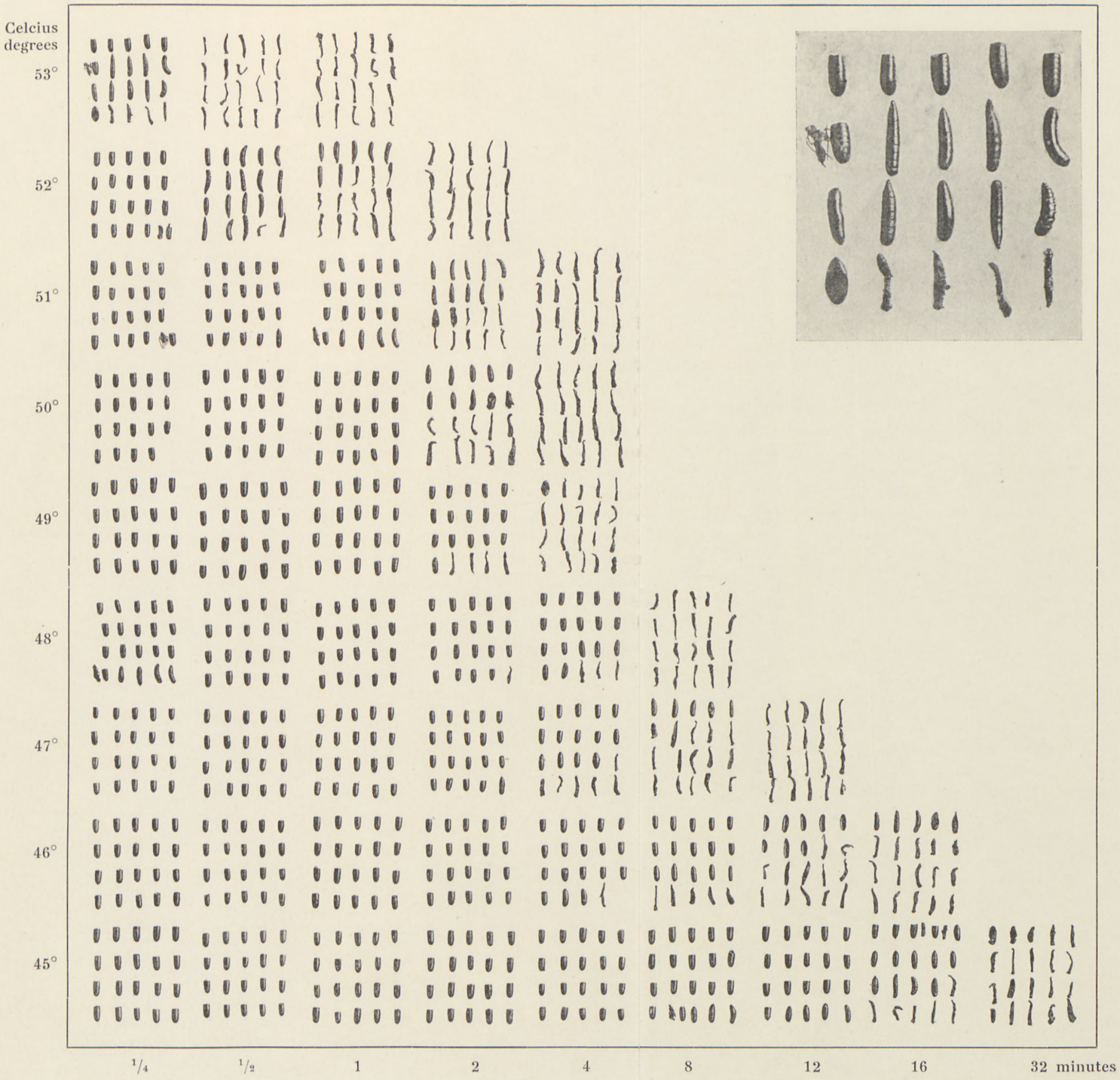
Photographic reproduction of the result of an experimental series with fully grown larvae, each group comprising 20 individuals in a single experiment.

The horizontal rows of groups show experiments at the same temperature, the vertical rows with the same period of exposure.

Within the single experimental groups the individuals have been arranged so as to show uppermost the larvae which have been able to carry through the development until the emergence of the flies, below them the individuals for which the formation of the puparia failed as a consequence of the exposure, and lowermost and last larvae which died immediately after cessation of exposure.

From the magnified detail picture, right (53°, 1/4 min.) it is seen that 5 larvae carried through the development until the flies emerged, 11 larvae made incomplete puparia, "larvaepupae", of which the first, however, contains a fully developed fly, while the eleventh is not hardened at all, for which reason it died soon. 4 larvae died immediately after the exposure.

If the exposure is weak, e. g. 46° and 1/4 min., all the larvae succeed in carrying through the development to flies; if the exposure is strong, e. g. 47° and 12 min., all the individuals die as larvae.


Celcius degrees
53°
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32 minutes

DET KGL. DANSKE VIDENSKABERNES SELSKAB
BIOLOGISKE MEDDELELSER, BIND XIX, NR. 4

EFFECT OF CORPUS CARDIACUM AND OTHER INSECT ORGANS ON THE COLOUR-CHANGE OF THE SHRIMP, *LEANDER ADSPERSUS*

BY

MATHIAS THOMSEN

KØBENHAVN
I KOMMISSION HOS EJNAR MUNKSGAARD
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CONTENTS

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I. Introduction.

During later years our knowledge of colour-change in the *Crustacea* has been much furthered by the discovery of hormones controlling the movement of pigments in the chromatophores (KOLLER 1925 and later, PERKINS 1928) and by the demonstration that the sinus-gland, which in most *Decapoda* is localized in the eye-stalk, produces such hormones (HANSTRÖM 1935 and 1937, CARLSON-CARSTAM 1935 and 1936). In the majority of the *Decapoda* (with the exception of the *Brachyura*), a removal of the eye-stalks causes maximum expansion of the commonly occurring red and yellow pigments. When subsequently an extract of the eye-stalks is injected into these blinded animals, the result is a rapid and complete contraction of the pigments making the chromatophores appear as dots, while the animal as a whole acquires a transparent whitish colour. A similar effect is obtained by injection of eye-stalk extract into shrimps adapted to a dark background. These results evidently mean that the sinus-gland normally produces a hormone causing contraction of the red and yellow pigments, while absence of the hormone results in expansion of the pigments. The white pigment which occurs in many species reacts in a different and somewhat irregular way.

The *Brachyura*, however, behave differently. These crustaceans possess melanin (which is said to be absent in other decapods save *Crangon*), and a removal of the eye-stalks is followed by contraction of the melanin, whereas injection of the hormone results in expansion of this pigment. On the other hand, the yellow and red pigments, also present in many crabs, react wholly (yellow) or partly (red) as in other decapods. For details, the reader is referred to the original papers (for the

Brachyura see especially CARSTAM 1942) and to the very useful review given by HANSTRÖM in his book "Hormones in Invertebrates" (1939).

In some interesting papers (1936, 1937 a and b, 1938, 1940 a), HANSTRÖM showed that extracts of the head of various insects, when injected into blinded shrimps *(Natantia)*, have a similar effect as the crustacean eye-stalk hormone, i. e. they cause a definite contraction of the red and yellow pigments, while the white chromatophores are not or only irregularly affected. In addition to this, CARLSON-CARSTAM (1935, 1936) and HANSTRÖM found that extracts of insect heads expand the black chromatophores of *Brachyura*. Thus, these extracts in their physiological effect show a striking conformity with the sinus-gland hormone.

The technique used by HANSTRÖM in these experiments was the following: The heads were crushed and extracted with seawater, the extract was boiled, filtered, and diluted to the original volume. In some cases it was found that unboiled extract had no (or only a slight) effect, while boiled extract had a considerable effect.

According to the insect species employed, the activity of the head extract showed a considerable variation. Extracts of heads of various species of *Saltatoria* had a vigorous effect, the colour of the test shrimps fading rapidly to a transparent white. The same applies to head extracts of blattids and phasmids. On the other hand, heads of some *Coleoptera*, *Hymenoptera*, and *Diptera* caused a less pronounced colour-change, and heads of certain *Lepidoptera*, adult *Odonata*, *Dermaptera*, and *Thysanura* had no effect at all (cf. HANSTRÖM 1940 a p. 10—11). In some species in which head extracts were very active, extracts of thorax and abdomen sometimes caused a weak reaction, sometimes none.

It was further proved that the insect head extract does not lose its activity when dissolved in alcohol or after being kept dry for 6 weeks; also in this respect it resembles the eye-stalk hormone (KALMUS 1938, HANSTRÖM 1940 a).

The far reaching similarity in the qualities of the two substances incited HANSTRÖM to infer that the insect head contains a hormone similar in kind to the chromatophorotropic hormone of the sinus-gland. The question of the localization of the in-

cretory organ inside the head was approached on the following line: Heads of *Dixippus* were cut transversely just behind the eyes, and extracts were made in the usual way from the anterior and the posterior parts, separately (HANSTRÖM 1938). When injected into blinded *Leander adspersus*, the extract of the hind portion caused a rapid and vigorous contraction of the chromatophores, while the anterior portion had little or no effect. Besides muscles and tracheae, the posterior half of the head contains the suboesophageal ganglion, the oesophageal (hypocerebral) ganglion, the corpora cardiaca, and the corpora allata. As the two last-mentioned organs are the only organs of the insect head which show the morphological characteristics of incretory glands, HANSTRÖM considers it likely that either the corpora allata or the corpora cardiaca are the source of the hormone in question.

In his later paper (1940 a), HANSTRÖM described some preliminary experiments with the isolated corpora cardiaca and corpora allata of blow-flies *(Calliphora)*. The organs were dissected in Copenhagen by Mrs. ELLEN THOMSEN, they were dried on filter paper and sent by mail to Lund, where Professor HANSTRÖM made the extracts and injected them into blinded *Leander*. The shrimps which had received extract of the corpora cardiaca showed an almost maximum contraction of the pigments, while the corpora allata produced a less convincing reaction. Extracts of total heads (without the organs mentioned) were ineffective.

Although these experiments pointed to the corpora cardiaca as the real source of the chromatophorotropic substance, they could not be regarded as conclusive. As Professor HANSTRÖM did not wish to pursue the problem himself, it was agreed between us that the author should do so. I take the opportunity of thanking Professor HANSTRÖM.

The injection experiments were made at the »Danmarks Akvarium«, Charlottenlund. I am indebted to the governing committee and to the staff, especially to Dr. A. F. BRUUN, leader of the biological department, for providing me with working-facilities, aquaria etc.

My thanks are due to Dr. H. BLEGVAD for a material of living *Crangon vulgaris* caught in the Øresund. As a test animal,

this species was found to be inferior to *Leander adspersus*, and the few experiments made will not be included in this paper. The results were in full accordance with those obtained with *Leander*.

I have received help from several persons who provided the great number of living insects necessary for the investigation. My special thanks are due to Dr. ANKER NIELSEN, Hillerød, for collecting the specimens of *Dytiscus marginalis*.

I am very grateful to Mr. ANKER HANSEN of the Laboratory of Normal Anatomy, Royal Veterinary and Agricultural College, for taking a number of colour photographs. The ordinary photographs have been taken by the author.

The Carlsberg Foundation has supported the work with a grant.

II. Insect species used in the experiments.

The insect organs used for preparing the extracts were mainly taken from *Orthoptera* (sens. lat.), as it was convenient to use insects of a reasonable size and species which could be bred in the laboratory without too much difficulty. The following species where chosen:

Saltatoria.

Tachycines asynamorus Adel.

An apterous, long-horned grasshopper frequently found in greenhouses. The majority of the experiments has been made with organs of this species.

Gryllus domesticus L.

Phasmida.

Dixippus (Carausius) morosus Br.

Blattariae.

Blatta orientalis L.

Coleoptera.

Dytiscus marginalis L.

Diptera.

Calliphora erythrocephala Meig.

III. Remarks on the corpora cardiaca, the corpora allata, and the stomatogastric nervous system.

It is not the scope of this paper to enter into a detailed discussion of the morphology and the function of these interesting organs which, during recent years, have attracted a good deal of attention of insect physiologists and anatomists. Reviews of our present knowledge have recently been given by HANSTRÖM (1939 and 1942) and PFLUGFELDER (1941) (cf. also HANSTRÖM 1940 b). Some remarks regarding the anatomy of the organs in the species of insects treated in this work may however be useful.

DE LERMA (1937) has described the anatomy of the corpora allata and corpora cardiaca ("corpi faringei") of *Gryllotalpa*, *Blatta orientalis*, and some species of grasshoppers. His figure of *Gryllotalpa* may be regarded as a diagram of the anatomical relations of these organs prevailing in the *Saltatoria*. The elongate corpora cardiaca are situated in the head dorsally to the oesophagus. The posterior part of each corpus cardiacum is connected with the wall of the aorta, the anterior end receives a nerv from the brain (protocerebrum), while lateral nerves pass to the corpora allata. These are ovoid and lie on the sides of the oesophagus. In the median line, between the corpora cardiaca and partially united with them, we find the ganglion hypocerebrale which belongs to the stomatogastric nervous system; it receives a nerve (nervus recurrens) from the frontal ganglion.

In numerous insects, including the orders mentioned above, HANSTRÖM (1940 b and 1942) found two paired nerves which pass from the brain to the corpora cardiaca: the nervi corporis cardiaci I and II. He gave a picture of the corpora cardiaca of *Tachycines* in which he designed both nerves. The nerve pictured by DE LERMA is the n. corporis cardiaci I.

The histology of the corpora cardiaca has been described by the same authors. According to DE LERMA, they contain numerous nuclei which resemble small nerve cells, and probably they contain neuropilem, so that they still retain the nature of ganglia. On the other hand, they also possess numerous cells with vacuolized cytoplasm and acidophilous granula, which

must be secretory cells; these two types of cells are to some degree separated in different parts of the organ ("parte glandolare", "parte nervosa"). There are signs that the secretion is chiefly discharged into the aorta, and to a smaller extent through the lateral walls of the corpora cardiaca, which are bathed by the haemolymph. From these observations DE LERMA concludes that the corpora cardiaca are incretory glands.

HANSTRÖM (1940 b, 1942) confirmed DE LERMA's observations and accepted his idea as to the function. Especially in *Tachycines*, he found the tissue of the corpora cardiaca virtually flooded with fuchsinophile drops, "deren Vorhandensein die Hypothese einer inkretorischen Tätigkeit der betreffenden Organe kräftig unterstützt. Diese Hypothese muss jedoch natürlich auf experimentellem Wege bestätigt werden."

In *Orthoptera* and blattids the corpora allata are paired organs. They consist of a great number of uniform cells with oval nuclei rich in chromatin; cell-boundaries are not visible. As is well known, the corpora allata are proved to be incretory organs which especially influence the metamorphosis and the ovarian development of insects (WIGGLESWORTH, WEED-PFEIFFER, PFLUGFELDER, BOUNHIOL, ELLEN THOMSEN, and others, vide E. THOMSEN 1942).

Own observations.

Tachycines asynamorus.

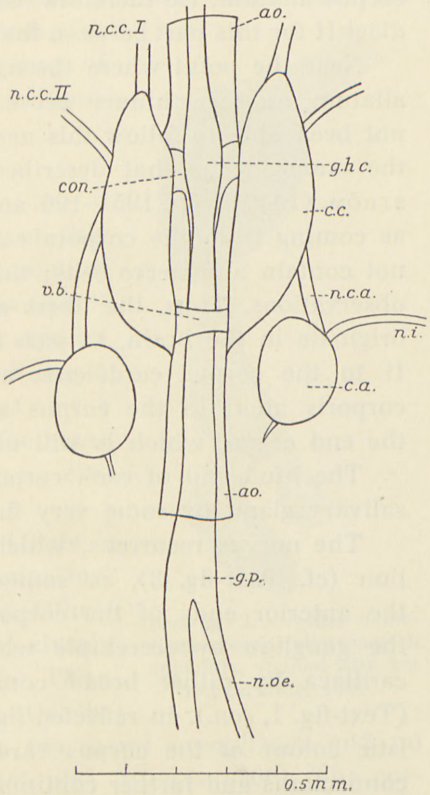
In several respects, the organs of *Tachycines* resemble those of *Gryllotalpa* as described by DE LERMA, but in some features the two species differ.

The corpora cardiaca (Text-fig. 1 and Plate I, fig. 1) are elongate organs measuring about 0.4—0.5 mm in the adult. They are situated behind the brain and are fastened to the dorsal side of the oesophagus by fine connective tissue and tracheae. Their caudal ends are fixed to the fat body by some very fine threads. The corpora cardiaca are intimately connected with the dorsal vessel, they may be described as thickened parts of the lateral walls of the aorta; in most of their length, the two corpora are separate, held together dorsally and ventrally by the ordinary thin wall of the aorta, but the posterior ends are united by a ventral bridge (Text-fig. 1, *v.b.*).

In dissections the corpora cardiaca are mostly conspicuous due to their bluish white colour. A similar colour is generally displayed by the corpora cardiaca of all other insect species I have dissected, and it is remarkable that, according to a personal communication by Professor HANSTRÖM, the sinus gland of *Decapoda* has the same bluish white hue.

The anterior end of each corpus cardiacum receives a well developed nerve from the brain; it enters the corpus cardiacum ventrally near the ganglion hypocerebrale (HANSTRÖM 1940 b, fig. 188). While, no doubt, this nerve is the nervus corporis cardiaci I, another thinner nerve, which is connected with the corpus cardiacum a little further behind (also ventrally), is probably the n. corporis cardiaci II.

The corpora allata are nearly spherical. Anteriorly each of them receives the nervus corporis allati, a rather stout nerve coming from the ventral side of the anterior part of the corpus cardiacum. It looks as if the nerve fibers really originate from the n. corporis cardiaci II and only pass through the ventral part of the corpus cardiacum for a short distance.



Text-fig. 1. *Tachycines asynamorus*. *ao.* aorta; *c.a.* corpus allatum; *c.c.*, corpus cardiacum; *con.*, connection between c. cardiacum and ganglion hypocerebrale (*g.hc.*); *g.p.* posterior ganglion; *n.c.a.*, nervus corporis allati; *n.c.c.I*, nervus corporis cardiaci I; *n.c.c.II*, nervus corporis cardiaci II; *n.i.* nerve from c. allatum; *n.oe.*, nervi oesophagei; *v.b.*, ventral bridge. — Dorsal view. 70×. Semi-diagrammatic.

In this connection, it should be remembered that PFLUGFELDER (1937) describes the n. corporis allati of *Dixippus* as coming directly from the brain, and only receiving a few nerve fibers from the corpus cardiacum (Text-fig. 3). HANSTRÖM (1940 b), however,

holds that this nerve has a similar course as in other insects, entering the corpus cardiacum, where some of its nerve fibers branch between the cells, while others possibly pass directly to the corpus allatum. He therefore retains the name of n. corporis cardiaci II for this part between the brain and the corpus cardiacum.

Near the point where the n. corporis allati enters the corpus allatum, another thinner nerve (Text-fig. 1, *n.i.*) issues. I have not been able to follow this nerve to its end, but it is probably the same one as that described by NABERT (1913) and HANSTRÖM (1940 b, pp. 195—196 and 226). These authors regard it as coming from the corpora cardiaca, as the corpora allata do not contain any nerve cells; this is in accordance with my own observations. Thus, the fibers of this unnamed nerve seem to originate in the brain, to pass through the n. corporis cardiaci II to the corpus cardiacum and, from there, through the n. corporis allati to the corpus allatum before they continue to the end organ, which is still unknown.

The hind end of each corpus allatum is connected with the salivary gland by some very fine and short threads.

The nervus recurrens, which comes from the frontal ganglion (cf. Text-fig. 3), is somewhat swollen at the height of the anterior ends of the corpora cardiaca. This thickening is the ganglion hypocerebrale which is joined with the corpora cardiaca by rather broad connections ventrally to the aorta (Text-fig. 1, *con.*). In reflected light, it is seen that the characteristic colour of the corpus cardiacum is also shown by these connections and further continues as a fine layer down the sides of the nervus recurrens. It seems possible that this colour indicates the occurrence of glandular cells.

In *Gryllotalpa* (DE LERMA), *Rhaphidophora* (ANDER 1939), and several species of *Saltatoria* investigated by BORDAS (1900), the nervus recurrens branches out at the ganglion hypocerebrale into two nerves which are sometimes called the nervi oesophagei. In *Tachycines*, however, the nerve continues undivided in a caudal direction, forming another ganglion somewhat behind the corpora allata, and then it branches into the nervi oesophagei. BORDAS found a similar course of the nervus recurrens in two species of *Mantidae* and three species of *Blattidae*. He

terms the posterior ganglion the "ganglion stomacal", while HOFER (1886) in *Blatta orientalis* describes it as "hinteres dreieckiges Ganglion des nervus recurrens" (cf. his fig. 1, Plate 1). In this species the nervus recurrens is much longer, and the ganglion is situated further behind. *Tachycines* seems to be the only genus of the *Saltatoria* in which such conditions hitherto have been observed.

Text-fig. 2. *Gryllus domesticus*. Corpus allatum with appendix, stained with borax-carmine. 100×.

Gryllus domesticus.

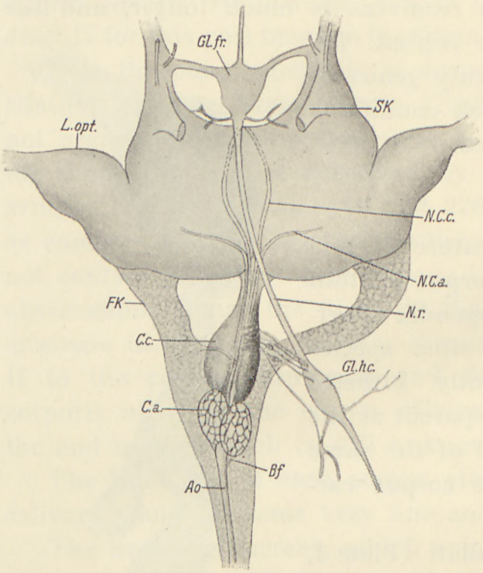
The most interesting feature of this species is shown by the corpus allatum which has a remarkable appendix (Text-fig. 2), not found in any other species investigated and apparently hitherto undescribed. It is not transparent as the corpus allatum proper, but of the same bluish white colour as the corpus cardiacum.

The nervus corporis allati (Plate I, fig. 2) leaves the corpus cardiacum further behind than in *Tachycines*. Otherwise, the relations of the organs closely resemble those found in *Gryllotalpa*. Thus, the nervus recurrens branches out at the ganglion hypocerebrale into the two nervi oesophagei, and no additional ganglion is found (cf. also BORDAS 1900). The corpora cardiaca unite posteriorly.

Dixippus (Carausius) morosus.

The stomatogastric nervous system and the corpora cardiaca and allata have been studied by several authors. The most detailed description has been given by PFLUGFELDER (1937) (cf. Text-fig. 3). I have nothing to add to his record, only it should be emphasized that the very close connection between the corpus cardiacum and allatum makes it almost impossible to separate these organs without injuring one or the other, most often the corpus cardiacum (cf. also Plate II,

fig. 3). This means that secretion may flow out into the 1% NaCl in which the insect is immersed for dissection or that even small bits of tissue adhere to the corpus allatum. This may involve impurity of the extract of the corpus allatum and even of other organs.



Text-fig. 3. *Dixippus morosus*. Brain and stomatogastric nervous system. Ventral view. *Ao*, aorta; *Bf*, fibrils; *C.a.*, corpus allatum; *FK*, fat-body; *Gl.fr.*, ganglion frontale; *Gl.hc.*, ganglion hypocerebrale; *L.opt.*, optic lobe; *N.C.a.* nervus corporis allati (= *n.* corporis cardiaci II after HANSTRÖM); *N.C.c.*, nervus corporis cardiaci I; *N.r.*, nervus recurrens; *Sk*, oesophageal connective. (After PFLUGFELDER).

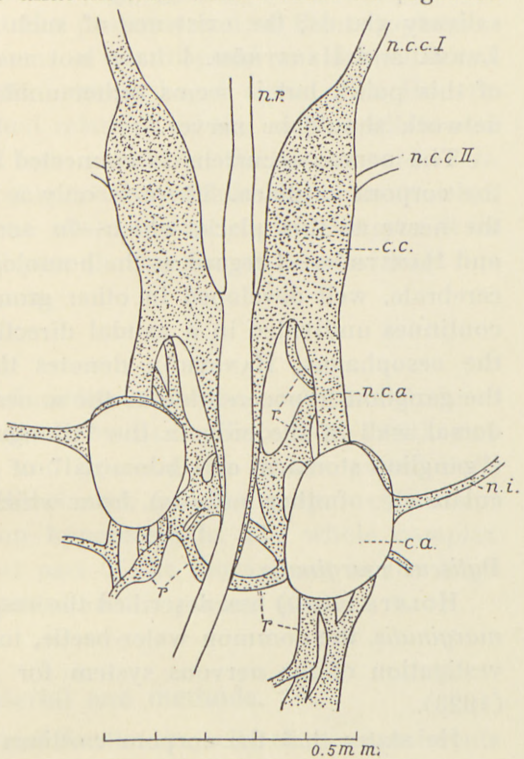
Blatta orientalis.

The corpora allata and cardiaca etc. of this species have been described by earlier authors (especially HOFER 1887, POLICE 1910, BRETSCHNEIDER 1914, and DE LERMA 1937), while BORDAS (1900), NABERT (1913), and HANSTRÖM (1940 b) have treated *Blattella germanica* and other species. The investigators disagree on certain points and, therefore, I think it necessary to give a short description of my own observations which are based mainly on dissections and partly on sections of the organs.

In dissected preparations the corpora cardiaca tend to diverge anteriorly (Text-fig. 4); this is probably due to a tearing of the thin wall of the aorta, as in sections they are found to adhere closely and to form the ventral wall of the aorta (Plate II, fig. 4, cf. also HANSTRÖM 1940 b, fig. 221). Posteriorly, they disjoin from the wall of the aorta which lies dorsally to them. Each corpus cardiacum receives two nerves; the foremost and coarsest is the n. corporis cardiaci I, while the other, which enters the corpus cardiacum further back, probably represents the n. corporis cardiaci II. Posteriorly, the corpora cardiaca are united with the nervus recurrens, continuing as narrow layers on each side

of this nerve almost as far as the hind end of the corpora allata.

From each corpus cardiacum comes a lateral band-like branch which goes to the corpus allatum and which must be regarded as the nervus corporis allati. It is remarkable that in fresh preparations this broad band has the same bluish white colour as the corpus cardiacum; it also contains many nuclei resembling those of the corpus cardiacum, so that its nervous character is not very obvious. It is likely, however, that it does contain nerve fibers which proceed through the corpus allatum to the fine nerve going out from this organ (Text-fig. 4, *n.i.*).



Text-fig. 4. *Blatta orientalis*. *n.r.*, nervus recurrens; *r.*, network between corpora allata and corpora cardiaca. Other letters as in Text-fig. 1. Dotted: organs of bluish colour. Dorsal view. 70×. Semi-diagrammatic.

The corpora allata are dorsoventrally flattened, disc-like organs, in sections tending to show a narrow lumen which may be an artefact. They are connected with the corpora cardiaca not only by the nervus corporis allati already referred to, but also by an irregular network of tissue between each corpus allatum and the caudal continuation of the corpus cardiacum on the same side (Text-fig. 4, *r*). The configuration of this network shows great individual variation. There is also a connection between the networks of the two sides, usually as a ventral bridge below the nervus recurrens. Similar, rather irregular threads connect the corpora allata with the salivary glands which lie just behind them.

The above description in most points agrees with the old account by HOFER (1886), while DE LERMA does not picture any network between the corpora allata. HOFER assumed that the corpora allata were ganglia which give off nerves to the salivary glands; the existence of such nerves is denied by DE LERMA and HANSTRÖM. I have not made any close inspection of this point, but it seems rather unlikely to me that the whole network should be nerves.

The nervus recurrens is connected with the caudal ends of the corpora cardiaca. There is only a very slight thickening of the nerve at this place, which—in accordance with DE LERMA and HANSTRÖM—I regard as the homologon of the ganglion hypocerebrale, well developed in other groups. The nervus recurrens continues undivided in a caudal direction on the dorsal side of the oesophagus, HANSTRÖM denotes the posterior part behind the ganglion hypocerebrale as the n. oesophageus. It ends on the dorsal wall of the crop in the "triangular ganglion" of HOFER ("ganglion stomacal ou abdominal" of BORDAS, ganglion ventriculare etc. of other authors), from which two branches originate.

Dytiscus marginalis.

HOLSTE (1910) has described the respective organs in *Dytiscus marginalis*, the common water-beetle, in connection with his investigation of the nervous system for KORSCHELT's monograph (1923).

He states that the corpora cardiaca ("Ganglien des Rückengefässes") are not very distinctly circumscribed ("ihre Form ist wenig scharf umrissen"); they do not resemble typical ganglia, but are more band- or pad-like in shape. They are intimately connected with the wall of the dorsal vessel and receive paired short nerves from the protocerebrum. They are said to innervate the dilatator muscles of the oesophagus through a very fine nerve on the median side posteriorly, to be connected with the nervus recurrens through a fine nerve, and to send out a lateral stouter nerve, which goes below the optic nerve and fuses with a branch of the maxillary nerve.

The corpora allata ("Tracheenganglien") are described as elongate-spherical bodies lying close to the oesophagus. From HOLSTE's fig. VIII it is seen that they are intimately connected

with the corpora cardiaca, almost as in *Dixippus*, without any intervening nerve.

The nervus recurrens is said to be devoid of a ganglion hypocerebrale, it continues as a strong unpaired nerve ending with a ganglion ventriculare at the posterior end of the oesophagus.

The number of specimens at my disposal was not sufficient for a detailed study of the organs, but I agree with HOLSTE as to the shape and mutual relations of the corpora allata and cardiaca. In my journal I have noted that the corpus cardiacum has an irregular, flattened shape, almost reminding of an amoeba; it adheres with the corpus allatum. Just as in *Dixippus* it is next to impossible to extirpate the corpus allatum without injuring the corpus cardiacum.

Calliphora erythrocephala.

The corpus allatum and cardiacum of the adult blow-fly have recently been studied in detail by ELLEN THOMSEN (1942), and I refer to the description and figures given in her paper. Both organs are unpaired in this insect, and the corpus cardiacum is fused with the ganglion hypocerebrale. The whole complex is situated in the foremost part of the thorax, not in the head as in the other species treated in the present paper.

IV. Material and methods.

The decapod species used for the majority of the experiments was the common edible shrimp *Leander adspersus* Rathke. The shrimps were bought at intervals on the fish-market of Copenhagen and taken to the aquarium. There was a rather high initial mortality, but after some days, the survivors had settled in the tank and—at least in the autumn and the winter—seemed to thrive well, they ate profusely and lived for weeks or even months. In some periods—especially during the warmer months and in the coldest periods of winter—it was impossible to procure living shrimps, and this is the cause of the limited number of animals in some of my experiments.

According to KOLLER (1936), *Crangon* has four clearly different pigments (melanin, white, yellow, and red), whereas *Leander adspersus* is said to show a remarkable chromatic variation in

the appearance of its pigments, which are described as white, yellowish white, chrome yellow, orange, rust coloured, red, brownish red, blackish brown, and blue. These are not all separate pigments, but with some reservation KOLLER combines them as follows: *a.* chrome yellow—rust coloured; *b.* red—blackish brown; *c.* white—yellowish white. He considers the first named colour of each group as representing only a dilute form of the second, and he even suggests the possibility that all colours of groups *a* and *b* represent one pigment only, while *c* is distinct from this. The diffuse blue pigment is different from all the others.

HANSTRÖM (1937) describes the occurrence of yellow and red pigments, besides the white and the blue. After extirpation of the eye-stalks, the yellow and the red pigments are maximally expanded, and the adaptation to the background disappears; the white pigment reacts to light as before the operation.

Similar results were obtained by CARSTAM (1942). He describes the red ("reddish brown") pigment as varying in appearance according to the degree of expansion; when maximally contracted, it looks almost black. The yellow pigment mostly occurs in the same chromatophores as the red one, sparsely in monochromatic yellow cells. The colour of the shrimps in the first stage of expansion is predominantly yellow, later the red pigment starts moving and then follows the same chromorhizae as the yellow. The same author also stresses the difference in the appearance of blinded shrimps and specimens adapted to a dark background. The former are reddish brown, all pigments are maximally expanded, the red forming a thin layer and thus appearing lighter red. The latter are darker, blackish brown, the yellow pigment is maximally expanded, while the reddish brown is only $^3/_4$ expanded and therefore appears darker. Experiments on the relation of time to degree of expansion lead CARSTAM to conclude that the yellow and the "brown" (red) pigments are controlled by two separate contracting hormones.

My investigations were not centered upon a study of the nature of the pigment; but my observations on a great number of living *Leander adspersus* mainly tend to confirm the view of CARSTAM, especially as to the difference in the reaction of the yellow and red pigments in normal and blinded animals. In a

few points, however, my opinion differs from his. First, I find the red pigment actually red, not "reddish brown" or even "brown". Secondly, I was at one time inclined to believe that besides the red, yellow, and white pigments—and the diffuse blue substance—a real black pigment existed in this species. It is true that the red pigment, when highly contracted, appears as an almost black lump in the center of the chromatophore, but there are also chromatophores which appear partly black in the expanded phase. Such blackish chromatophores are present on the ventral side of the eye-stalks; they are of large size and the pigment has a peculiar bluish black colour. By re-examination I found that the dilute pigment in the finer branches of the cells looks really blue, so that it may be a case of intracellular occurrence of the usual blue pigment. As to the blackish looking cells which often occur along the median dorsal line of the carapace and the abdomen (Plate III, fig. 5) these probably contain condensed red pigment.

As stated by CARSTAM, most of the chromatophores contain red and yellow pigments. A smaller number contains red and white pigments; it is remarkable that the white chromorhizae lie nearer to the cuticle than the others.

Of the newly caught shrimps, the bigger ones are often rather dark, brownish, the yellow pigment is highly, the red one only partly expanded. The smaller specimens are generally of a lighter, yellow colour. Some specimens are greyish or greenish grey; in these, the diffuse blue pigment occurs as circular areas round the chromatophores in which the red pigment is wholly contracted, while the yellow one is more or less expanded.

The blue pigment is often, but not always, observed when the red pigment contracts after injection of certain extracts (cf. below). It appears first on the antennae, especially on the inner edge of the two basal joints of the first antenna and on the inner border of the exopodite of the second antenna, and at the same time on the sides of the rostrum, which are parallel to the basal joints of the antennae. The uropods may begin to show the blue colour simultaneously, and in such individuals the rest of the body soon follows. Much individual variation occurs, in some specimens only parts of the antennae and the

mouth-parts turn faintly bluish, and in many shrimps no trace of blue colour is observed; in well-defined cases it is clearly seen that the blue pigment is formed as dots around the rapidly contracting red chromatophores. These observations are in accordance with the view of KEEBLE and GAMBLE that the blue substance is formed by the red pigment during contraction.

The shrimps were kept in a large tank with light quartz sand on the bottom, the temperature of the water being about 10° C. They showed a rapid adaptation to the light bottom, becoming conspicuously paler than they were at their arrival (Plate III, fig. 5). In preparation for the injection, the eyes of a number of shrimps (e. g. 100) were cut off by means of fine scissors; each eye was taken separately, generally on two consecutive days. In spite of this, the mortality following the amputation was considerable, often about 40 per cent. Possibly, the losses might have been reduced by more elaborate methods, but in view of saving time the simple technique also used by previous investigators was maintained. The individuals which survived the first one or two days after the last operation often lived for weeks in the tank.

On the day after the removal of the second eye, the shrimps had generally adopted a more or less marked red colour (Plate III, fig. 6). However, a considerable individual variation existed, as some specimens were only yellowish, others (the majority) lighter or darker red. In the latter, the red chromorhizae form a very delicate network all over the surface making the animal appear brilliantly red (Plate IV, fig. 8); as far as possible only such individuals were used for the injections.

The insects were dissected in the Zoological Laboratory of the Royal Veterinary and Agricultural College. The organs were placed on filter paper, labelled, dried for one or two days in an incubator at 60° C., and kept in a desiccator for later use. Mostly, organs from five individuals were placed on each paper (e. g. 5 brains or 10 corpora allata); in some cases, it was necessary to use a smaller number, rarely a greater one.

On the day fixed for the injection, the central part of the filter paper was cut out, and 1 cm^3 sea-water (from the aquarium) was added. Under the binocular the filter paper was divided into small bits and fibers and was left for about one

hour to extract. Then, the fluid with its content was transferred to a small test tube and was heated in a water bath to 100° C. After cooling it was filtered and was now ready for use. Generally, the fluid appeared quite clear and colourless, only extracts of whole heads, thoraces, and abdomina were more or less yellow or brown even after repeated filtration.

In most cases, the extracts were made a few hours before the injections took place, but, a few times, an extract was kept in a frigidaire till the next day, which did not involve any discernible reduction of the activity, while storing at room temperature resulted in a destruction of the active substance.

Before injection, the extract was cooled by keeping the filled syringe in cold water for a few minutes. The shrimps only stand the injection if the temperature of the fluid is about the same (not higher) as that of their own body, but when this is the case, they generally recover very rapidly and may live for a long time afterwards. Each individual received 0.1 cm^3 of the extract which was injected into the dorsal part of one of the first abdominal segments. The number of shrimps used for each experiment was generally 4—6, as the changes could not be followed exactly when a greater number was used. The effect of the treatment was controlled by regular inspection every 5 minutes; the general colour-tone and the shape of the chromatophores, as seen under the binocular, were noted. The observations were mostly confined to the red and "blackish" chromatophores which are easily observed. Already 2 minutes after injection, the most active extracts had caused a distinct fading of the colour, and after 10 minutes the shrimps were pale and transparent, simultaneously the pigments showed a maximum or submaximum contraction.

It is difficult to measure the effect in a really exact way. After some practice, however, I found it possible to characterize the momentary degree of contraction by such descriptive terms which allow us to compare the results of single experiments. For further simplification, each experiment was given a "mark" expressed as from one to three + or as ÷, (+) indicating a smaller effect than +. The whole scale thus comprises 7 degrees of effect, *viz.* +++, ++(+), ++, +(+), +, (+), and ÷.

It should be added that the lower plus degrees differ from

the higher ones not only in the stage of contraction reached but also in the slower reaction. (+) means a just discernible contraction of the pigment; + designates a definite, but faint contraction, the shrimp generally showing a yellowish colour, and so on.

In the following, a description of an experiment from my journal is given.

Experiment P 23. *Tachycines* 14. $^{21}/_{11}$ 1941.

Strength: 10 corpora cardiaca in 100 cm^3 sea-water.

16 h^{05}. 0.1 cm^3 of the extract is injected into each of 4 blinded (red) *Leander adspersus*.

16 h^{07}. Colour distinctly fading.—Red pigment showing beginning contraction (cf. Plate IV, fig. 9).

16 h^{10}. The biggest individual, originally dark red, is now greyish; its red pigment is distinctly contracted (as Plate IV, fig. 10). Three smaller specimens (originally somewhat lighter red) are now pale (whitish), their pigments are even more contracted, blue colour is beginning to appear.

16 h^{15}. Biggest individual pale (whitish); its pigment highly contracted (as Plate V, fig. 11). The three others are quite transparent; pigment showing submaximal contraction.

16 h^{20}. Biggest individual quite transparent; pigment submaximally contracted (as Plate V, fig. 12). Three others as before.

No further change. +++

V. Experiments.

The results of the experiments are summarized in tables 1—6.

1. *Tachycines asynamorus.*

As I had a rather abundant supply of this grasshopper, most of the experiments were made with organs of this species (cf. Table 1).

Extracts of entire heads (5 heads to 1 cm^3 sea-water) injected into blinded (red) *Leander adspersus* (0.1 cm^3 per individual) caused a rapid contraction of the red pigment, reaching

Table 1.

Tachycines asynamorus.

Organ	Strength (number of organs/cm³)	Number of shrimps inj.	Results	
Head (total)	5/1	10	+++	..
Thorax	5/2	6	+(+)	..
—	-	2	+	..
—	-	2	?	..
—	-	6	..	÷
Abdomen	5/2	6	+	..
—	-	5	(+)	..
—	-	4	?	..
—	-	3	..	÷
Brain	5/1	23	..	÷
—	4/1	3	..	÷
Suboesophageal ganglion	5/1	24	..	÷
Optic lobe	10/1	16	..	÷
—	9/1	6	..	÷
—	8/1	4	..	÷
Frontal ganglion	5/1	22	..	÷
—	-	2	?	..
Oesophageal connectives	10/1	7	..	÷
Corpus allatum	19/1	3	..	÷
—	10/1	29	..	÷
—	-	2	(+)	..
—	-	5	+	..
Corpus cardiacum ...	10/1	33	+++	..
— ...	10/10	4	+++	..
— ...	10/100	18	+++	..
— ...	10/1000	4	+++	..
— ...	-	6	++	..
— ...	-	8	+(+)	..
— ...	10/10,000	4	++	..
— ...	-	11	+	..
— ...	-	9	..	÷
— ...	10/100,000	4	+	..
— ...	-	2	(+)	..

Table 1 (continued).
Tachycines asynamorus.

Organ	Strength (number of organs/cm³)	Number of shrimps inj.	Results	
Corpus cardiacum ÷ G. hypocerebrale	10/1	9	+++	..
— ...	10/100	9	+++	..
— ...	-	2	++(+)	..
— ...	10/1000	2	+(+)	..
— ...	10/10,000	2	..	÷
Head ÷ C. card. ÷ C. allat. ÷ G. hypocerebr. ÷ Suboes. ggl.	5/1	2	++	..
—	-	2	+(+)	..
—	-	2	(+)	..
—	5/10	2	(+)	..
Ganglion hypocerebrale	5/1	7	+(+)	..

its maximum in about 10 minutes. This is in agreement with the results obtained by HANSTRÖM (1940 a) with head extracts of 7 other species of grasshoppers belonging to as many genera.

Extracts of thorax and abdomen[1] gave varying results, in some cases the effect being definitely positive, in other cases doubtful or even negative. The most probable explanation is that the active substance is carried with the blood from the head to other regions of the body, where it is found in varying concentration.

Extracts were then made of all organs of the head which might be imagined as possible sources of the chromatophorotropic substance.

Extracts of the brain, the suboesophageal ganglion, the optic lobe, and the frontal ganglion, when injected into red shrimps, showed no effect on the chromatophores. Only 2 out of 24 shrimps tested with extract of the frontal ganglion were dubious. As BROWN and EDERSTROM (1940) state

[1] It was necessary to use more fluid to extract these voluminous parts of the body (2 cm³ for every 5 individuals).

that the oesophageal connectives of *Crangon* produce a hormone which influences the colour-change of this shrimp causing an expansion of the black pigment in the telson and uropods, I have in a few experiments tried to apply an extract of the oesophageal connectives of *Tachycines*; injected into blinded *Leander adspersus* this substance was without any effect.

It was of special interest to test extracts of the corpus allatum, as this organ is known to be an incretory gland of great importance in the developmental physiology of insects, producing hormones which control the metamorphosis and the ovarian development. So, the corpus allatum might a priori be suspected as the possible cause of the chromatophorotropic effect of the head. However, 32 out of 39 test animals did not react at all to the injection of this extract, while 7 individuals showed a weak contraction of the red pigment. It is obvious, therefore, that the substance causing the very conspicuous effect following injection of extracts of entire heads cannot have its origin in the corpus allatum.

The results of all experiments hitherto mentioned contrast sharply with those obtained with the corpus cardiacum. An extract made of 10 corpora cardiaca (i.e. organs from 5 individuals) in 1 cm^3 sea-water was injected into blinded shrimps, each specimen as usual receiving 0.1 cm^3, corresponding to 1 organ. This extract was tested on 33 red shrimps which all showed a very rapid and complete contraction of the pigment, accompanied by a prompt colour-change from red to a transparent white (Plate III, fig. 7). Already 2 minutes after the injection, a definite fading was noticeable, and after about 10 minutes the colour-change was complete, the red, yellow, and "black" chromatophores being in a state of maximum contraction. At this stage, most of the chromatophores, when regarded with a weak objective, appeared as rounded dots, but a higher magnification revealed that many of them, especially the larger ones, actually were irregular, verrucous lumps (Plate V, fig. 13). During contraction some of the larger chromatophores, especially the large cells along the median line of the carapace, passed through a stage in which the pigment was condensed in the proximal parts of the chromorhizae, while nothing could be seen in the center of the cell; these stages presented a picture somewhat resembling the

metaphase of a mitosis (Plate V, fig. 12). In some specimens the diffuse blue pigment appeared to a varying extent (cf. p. 17). The changes following injection of this extract corresponded completely to those produced by extracts of the whole head.

It should, however, be noted that in these experiments no special care was taken to separate the corpora cardiaca from the ganglion hypocerebrale which is intimately connected with them (Text-fig. 1). The corpora cardiaca are plainly glandular, in dissections they are seen to be actually distended by fluid, while the ganglion does not show any histological or other signs of secretion; so it is very unlikely that the ganglion has anything to do with the effect on the crustacean chromatophores. Later, I succeeded in severing the ganglion from the corpora cardiaca, and it could be proved that the extract of the corpora cardiaca had still a very strong effect. In a single experiment with an extract of the isolated ganglion hypocerebrale (5 organs per 1 cm^3) a weak effect was found, but in accordance with other experiences (cf. below) I cannot consider this a proof of a secretion of the ganglion; I am inclined to regard it as due to admixture of a small quantity of the contents of the corpus cardiacum.

In some other experiments the suboesophageal ganglion, the corpora allata, the corpora cardiaca, and the ganglion hypocerebrale were removed, and an extract was made of the rest of the head. This extract had a varying but definitely positive effect. The organs remaining in the head were the brain, the optic lobes, and the frontal ganglion, which, when tried separately, had no effect on the chromatophores; apart from these, the head contained the oesophagus, muscles, tracheae, fat cells etc. which are not likely to be concerned with the effect. It is probable, therefore, that the response to this extract is due to secretion of the corpus cardiacum present in the blood or absorbed in the tissues of the head. Obviously, if this extract of "the rest of the head" would not show any effect on the chromatophores, the explanation given above (p. 22) of the positive reaction obtained with extracts of thorax and abdomen could hardly hold.

The above conclusion is supported by the fact that the extract of the corpora cardiaca remains active even after extreme

dilution. In Table 1, several experiments (4) are combined and arranged according to a decreasing concentration. The difference in the results of the single experiments may either be due to a variation—individual or temporary—in the production of the active substance by the corpora cardiaca, or to differences in the threshold value of the test specimens, or to technical circumstances.

Dilution to 1 : 100 of the original strength, i. e. 10 organs in 100 cm^3 water, showed no appreciable decrease in the effect. In some cases, even 1 : 1000 gave the same result, in other experiments, however, this strength showed a somewhat reduced activity. After dilution to 1 : 10,000 of the basic extract the fluid was in most cases still active, but on 9 test specimens no change could be observed. Even after diluting to 1 : 100,000 of the original strength there was in some cases a faint reaction, while in other cases no reaction could be observed.

The real dilution is, however, not correctly expressed by these figures, since already the basic extract represents a considerable dilution of the active substance. The size of a corpus cardiacum is about 0.25 mm × 0.37 mm × 0.12 mm (measured on sections); the volume is then at the most 0.01 mm^3. If the whole volume is considered to be an active secretion, the basic extract of 10 organs per 1 cm^3 represents a dilution of 1 : 10,000. This means that the figures should be multiplied by 10,000, so that the secretion of the corpus cardiacum should be active after a dilution of 1 : 100 millions or even 1 : 1000 millions. The sensitivity of the chromatophores to the active substance of the corpus cardiacum would thus be extremely high. In this connection it should be mentioned that the crustacean eye-stalk hormone is stated to be active after a dilution of at least 1 : 500,000 (quoted from Hanström 1939, p. 107). Krogh and Rehberg (*vide* Krogh 1930, p. 185), in perfusion experiments with frogs, found that 1 part pituitrine to 50,000—1 million parts perfusion fluid was still able to maintain the tonus of the capillaries.

2. *Dixippus morosus.*

The stick-insect *Dixippus morosus* has been the object of several investigations by Pflugfelder, who produced a considerable amount of evidence regarding the function of the cor-

Table 2.
Dixippus morosus.

Organ	Strength (number of organs/cm³)	Number of shrimps inj.	Results	
Head (total)	5/1	10	+++	..
Thorax	5/2	10	++	..
Abdomen	5/2	10	+	..
Brain	6/1	8	..	÷
—	5/1	4	..	÷
—	-	9	++(+)	..
—	4/1	6	+	..
Suboesophageal ganglion	5/1	12	..	÷
—	4/1	6	?	..
Optic lobe	12/1	7	..	÷
—	9/1	6	+	..
—	8/1	12	..	÷
Frontal ganglion	5/1	10	+	..
—	-	10	..	÷
Corpus allatum	10/1	14	+	..
—	-	6	++	..
—	10/10	4	+	..
—	10/100	4	..	÷
Corpus cardiacum	10/1	11	+++	..
—	10/2	3	+++	..
—	10/4	3	+++	..
—	10/8	4	+++	..
—	10/16	3	+++	..
—	10/64	3	+++	..
—	10/640	3	++	..
—	10/6,400	3	+	..

pora allata. The physiology of the corpora cardiaca is completely unknown.

As already found by HANSTRÖM, extracts of the whole head of this species cause a rapid and complete contraction of the red pigment of blinded *Leander adspersus* (Table 2). A somewhat

weaker effect is produced by extract of the thorax, and a still weaker one by the abdomen.

Extracts of the isolated organs of the head gave somewhat surprising results. As in the case of *Tachycines*, extracts of the corpora cardiaca were very active and in no way inferior to the entire head. When diluted, the extract retained its activity down to a dilution of 1 : 6,400 of the basic extract. However, at least in some experiments, extracts of the other organs also gave positive results, i. e. they caused a more or less pronounced contraction of the pigment. Thus, brain extract was negative in 12 cases, but in 15 cases it caused a distinct contraction. The optic lobe and the frontal ganglion showed a similar effect, while extract of the suboesophageal ganglion had no or at the most a very doubtful effect. On the other hand, extract of the corpus allatum always caused contraction of the chromatophores, though the effect disappeared when the fluid was diluted to 1 : 100 of the original strength.

These observations claim an explanation. One might infer that the above-mentioned organs really produce a substance similar in effect to the hormone of the corpus cardiacum. In the case of the corpus allatum this might be true, but the very irregular results obtained with the four other organs hardly warrant such a conclusion. It seems much more likely that the effect is due to admixture of some secretion of the corpus cardiacum. As already mentioned above (cf. p. 11 and Plate II, fig. 4), it is hardly possible to remove the corpus allatum from the corpus cardiacum without injuring the latter, and this involves that the secretion flows out among the organs still present in the head under dissection, i. e. the brain, the optic lobe, and the frontal ganglion, so that these organs + the corpus allatum may happen to contain traces of the active hormone, even if they are washed in sea-water before being dried. Of the organs in question, only the suboesophageal ganglion is removed before the corpus allatum and the corpus cardiacum are dissected, and the fact that the extract of this ganglion has practically no effect seems to support the hypothesis given above. It is further in agreement with the experience that the extract of the corpus cardiacum retains its effect even when highly diluted.

3. *Blatta orientalis.*

The experiments with the cockroach organs (Table 3) give a simpler and clearer picture than did the preceding ones. The extracts of the brain, the suboesophageal ganglion, and the optic lobe had no effect on the chromatophores, while extract of the

Table 3.
Blatta orientalis.

Organ	Strength (number of organs/cm³)	Number of shrimps inj.	Results	
Head (total)	5/1	10	+++	..
Brain	5/1	8	..	÷
Suboesophageal ganglion	7/1	4	..	÷
—	5/1	10	..	÷
Optic lobe	6/1	4	..	÷
Corpus allatum	16/1	4	..	÷
—	10/1	4	..	÷
—	-	6	+	..
Corpus cardiacum	16/1	5	+++	..
—	16/2	3	+++	..
—	16/10	3	+(+)	..
—	10/1	6	+++	..
—	-	4	++(+)	..
—	10/2	2	+(+)	..
—	10/10	4	(+)	..

corpus allatum was negative in 8 cases, and in 6 others caused a slight contraction of the pigment.

The extract of the corpus cardiacum induced a rapid and complete discoloration of the injected shrimps. This only applies to the basic strength, as a dilution to 10/10 already reduces the effect to a just observable reaction. Thus, the hormone of *Blatta* is either much less effective than that of *Dixippus* and *Tachycines*, or it is produced in a smaller quantity.

4. *Gryllus domesticus.*

Extracts of the corpus cardiacum of the cricket showed a surprisingly small effect on the pigment of *Leander* (Table 4); in one experiment even no change of the chromatophores or

Table 4.
Gryllus domesticus.

Organ	Strength (number of organs/cm³)	Number of shrimps inj.	Results	
Head (total)	5/1	10	(+)	..
Brain	5/1	6	..	÷
Suboesophageal ganglion	5/1	4	..	÷
Corpus allatum + appendix	20/1	6	+	..
—	10/1	7	..	÷
Corpus allatum ÷ appendix	10/1	4	..	÷
Appendix	10/1	4	..	÷
Corpus cardiacum ...	20/1	6	+	..
— ...	10/1	5	+	..
— ...	10/1	4	..	÷

of the colour was obtained, this result being unique among the species examined. It should be noticed that the extract of entire heads had also a small effect only.

5. *Dytiscus marginalis.*

The few experiments performed with this representative of the *Coleoptera* (Table 5) led to results which are apparently contradictory to those hitherto regarded, since the extract of the corpus allatum had almost the same effect on the chromatophores as the corpus cardiacum. This might indicate that the corpus allatum of this beetle contains a pigment-activating hormone; however, this is not the only possible explanation, as

Table 5.
Dytiscus marginalis.

Organ	Strength (number of organs/cm³)	Number of shrimps inj.	Results	
Brain.....	5/1	2	..	÷
—	-	4	?	..
—	-	2	(+)	..
—	3/1	6	(+)	..
Suboesophageal ganglion.....	5/1	13	..	÷
Corpus allatum.....	10/1	8	+++	..
—	-	1	++	..
—	10/10	2	++	..
—	10/100	4	+	..
—	10/1000	2	..	÷
Corpus cardiacum...	10/1	16	+++	..
— ...	10/100	2	++	..
— ...	10/1000	3	+	..
— ...	10/10,000	2	..	÷

in this species—just as in *Dixippus*—the two organs are so intimately connected that I have not been able to remove the corpus allatum without injuring the corpus cardiacum. The positive result of the corpus allatum may therefore be due to an admixture of the hormone of the corpus cardiacum.

The extract of the corpus allatum retained its activity after dilution to 1 : 100 of the original strength, while the extract of the corpus cardiacum was still active when diluted to 1 : 1000; however, these facts are only based on a single experiment, as only few beetles were available.

6. *Calliphora erythrocephala.*

Finally, I have made some experiments with organs of blowflies. I owe thanks to Dr. Ellen Thomsen for dissecting the flies and excising the organs. In *Calliphora* the corpus allatum and the corpus cardiacum are unpaired and extremely small; in mature females the corpus allatum measures only about 80 × 100 × 80 µ (E. Thomsen 1942). For this reason it was deemed

Table 6.
Calliphora erythrocephala.

Organ	Strength (number of organs/cm³)	Number of shrimps inj.	Results	
Corpus allatum.....	20/1	20	..	÷
—	-	4	?	..
Corpus cardiacum...	20/1	23	++	..
— ...	-	2	+	..
Head.....	20/1	8	+(+)	..
—	-	15	+	..

necessary to use 20 organs (corresponding to 20 flies) to 1 cm³ of sea-water, each shrimp as usual receiving 0.1 cm³ of the extract, but even then the concentration is weaker than that used as basic strength in the other experiments.

Table 6 shows that the extract of the corpus allatum injected into 20 shrimps had no effect at all, while 4 cases were dubious. On the other hand, the extract of the corpus cardiacum as usual caused a rapid colour-change; the shrimps became pale, and the pigment contracted, though not quite to the maximum degree shown in Plate V, fig. 13.

These results agree well with the general trend of the previous experiments. On the other hand it seems surprising that entire heads also gave positive results, in spite of the fact that they do not contain the corpus cardiacum. In *Calliphora* and other flies, both this organ and the corpus allatum lie further back, in the foremost part of the thorax (E. Thomsen 1942). I have not attempted to investigate whether any single organ really present in the head (e. g. the brain) causes a similar effect, but the experiments with *Tachycines* previously reviewed make it probable that the positive reaction after injection of extract of the entire head is caused by the hormone of the corpus cardiacum present in the blood and tissues of the head.

These results differ somewhat from those obtained by Hanström (1940 a) with the same species. In his experiments, extract of the corpus cardiacum (14/1) injected into blinded *Leander adspersus* brought about a conspicuous reaction "bis

zur beinahe Maximalkontraktion der Mehrzahl der Chromatophoren". Extract of the corpus allatum (same strength) in 3 injected specimens induced a weaker positive response, while the reaction of 2 others was doubtful. Finally, extract of entire heads (14/1) in 5 treated shrimps had no effect on the chromatophores.

VI. Discussion.

The experiments recorded in this paper prove that the colour-change which is seen in *Leander adspersus* after injection of extracts from entire heads of certain insects is primarily induced by a substance present in the corpus cardiacum. In five species of insects, extracts of the isolated corpora cardiaca caused a contraction of the red, yellow, and "black" pigments, which, both as to the rate of the process and to the final stage reached, did not differ from that obtained by injection of extracts of the entire head. In four of the species, the effect must be described as a very conspicuous and rapid one, leading to a maximum contraction of the red pigment in the course of about 10 minutes. Only in *Gryllus*, the contraction stopped at a rather early stage, but it is very significant that in this species also the extract of the entire head had only a small effect, so that in all species there is full accordance between the corpus cardiacum and the head as a whole with regard to their pigment-activating effect.

Also the sixth species studied, *Calliphora erythrocephala*, agreed with the others, extract of the corpus cardiacum causing a corresponding contraction of the pigment of *Leander*. In this species only, the effect of the corpus cardiacum cannot be compared with that of the whole head, since the corpus cardiacum is not situated in the head, but in the thorax. As it was found that head extract of *Calliphora* nevertheless induced contraction of the pigment, some experiments were performed with extracts of *Tachycines* heads, from which the corpora cardiaca and the corpora allata had been removed (p. 24). These extracts also gave a positive effect, though much weaker than that produced by extracts of the corpora cardiaca of the same species. The only possible explanation seems to be that secretion of the corpus cardiacum, present in the blood and tissues of the head, is the

cause of the positive reaction of the head without the corpora cardiaca and allata, both in the case of *Tachycines* and *Calliphora*. If this holds, it is possible to explain the weak positive response sometimes obtained with extracts of the thorax and the abdomen of *Tachycines* in a similar way.

The experiments with *Tachycines* gave an unequivocal result: extracts of various other organs of the head did not produce any change of the chromatophores; only in a few cases did extract of the corpora allata cause a slight contraction. Hence, there can be no doubt that in this species the pigment-activating effect of the entire head is due solely to the corpora cardiaca.

In *Blatta*, the results are equally clear, as the corpora cardiaca in all experiments had a positive effect, while other organs (with the exception of a single experiment) were negative. Almost the same applies to *Gryllus*, but with *Dixippus* and *Dytiscus* the results were more ambiguous, as not only extracts of the corpora cardiaca but also those of other organs, notably the brain and the corpora allata, sometimes induced a positive response of the chromatophores. As to the brain, the results were irregular, sometimes negative, in other experiments positive but varying in degree; so this case can most likely be explained in the same way as the results with "the rest of the head" of *Tachycines* (cf. above). Extracts of the corpora allata, however, in all experiments with *Dixippus* and *Dytiscus* gave a positive result (down to a certain dilution). It is possible, therefore, that these organs really produce (or contain) a substance similar in its effect to that produced by the corpora cardiaca, but this conclusion is not cogent. It should be remembered that, among the types investigated, only in *Dixippus* and *Dytiscus* it was impossible to sever the corpora cardiaca from the corpora allata without injuring the former or both organs. Presumably, the extirpated corpora allata were more or less smeared with the secretion of the corpora cardiaca, or even tiny bits of the latter in some cases adhered to the corpora allata.

The probability that such admixture may suffice to involve a positive effect of the whole extract is supported by the experiments with diluted extracts of the corpus cardiacum, which show that even highly diluted fluids may retain their positive effect. In the case of *Dixippus*, it is significant that extract of

the corpus allatum loses its pigment-concentrating power already at a dilution of 10/100 (10 organs in 100 cm^3), while the corpus cardiacum is active even at a dilution of 10/6,400. These facts involve that the content of active substance in the basic extract of the corpus cardiacum must be at least 600 times as great as in that of the corpus allatum. This is in good agreement with the hypothesis given above, which, after all, I consider the most likely one. In corresponding experiments with *Dytiscus* the difference is not so pronounced.—In the case of *Calliphora* the extract of the corpus allatum was mainly negative, while the corpus cardiacum had always a positive effect.

Thus, all evidence so far available points to the corpus cardiacum as the source of the chromatophorotropic substance of the insect head. Regarding the nature of this substance, the following facts are at hand: It is resistant to boiling, soluble in water and—as shown by other investigators—in alcohol. It does not lose its activity after being kept dry for a long period; I have found pronounced effect of preparations which had been kept in a desiccator for 15 months. In the case of some species (as *Tachycines asynamorus*) the substance retains its activity even after extreme dilution. The activity is not specific, but extends to members of another systematic class. Finally, it may be noted that the corpus cardiacum shows the anatomical characteristics of a ductless gland, the secretion of which, according to DE LERMA, is discharged into the blood. These facts apparently justify to regard the active substance of the corpus cardiacum as a hormone. HANSTRÖM reached the same conclusion with regard to the active principle of the entire head.

As the hormone of the corpus cardiacum, when injected into blinded shrimps, influences the chromatophores in exactly the same way as the hormone of the sinus-gland, it seems likely that the two substances are chemically related, though they need not be identical.

Several authors (HANSTRÖM, E. THOMSEN, and others) have stressed the anatomical and physiological resemblance existing between the corpora allata of insects and the hypophysis of vertebrates. HANSTRÖM (1941) extended the analogy to cover also the corpora cardiaca which he compared with the neurohypophysis (pars posterior), while the corpora allata were com-

pared with the adenohypophysis (pars anterior); this comparison was based on the ontogeny and the anatomical relation of the two organs. As also emphasized by HANSTRÖM, the analogy should not be carried too far, but it is useful as far as the better known conditions of the vertebrates can guide us in the study of the invertebrates. In this respect, the experiments recorded in the present paper may be of some interest, the results showing a certain analogy to conditions in the vertebrates. The corpus cardiacum in insects produces a hormone which, at the time being, is only known through its action on the chromatophores of crustaceans. The hypophysis of the lower vertebrates forms one or more hormones which influence the movement of pigments in the chromatophores. In mammals, however, the pars intermedia of the hypophysis produces a hormone ("intermedin") which, when injected into frogs or fishes, induces quite similar changes of the chromatophores, while its rôle in the physiology of mammals is unknown. The analogy is not complete, since the pars intermedia is regarded as belonging to the adenohypophysis, whereas the corpus cardiacum—according to HANSTRÖM's interpretation—should be compared with the neurohypophysis. However that may be, the resemblance is rather striking.

It is evident that the part played by the corpus cardiacum in the physiology of insects themselves has not been elucidated by this investigation. It might be imagined that the colour-change of insects in some way should be regulated by the corpora cardiaca, though available evidence is not much in favour of this view. Even if this could be proved, the corpora cardiaca must have other additional functions, as they occur and are well-developed in all insects (except some apterygotes), while only a few insects are able to change their colour.

Summary.

1. It has previously been shown by HANSTRÖM that extracts of the head of various insects, when injected into blinded shrimps, cause a contraction of the pigment similar to that produced by the sinus-gland hormone. In the present paper a closer analysis of this phenomenon has been attempted.

2. The following species were used: *Tachycines asynamorus* Adel., *Gryllus domesticus* L., *Dixippus* (*Carausius*) *morosus* Br., *Blatta orientalis* L., *Dytiscus marginalis* L., and *Calliphora erythrocephala* Meig. Most of the investigations were made on the first named species.

3. The corpora allata, corpora cardiaca, and the stomatogastric nervous system of *Tachycines* and *Blatta* are described in some detail.

4. Extracts were made of several organs of the head, comprising the corpora cardiaca and allata. They were tested by injection into shrimps (*Leander adspersus*) which through amputation of the eye-stalks had been deprived of their own pigment-contracting hormone. Such individuals are red, owing to the maximum expansion of the predominant red pigment.

5. In all experiments, extracts of the corpora cardiaca induced a contraction of the pigment equivalent to that caused by the entire head. In the case of *Tachycines* even extremely dilute extracts still produced an observable effect, in the other species the activity disappeared at somewhat higher concentrations. The order was: *Tachycines, Dixippus, Dytiscus, Blatta, Calliphora, Gryllus.*

6. The positive results sometimes obtained with extracts of the brain, "the rest of the head", i. e. the head without corpora cardiaca and allata, the thorax, and the abdomen are most likely caused by secretion of the corpus cardiacum present in the blood and tissues. The positive effect of the corpora allata in *Dixippus* and *Dytiscus* is considered due to admixture of substance of the corpus cardiacum, owing to the difficulty of completely separating the two organs in these species.

7. The conclusion is drawn that the chromatophorotropic effect of extracts of entire insect heads is most probably solely due to a substance of the corpus cardiacum. This substance is regarded as a hormone.

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Addendum.

In a review by B. Scharrer (Physiol. Rev. 21,3, 1941) I have recently found a brief reference to a paper by Brown and Meglitsch (Biol. Bull. 79, 1940), at present inaccessible to me, in which the authors state an effect of corpora cardiaca, cerebral and frontal ganglia of insects on the chromatophores of crustaceans.

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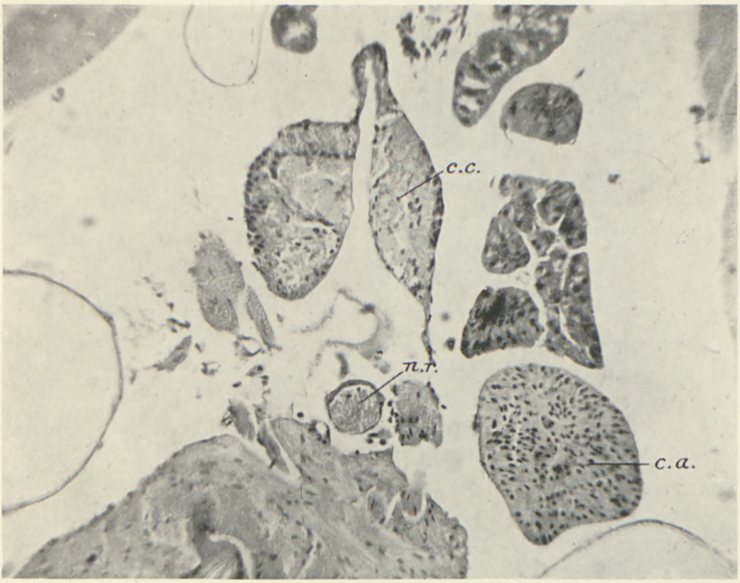

Fig. 1. *Tachycines asynamorus*. Transverse section of head. *c.a.*, corpus allatum; *c.c.*, corpus cardiacum; *n.r.*, nervus recurrens. 105 ×.

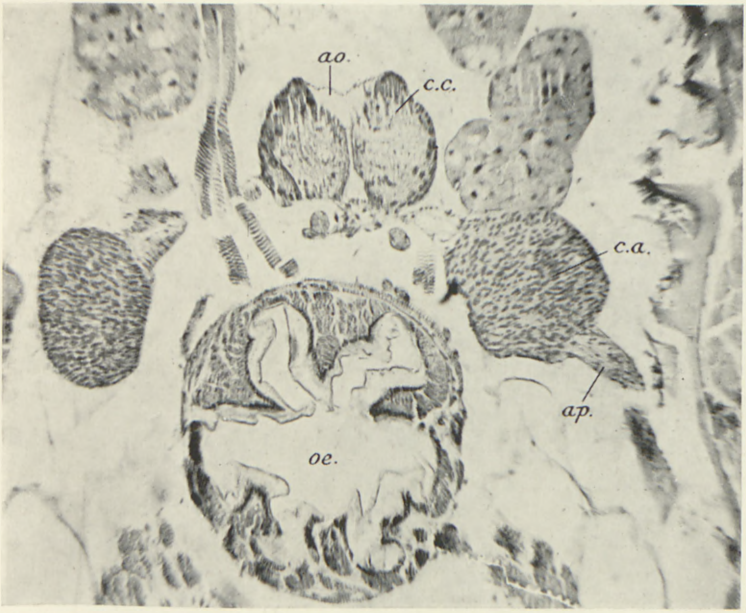

Fig. 2. *Gryllus domesticus*. Transverse section of head. *ao.*, aorta; *c.a.*, corpus allatum; on the right side the appendix (*ap.*) is partly visible; *c.c.*, corpus cardiacum; *oe.*, oesophagus. 105 ×.

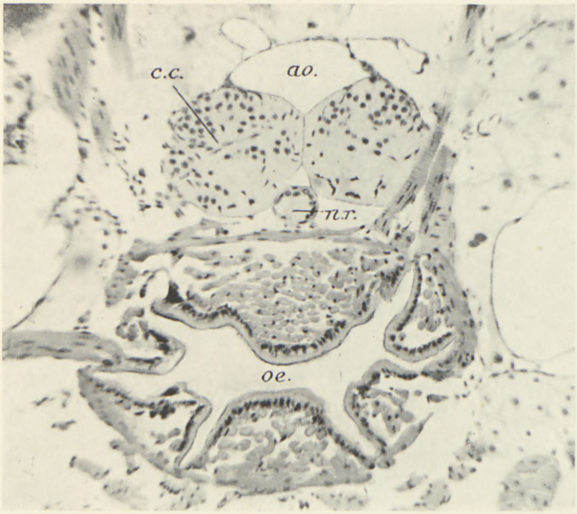

Fig. 3. *Blatta orientalis.* Transverse section of head. Letters as in preceding figures. 105×.

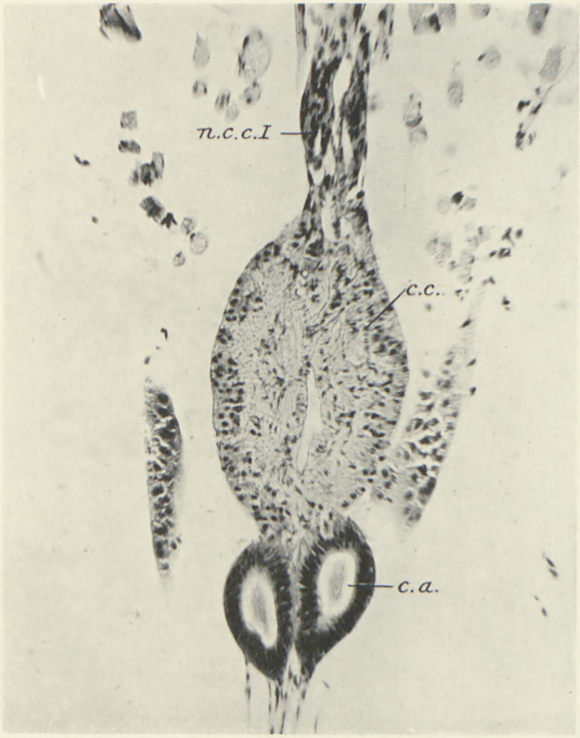

Fig. 4. *Dixippus morosus.* Horizontal section of head. *n.c.c.I*, nervus corporis cardiaci I. Note the intimate connection between the corpora cardiaca (*c.c.*) and the c. allata (*c.a.*) 105 ×.

Fig. 5. *Leander adspersus*. Living specimen from light sand bottom. $1^1/_2 \times$.

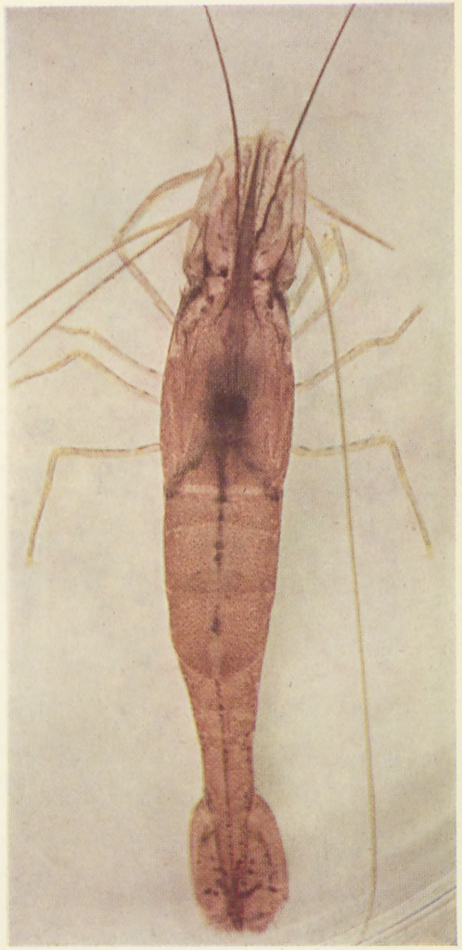

Fig. 6. Maximal expansion of pigment after amputation of eye-stalks; living specimen. $1^1/_2 \times$.

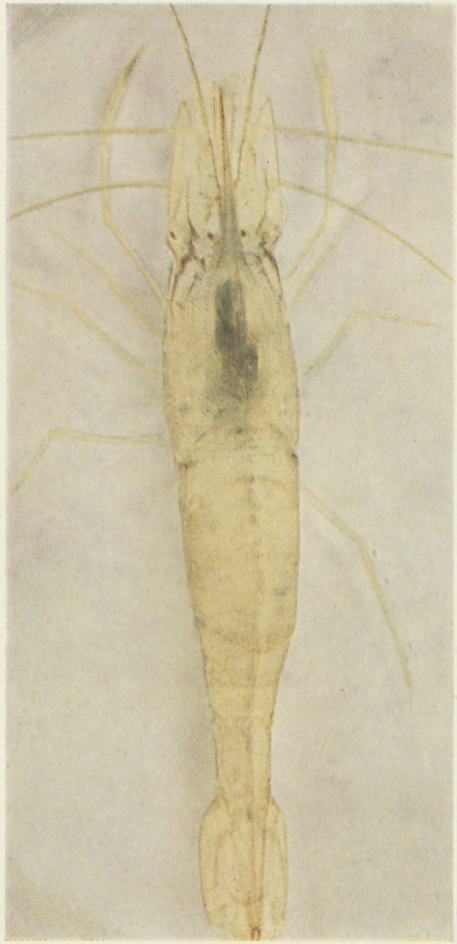

Fig. 7. Effect of injection of corpus cardiacum extract: complete contraction of pigment; living specimen. $1^1/_2 \times$.

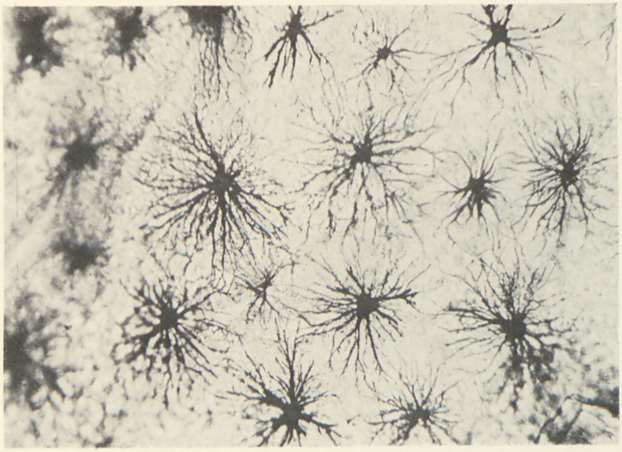

Fig. 8. *Leander adspersus*. Maximally expanded red chromatophores after amputation of eye-stalks. 75 ×.

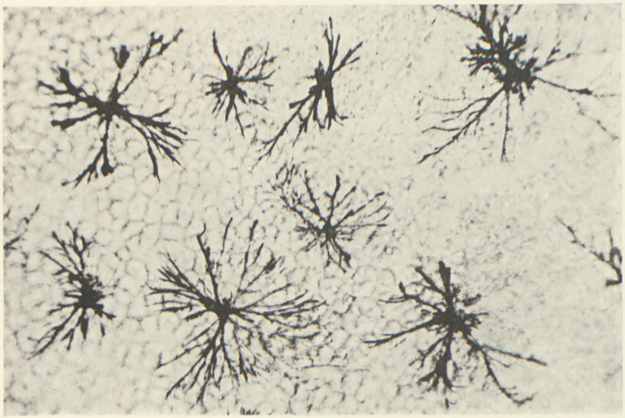

Fig. 9. Beginning contraction of red chromatophores. 75 ×.

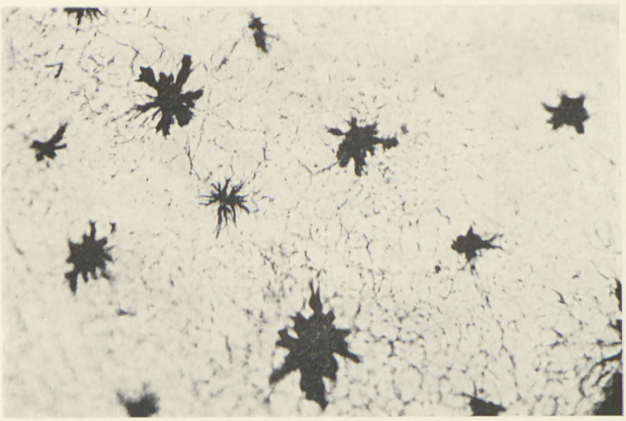

Fig. 10. Red chromatophores halfway contracted: early stage after injection of extract of corpus cardiacum of *Tachycines*. 75 ×.

Fig. 11. Proceeding contraction of red pigment; extract of corpus cardiacum of *Tachycines*. 75 ×.

Fig. 12. Chromatophores showing submaximal contraction of red pigment. Left: large chromatophore, in which the pigment is arranged in a figure resembling a mitotic metaphase. Extract of corpus cardiacum of *Tachycines*. 75 ×.

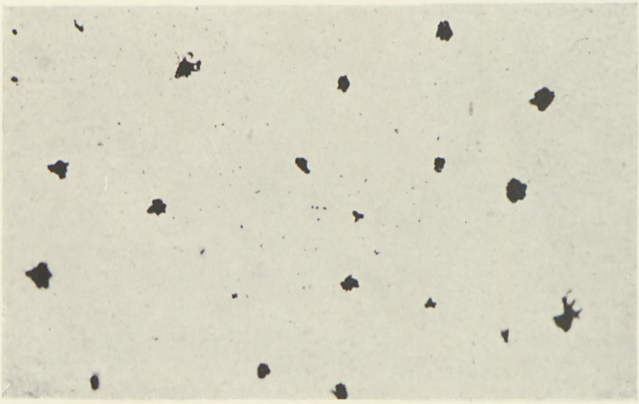

Fig. 13. Maximum contraction of red pigment: final stage after injection of extract of corpus cardiacum of *Tachycines*. 75 ×.

DET KGL. DANSKE VIDENSKABERNES SELSKAB
BIOLOGISKE MEDDELELSER, BIND XIX, NR. 5

CONTRIBUTIONS TO THE DISCUSSION OF THE AGGLUTINATION-INHIBITION METHOD

BY

JUL. HARTMANN.

KØBENHAVN
I KOMMISSION HOS EJNAR MUNKSGAARD
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INTRODUCTION

1. The Agglutination-Inhibition Method.

As is well known, the Agglutination-Inhibition Method for the measurement of the strength of a group-antigen is based on the following facts:

1° that the antibody contained in the serum from a person of the blood group *A* will cause blood corpuscles from a person of the blood group *B* to agglutinate and

2° that antigen from a person of the blood group *A* or any antigen of the same type will "fix" the serum antibody from a person of blood group *B*, thereby inhibiting more or less the agglutination of blood corpuscles of group *A* brought into touch with the serum antibody.

If in 1° and 2° *A* is replaced by *B* and *B* by *A* the statements thus formulated are likewise true. It may be noted that the serum from a person of blood group *B* is called anti-*A* because it reacts with the antigen from a person of blood group *A*. Similarly the serum antibody from a person of blood group *A* is called anti-*B*.

Now let the problem be to measure the strength of a group-antigen of a known blood group, say *A*. The antigen may be contained in an aqueous extraction from an organ of a person of this blood group or in a solution of a secretion from such a person. The agglutination inhibition method of measurement then generally takes the following shape. A series of small test tubes arranged in a stand is used. Into the first tube of the series 0.1 cm^3 of the original antigen extraction or solution is introduced; its concentration may be denoted by 1. Into the second tube 0.1 cm^3 of a solution of the concentration $^1/_2$ is inserted, into the third tube 0.1 cm^3 of a solution of the con-

centration $^1/_4 = {}^1/_2{}^2$ etc. Hence into the tube no. n 0.1 cm^3 of a solution of the concentration $^1/_2{}^{n-1}$ is introduced. Then 0.1 cm^3 of a serum anti-A is added to the contents of alle the tubes. Thus after this operation the antigen concentrations in the series of tubes are as $^1/_2$, $^1/_2{}^2$, $\cdots$ $^1/_2{}^n$. After the introduction of the serum the test tubes are kept at about 20° C for an hour in order that there may be time for the fixation of the serum antibody to take place. Then one drop of a 5 p.c. suspension in saline of washed blood corpuscles of the group A (A_1) is added to all the tubes. The tubes are again left to themselves for about 2 hours, when they are shaken and the effect of this shaking observed. It will generally be found that in all the tubes up to and including a certain number, no. n, there is no agglutination, while in tube no. $n+1$ and in all the following tubes agglutination has taken place. The explanation of this observation is fairly evident. In all the tubes up to tube no. n the concentrations of the antigen have been high enough to fix the serum antibody to such an extent that the remainder is unable to produce any appreciable agglutination. From tube no. $n+1$ and above, the fixation is no longer complete and so free antibody is present, causing agglutination of the blood corpuscles. Obviously the number n of the last tube in which there is still no agglutination will be the higher, the higher the concentration of the antigen in the original solution or extraction. So n may appropriately be taken as a measure for the strength or concentration of the antigen in question.

The series of tubes with their contents of antigen solution or extraction, serum and blood corpuscles may obviously be vizualised as a scale, the titer scale, on which the strength of the antigen extraction or solution in question is characterized by the number n of the last tube in which no agglutination is perceptible. Let $C_{A\cdot 0}$ be the concentration of the original extraction or solution of antigen, then in tube no. n the antigen concentration will be $\frac{C_{A\cdot 0}}{2^n}$. Let C_s be the concentration of the serum in the tubes and let $C_{s\cdot m}$ be the highest value of the serum concentration without any appreciable effect of agglutination, then the relation between the titer reading n and the antigen concentration $C_{A\cdot 0}$ in question may be written

(1) $$\frac{C_{A\cdot 0}}{2^n} = k\,(C_s - C_{s\cdot m})$$

expressing that in tube no. n all the serum short of the amount $C_{s\cdot m}$ has been fixed by the antigen. The factor k need not be a real constant but may depend on the concentrations of the antigen or of the serum antibody or of both. From (1) it follows that if two antigens of the same type give the readings n_1 and n_2 on two scales with the same serum then the concentration $C_{A\cdot 1.0}$ and $C_{A\cdot 2.0}$ of the antigens must satisfy the relation

(2) $$\frac{C_{A\cdot 1.0}}{C_{A\cdot 2.0}} = 2^{n_1 - n_2}$$

for in the two tubes no. n_1 and n_2 of the two titer scales the antigen concentrations as well as the serum concentrations are in this case the same, and so the factor k must have the same value.

2. The experimental Basis of the present Discussion.

The discussion which will be reported below is based on certain experiments to which the use of the agglutination inhibition method gave rise. When suitably treated these experiments

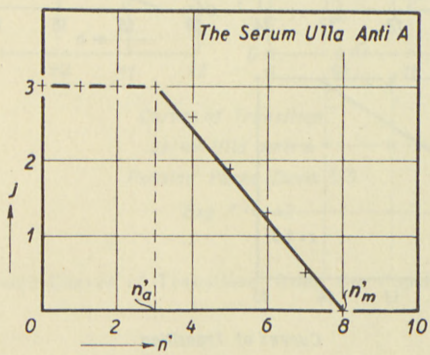

Fig. 1. Serum Titration Curve for Serum Ulla Anti *A*.

turn out to yield results of such a simple and characteristic nature that it occurred to the author that valuable information as to the process of fixation between antigen and serum antibody

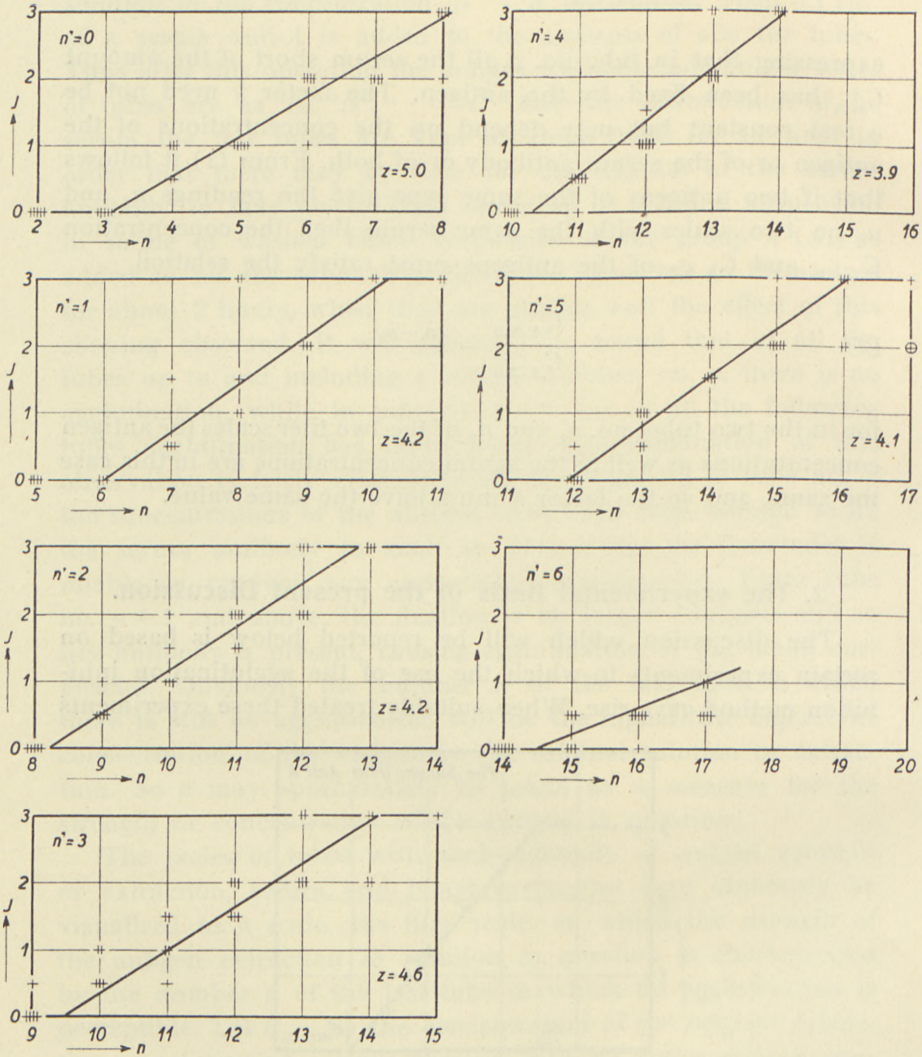

Fig. 2. Smoothed-out Curves of Transition from the Kaas-Ulla Experiment.

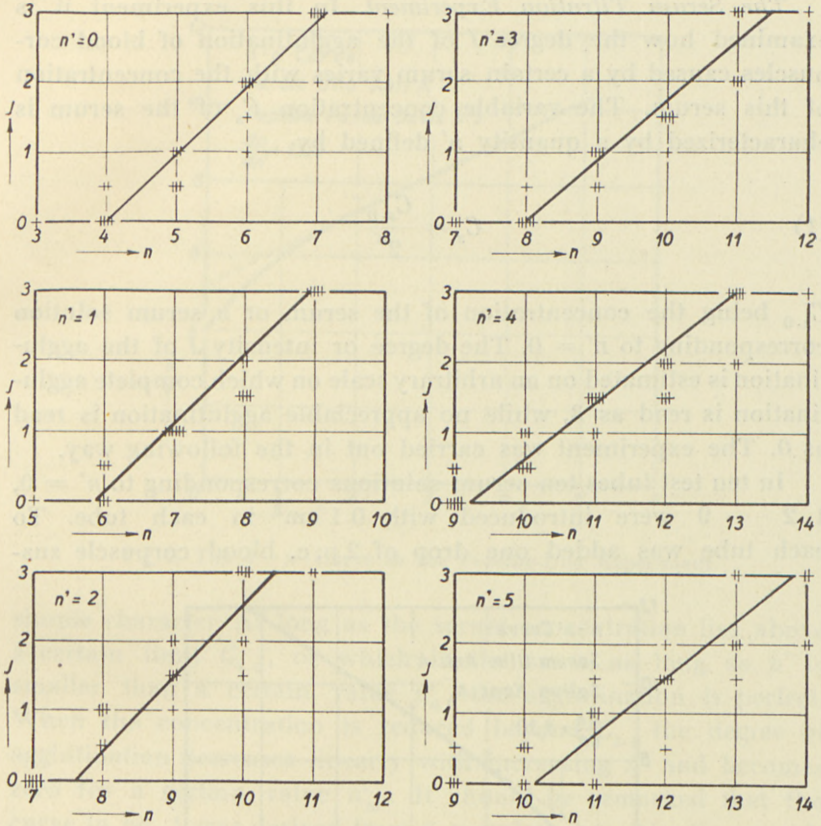

Fig. 3. Smoothed-out Curves of Transition from the Pepsine-Ulla Experiment.

might probably be derived from these results by mathematical analysis. The experiments referred to will now be considered.

The Serum Titration Experiment. In this experiment it is examined how the degree J of the agglutination of blood corpuscles caused by a certain serum varies with the concentration of this serum. The variable concentration C_s of the serum is characterized by a quantity n' defined by

$$C_s = \frac{C_{s.0}}{2^{n'}} \tag{1}$$

$C_{s.0}$ being the concentration of the serum or a serum solution corresponding to $n' = 0$. The degree or intensity J of the agglutination is estimated on an arbitrary scale on which complete agglutination is read as 3, while no appreciable agglutination is read as 0. The experiment was carried out in the following way.

In ten test tubes ten serum solutions corresponding to $n' = 0, 1, 2 \cdots 9$ were introduced with 0.1 cm^3 in each tube. To each tube was added one drop of 2 p. c. blood corpuscle sus-

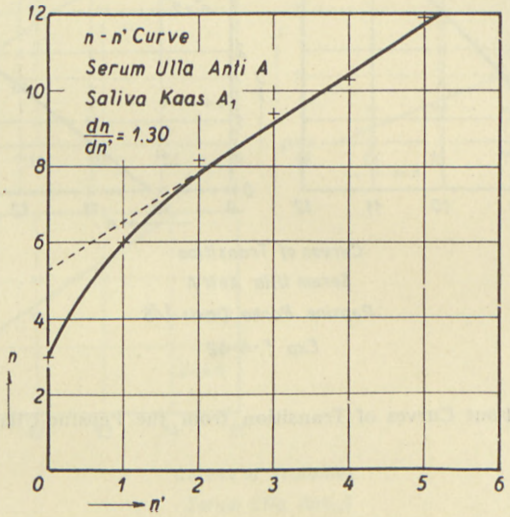

Fig. 4. The $n - n'$-Curve in the Kaas-Ulla Experiment.

pension of the type able to agglutinate under the influence of the serum. After two hours the intensity of the agglutination

was read. Fig. 1 shows the result of an experiment with a serum "Ulla Anti-*A*". It will be noted that the $J-n'$-curve is of a very

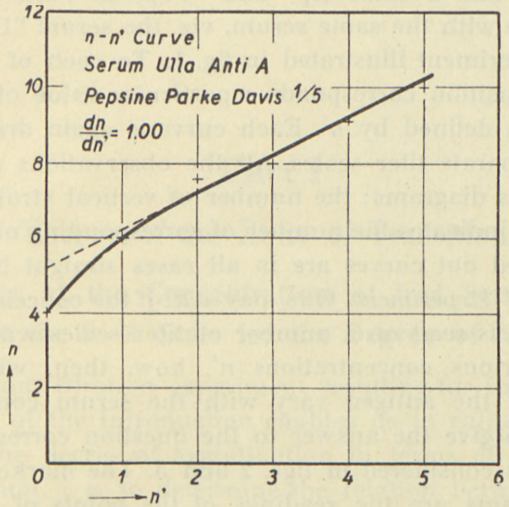

Fig. 5. The $n-n'$-Curve in the Pepsine-Ulla Experiment.

simple character. As long as the serum concentration lies above a certain limit $C_{s.a}$, or which is the same, as long as n' is smaller than a certain value n'_a, the agglutination is perfect. When the concentration is reduced beyond $C_{s.a}$ the degree of agglutination decreases linearly with increasing n' and becomes zero for a certain value n'_m. It should be remarked that the curve in fig. 1 was derived from 6 complete determinations, each point being thus the average of 6 independent observations.

The Zone of Transition. In the reading of the titer scale in the agglutination inhibition measurement one does not, as a rule, confine oneself to simply finding the last tube n in which no trace of agglutination is perceptible. The degree of agglutination in the following tubes $n+1, n+2, \cdots n+\Delta \cdots$ is made the subject of observation, the same scale for the degree of agglutination being used as in the case of the serum titration just described. In tube no. n the degree of agglutination is zero; in the following tubes the degree gradually rises, generally to complete agglutination, read as 3. The zone within which this rise takes place we shall call the zone of transition. Figs. 2 and 3

show a number of curves of transition originating from two comprehensive investigations carried out with two different antigens, viz. "Saliva Kaas A_1" and "Pepsine Parke Davis $^1/_5$", but otherwise with the same serum, viz. the serum "Ulla Anti-A" from the experiment illustrated in fig. 1. To each of the several curves of transition corresponds a particular value of the serum concentration defined by n'. Each curve is again drawn on the basis of 6 separate titer scales. All the observations are marked in the various diagrams; the number of vertical strokes in each of the points indicates the number of corresponding observations. The smoothed out curves are in all cases straight lines.

The $n - n'$-Experiment. One may ask: if the concentration of a given antigen is read on a number of titer scales with the same serum in various concentrations n', how, then, will the titer reading n of the antigen vary with the serum concentration? Figs. 4 and 5 give the answer to the question corresponding to the two cases considered in figs. 2 and 3. The marked ordinates of the diagrams are the readings of the points of intersection between the various curves of transition and the axes of abscissa in the diagrams of figs. 2 and 3. It will again be noted that the observations determine rather smooth and regular curves.

The experimental material recorded in figs. 1—5 forms the direct basis of the discussion that follows.

Part I

Theory of the Zone of Transition.

1. Variation of the Concentration of free Serum with the Reading on the 0—3 Scale for the Degree of Agglutination.

The serum titration experiment resulting for instance in the curve fig. 1 of the Introduction enables us to calibrate our 0—3 scale for the degree of agglutination in terms of concentration of free serum, i. e. to determine the relation between this concentration C_s and the reading J on the scale. Let fig. 6 represent the titration curve. Then the relation between J and the figure n' characterizing the concentration of the serum in the titration experiment (where the concentration of serum is identical with the concentration of free serum) is obviously

$$J = J_a \frac{n'_m - n'}{n'_m - n'_a}. \tag{1}$$

We introduce instead of the qualities n' the corresponding serum concentrations determined by

$$C_{s.a} = \frac{C_{s.0}}{2^{n'_a}}, \quad C_s = \frac{C_{s.0}}{2^{n'}}, \quad C_{s.m} = \frac{C_{s.0}}{2^{n'_m}} \tag{2—4}$$

from which

$$n'_a = \frac{1}{l_e 2} \cdot l_e\left(\frac{C_{s.0}}{C_{s.a}}\right), \; n' = \frac{1}{l_e 2} \cdot l_e\left(\frac{C_{s.0}}{C_s}\right), \; n'_m = \frac{1}{l_e 2} \cdot l_e\left(\frac{C_{s.0}}{C_{s.m}}\right). \tag{2a—4a}$$

If the latter expressions are introduced into (1) we get

$$J = J_a \frac{l_e\left(\frac{C_s}{C_{s.m}}\right)}{l_e\left(\frac{C_{s.a}}{C_{s.m}}\right)} \tag{5}$$

from which it follows that

$$\frac{C_s}{C_{s.m}} = \left(\frac{C_{s.a}}{C_{s.m}}\right)^{\frac{J}{J_a}}. \tag{6}$$

In the case of serum Ulla it appears from fig. 1 that $n'_a = 3$, $n'_m = 8$. Seeing that $\frac{C_{s.a}}{C_{s.m}} = 2^{n'_m - n'_a}$ and noting that J_a stands for the reading 3 on the 0—3 scale it follows that for this serum

$$\frac{C_s}{C_{s.m}} = 32^{\frac{J}{3}} = 2^{\frac{5}{3}J}. \tag{7}$$

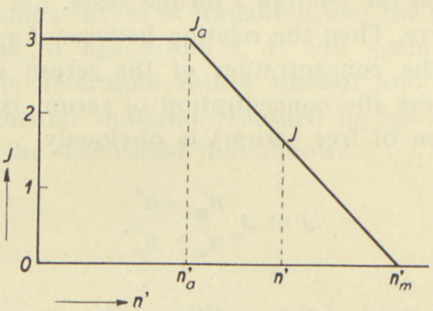

Fig. 6. The Serum Titration Curve (Diagram).

2. Variation of free and fixed Serum within the Zone of Transition.

Employing now our calibrated scale of agglutination we may be able to tell how the concentration of free serum varies within the zone of transition, seeing that we know how the degree of agglutination varies.

It appears from figs. 2 and 3 that the curves of transition may generally be considered as straight lines. If $n+\Delta$ is the

number of a tube within the zone we may appropriately take Δ as abscissa in the representation of the curve of transition when we may write the relation between J and Δ as

$$J = J_a \cdot \frac{\Delta}{\Delta_m}, \tag{1}$$

Δ_m being the width of the zone. It follows from (1) and from (6) paragraph 1 that the concentration of free serum C_s varies with Δ according to the expression

$$\frac{C_s}{C_{s \cdot m}} = \left(\frac{C_{s \cdot a}}{C_{s \cdot m}}\right)^{\frac{\Delta}{\Delta_m}}. \tag{2}$$

In the case of the Kaas-Ulla experiment, fig. 2, Δ_m is on an average found to be 4.6 titers and so $\frac{C_s}{C_{s \cdot m}} = 2^{\frac{5}{4.6}\Delta} = 2^{1.09\Delta}$.

The concentration of fixed serum $C_{s \cdot f}$ is hereafter readily derived from the concentration of free serum C_s and from the (total) concentration of serum used in the setting up of the titer scale. It has been suggested that for the latter concentration the value $C_{s \cdot a} = \frac{C_{s \cdot 0}}{2^{n'_a}}$, comp. fig. 1[1], should be adopted. Assuming this choice the concentration of fixed serum is determined by

$$C_{s \cdot f} = C_{s \cdot a} - C_s \tag{3}$$

or by

$$\frac{C_{s \cdot f}}{C_{s \cdot m}} = \frac{C_{s \cdot a}}{C_{s \cdot m}} - \frac{C_s}{C_{s \cdot m}} \tag{3a}$$

and so by virtue of (2)

$$\frac{C_{s \cdot f}}{C_{s \cdot m}} = \frac{C_{s \cdot a}}{C_{s \cdot m}}\left[1 - \left(\frac{C_{s \cdot a}}{C_{s \cdot m}}\right)^{\frac{\Delta}{\Delta_m}-1}\right]. \tag{4}$$

[1] It should here be noted that if the concentration $C_{s \cdot a}$ is aimed at in the production of the titer scale, a serum of concentration $n'_a - 1$ should be used, seeing that the concentration of serum is reduced to half its former value by being added to the antigen solution in the titer scale.

Now, this expression is derived on the assumption that the concentration of the serum in the titer scale (before fixation) is just $C_{s.a}$ (corresponding to n'_a in fig. 1). If the concentration is $C_{s.n'}$ corresponding to n', where $n' < n'_a$, the formula is slightly modified. In that case

$$\frac{C_{s.f}}{C_{s.m}} = \frac{C_{s.n'}}{C_{s.m}} - \frac{C_s}{C_{s.m}} \tag{3b}$$

leading to

$$\frac{C_{s.f}}{C_{s.m}} = \frac{C_{s.n'}}{C_{s.m}} \left[1 - \frac{C_{s.a}}{C_{s.n'}} \cdot \left(\frac{C_{s.a}}{C_{s.m}} \right)^{\frac{\Delta}{\Delta_m} - 1} \right] \tag{4a}$$

where it is assumed that the curve of transition is still a straight line and where Δ_m is the width of the zone, which may or may not be equal to that of the zone with the serum concentration $C_{s.a}$.

3. Relative Variation of fixed Antigen within the Zone of Transition.

We will now derive a formula for the relative variation of the fixed antigen within the zone of transition. We shall write the concentration of fixed antigen in tube no. $n + \Delta$ under the form

$$C_{A.f} = \frac{1}{k_\Delta} \cdot \frac{C_{A.0}}{2^{n+\Delta}} \tag{1}$$

thus a certain fraction $\frac{1}{k_\Delta}$ of the antigen present in the said tube. Most likely $\frac{1}{k_\Delta}$ is a function of Δ, i. e. of the concentration of the antigen. In deriving the function we shall make the sole assumption that the antigen will fix serum in a definite ratio, i. e. that the amount of antigen required to fix a certain amount of serum is proportional to the latter amount. This assumption is expressed in the equation

$$C_{A.f} = c \cdot C_{s.f} \tag{2}$$

where c is a constant. Introducing the value for $C_{s\cdot f}$ derived from (4) in the preceding paragraph we find

$$(3) \qquad C_{A\cdot f} = cC_{s\cdot a}\left[1-\left(\frac{C_{s\cdot a}}{C_{s\cdot m}}\right)^{\frac{\Delta}{\Delta_m}-1}\right]$$

and finally from (3) and (1)

$$(4) \qquad \frac{1}{k_\Delta} = \frac{cC_{s\cdot a}}{C_{A\cdot 0}}\cdot 2^n\left[1-\left(\frac{C_{s\cdot a}}{C_{s\cdot m}}\right)^{\frac{\Delta}{\Delta_m}-1}\right]\cdot 2^{\Delta}.$$

The factor preceding the brackets is with a given titer scale a constant, and a formula for the relative variation of $\frac{1}{k_\Delta}$ with Δ may thus be written

$$(5) \qquad \frac{1}{k_\Delta} = \left[1-\left(\frac{C_{s\cdot a}}{C_{s\cdot m}}\right)^{\frac{\Delta}{\Delta_m}-1}\right]\cdot 2^{\Delta}.$$

Here it is supposed that the concentration of the serum in the titer scale is $C_{s\cdot a}$ corresponding to n'_a in fig. 1. If the concentration is $C_{s\cdot n'}$ corresponding to $n' < n'_a$, then the expression (4a) of paragraph 2 for $C_{s\cdot f}$ should be introduced into (2), when we get instead of (5)

$$(5\,a) \qquad \frac{1}{k_\Delta} = \left[1-\frac{C_{s\cdot a}}{C_{s\cdot n'}}\cdot\left(\frac{C_{s\cdot a}}{C_{s\cdot m}}\right)^{\frac{\Delta}{\Delta_m}-1}\right]\cdot 2^{\Delta}.$$

4. The Question of a Law of Mass-Action for the Fixation within the Zone of Transition.

Let us assume that one molecule of antigen unites with one molecule of the antibody to form one single molecule of the combination product. Then we might presumably expect a law of mass-action of the form

$$(1) \qquad \frac{C_s C_A}{C_{A\cdot f}} = K \quad \text{(constant)}$$

C_s being the concentration of free serum, C_A the concentration of free antigen, and $C_{A \cdot f}$ the concentration of fixed antigen (equal or proportional to the concentration of fixed serum and to the concentration of the combination product). Introducing for the concentrations the following expression

$$C_s = \left(\frac{C_{s \cdot a}}{C_{s \cdot m}}\right)^{\frac{\Delta}{\Delta_m}} \cdot C_{s \cdot m} \tag{2}$$

$$C_{A \cdot f} = \frac{1}{k_\Delta} \frac{C_{A \cdot 0}}{2^{n+\Delta}} \tag{3}$$

$$C_A = \frac{C_{A \cdot 0}}{2^{n+\Delta}} \left(1 - \frac{1}{k_\Delta}\right) \tag{4}$$

we may rewrite (1) in the shape

$$K = \frac{C_s C_A}{C_{A \cdot f}} = \frac{1 - \frac{1}{k_\Delta}}{\frac{1}{k_\Delta}} \cdot \left(\frac{C_{s \cdot a}}{C_{s \cdot m}}\right)^{\frac{\Delta}{\Delta_m}} \cdot C_{s \cdot m}. \tag{5}$$

Now, obviously, $\frac{1}{k_\Delta}$ here stands for the absolute value of the fraction of antigen fixed by the serum. Our theory, as expressed by the formulae (5) and (5a) of paragraph 3, yields relative values only. If the figures derived from the formulae are all divided by the value of $\frac{1}{k_\Delta}$ for $\Delta = 0$, i. e. if the formulae (5) and (5a) of the said paragraph are transcribed to

$$\frac{1}{k_{\Delta \cdot 1}} = \frac{1 - \left(\frac{C_{s \cdot a}}{C_{s \cdot m}}\right)^{\frac{\Delta}{\Delta_m} - 1}}{1 - \left(\frac{C_{s \cdot a}}{C_{s \cdot m}}\right)^{-1}} \cdot 2^\Delta \tag{6}$$

and

$$\frac{1}{k_{\Delta\cdot 1}} = \frac{1-\frac{C_{s\cdot a}}{C_{s\cdot n'}}\left(\frac{C_{s\cdot a}}{C_{s\cdot m}}\right)^{\frac{\Delta}{\Delta_m}-1}}{1-\frac{C_{s\cdot a}}{C_{s\cdot n'}}\left(\frac{C_{s\cdot a}}{C_{s\cdot m}}\right)^{-1}}\cdot 2^{\Delta} \tag{6a}$$

we arrive at relative values of $\frac{1}{k_\Delta}$ defined by $\frac{1}{k_\Delta}$ being 1 for $\Delta = 0$. We shall make these values our starting point in the following test and denote the absolute value of $\frac{1}{k_\Delta}$ to be introduced into (5) by $a \cdot \frac{1}{k_{\Delta\cdot 1}}$, where a is a constant so far unknown. What we can now do is to try whether the quantity $K = \frac{C_s C_A}{C_{A\cdot f}}$ may assume a constant value with a suitably chosen value of a.

If there be such a value it should satisfy the equation

$$\frac{dK}{d\Delta} = 0 \quad \text{for} \quad \Delta = 0. \tag{7}$$

Conversely we may try whether the value of a derived from this relation will make K a constant within the zone of transition or a greater or smaller part of that zone.

Confining ourselves to the case $C_{s\cdot n'} = C_{s\cdot a}$ we may for the sake of simplicity write (6) as

$$\frac{1}{k_{\Delta\cdot 1}} = \frac{1-z^{\frac{\Delta}{\Delta_m}-1}}{1-\frac{1}{z}}\cdot 2^{\Delta} \tag{8}$$

where $z = \frac{C_{s\cdot a}}{C_{s\cdot m}}$. Introducing $a \cdot \frac{1}{k_{\Delta\cdot 1}}$ for $\frac{1}{k_\Delta}$ in (5) we get

$$K = \frac{z-1-a\left(z-z^{\frac{\Delta}{\Delta_m}}\right)\cdot 2^{\Delta}}{a\left(z-z^{\frac{\Delta}{\Delta_m}}\right)\cdot 2^{\Delta}}\cdot z^{\frac{\Delta}{\Delta_m}}. \tag{9}$$

Differentiating K with regard to Δ and putting $\frac{dK}{d\Delta} = 0$ for $\Delta = 0$ we find, after a series of rather tedious transcriptions, the following expression for a

$$a = \frac{z}{z-1} - \frac{l_e 2}{l_e z} \cdot \Delta_m. \tag{10}$$

Generally z is large compared to 1 and thus, approximately, we may write

$$a = 1 - \frac{l_e 2}{l_e z} \cdot \Delta_m. \tag{10a}$$

The latter formula is also found if an approximate expression is derived for K on the assumption of small values of Δ. On this assumption and assuming that z is large compared to 1 (9) may be replaced by

$$K = \frac{1 - a \cdot 2^{\Delta}}{a \cdot 2^{\Delta}} \cdot z^{\frac{\Delta}{\Delta_m}}. \tag{9a}$$

If K is here differentiated with regard to Δ and if $\frac{dk}{d\Delta}$ is equalised with zero for $\Delta = 0$ we can immediately write down the expression for a in (10a). Again it may be noted that the value of K corresponding to $\Delta = 0$ is found to be

$$K = \frac{1-a}{a} \tag{11}$$

both from (10) and from the approximate expression (10a). Now our deduction is based on the formula (6) for $\frac{1}{k_{\Delta \cdot 1}}$, i. e. a formula applying only to the case where the serum concentration in the tubes of the titer scale is just identical with $C_{s \cdot a}$. If the said concentration is larger, viz. $C_{s \cdot n'}$ $(n' < n'_a)$ the formula (6a) should be used. Using the abbreviations $\frac{C_{s \cdot a}}{C_{s \cdot m}} = z$ and $\frac{C_{s \cdot a}}{C_{s \cdot n'}} = u$ (6a) may be rewritten as

$$\frac{1}{k_{\Delta \cdot 1}} = \frac{1 - uz^{\frac{\Delta}{\Delta_m} - 1}}{1 - u \cdot \frac{1}{z}} \cdot 2^{\Delta}. \tag{12}$$

If this expression is introduced into the formula (5) we get

$$K = \frac{z - u - a\left(z - uz^{\frac{\Delta}{\Delta_m}}\right) \cdot 2^{\Delta}}{a\left(z - uz^{\frac{\Delta}{\Delta_m}}\right) \cdot 2^{\Delta}} \cdot z^{\frac{\Delta}{\Delta_m}}. \tag{13}$$

Now, it should here be noted that u is a fraction such as $\frac{1}{2}$ or $\frac{1}{4}$ or $\frac{1}{5}$ etc. thus smaller than unity. Hence for suitably small values of Δ we may use the approximate formula

$$K = \frac{1 - a \cdot 2^{\Delta}}{a \cdot 2^{\Delta}} \cdot z^{\frac{\Delta}{\Delta_m}}, \tag{14}$$

i. e. exactly the same formula as in the particular case of $C_{s \cdot n'} = C_{s \cdot a}$. Hence, in the more general case also, we may calculate an approximate value a corresponding to $\frac{dK}{d\Delta} = 0$, $\Delta = 0$ from (10a), and the value of K for $\Delta = 0$ from the formula (11).

5. Application of the Formulae to the Kaas-Ulla- and the Pepsine-Ulla Experiment.

We will apply our formulae to the experiments described in paragraph 2 of the Introduction. We shall illustrate the mode of calculation by the Kaas-Ulla experiment. The various steps are given in Table I. We consider the case $n' = n'_a = 3$ (comp. fig. 1 Introduction). With serum Ulla $n'_m = 8$ and so $C_{s \cdot a}/C_{s \cdot m} = 2^{8-3} = 2^5 = 32$. Again in the Kaas-Ulla experiment $\Delta_m = 4.6$ (comp. fig. 2 Introduction). With these values the formula (5) of paragraph 3 becomes

$$\frac{1}{k_{\Delta}} = \left[1 - 32^{\frac{\Delta}{\Delta_m}-1}\right] \cdot 2^{\Delta}.$$

The first 6 columns of Table I show the calculation of $\frac{1}{k_{\Delta}}$ according to this formula. In the following column that of $\frac{1}{k_{\Delta \cdot 1}}$ is given. Now from the formula (10) of paragraph 4 we find $a = \frac{32}{31} - 0.2 \cdot \Delta_m = 0.111$. In column 8 the values of $a \cdot \frac{1}{k_{\Delta \cdot 1}}$ are stated, in column 9 then the values of $\frac{1 - a\frac{1}{k_{\Delta \cdot 1}}}{a\frac{1}{k_{\Delta \cdot 1}}}$ and finally in column 10 the figures found for the quantity

$$K = \frac{1 - a\frac{1}{k_{\Delta \cdot 1}}}{a\frac{1}{k_{\Delta \cdot 1}}} \cdot 32^{\frac{\Delta}{\Delta_m}}$$

derived from (5) paragraph 4 where we have neglected the constant quantity $C_{s \cdot m}$. Table I further comprises a calculation of K based on another value of a; the meaning of this calculation will be explained below.

A similar set of calculations was carried out from the data of the Pepsine-Ulla experiment. Here the width of the zone of transition Δ_m was 3.3 titers, from which the value of a defined by (7) or (10), paragraph 4, is found to be 0.371.

Again for both of the two experiments the variation of the quantity K with Δ was calculated corresponding to other values of a. The results of all the calculations here referred to will now be considered.

In fig. 7 the values of $\frac{1}{k_{\Delta \cdot 1}}$ from Table I have been plotted against Δ, curve a. The curve corresponds to a serum concentration n' (in the tubes of the titer scale) equal to $n'_a = 3$ (comp. fig. 1). It is seen that $\frac{1}{k_{\Delta \cdot 1}}$ rises to a maximum and then drops rapidly to zero at $\Delta = \Delta_m$. In the same figure curve b

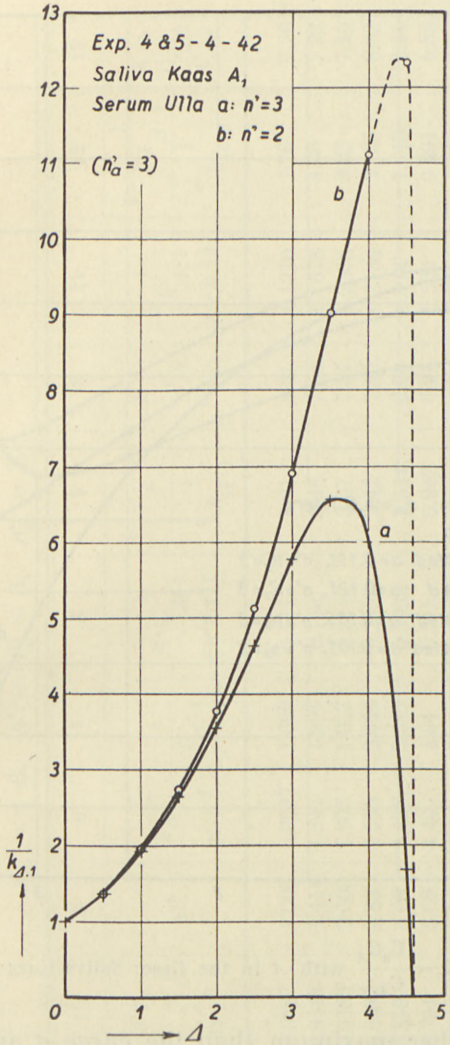

Fig. 7. Variation of $\frac{1}{k_{\Delta \cdot 1}}$ with Δ in the Case: Saliva Kaas A_1, Serum Ulla, $n' = n'_a = 3$, Curve a and $n' = 2$, Curve b.

represents the variation of $\frac{1}{k_{\Delta \cdot 1}}$ with Δ, corresponding to a titer scale with a serum concentration $n' = 2$. The curve has been calculated from formula (6a), paragraph 4, where $\frac{C_{s \cdot a}}{C_{s \cdot n'}} = \frac{1}{2}$.

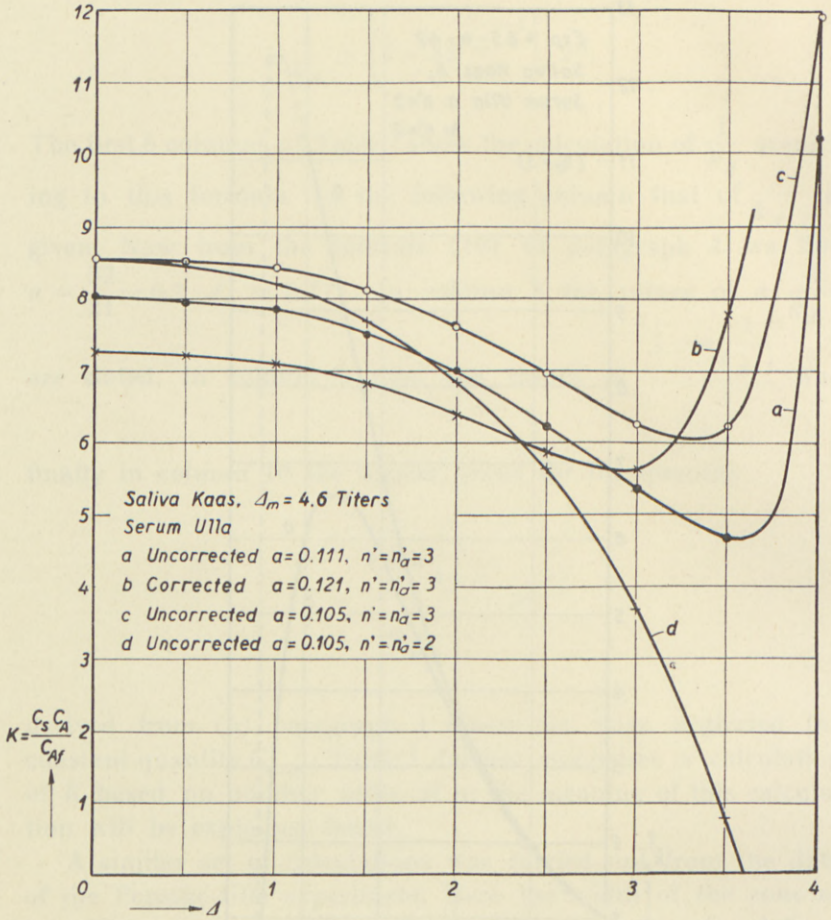

Fig. 8. Variation of $K = \frac{C_s C_A}{C_{A \cdot f}}$ with Δ in the Case: Saliva Kaas A_1, Serum Ulla.

It rises to a higher maximum than the curve *a* and the maximum is located closer to Δ_m than in the latter case. In fig. 8 the variations with Δ of K from Table I have been represented in the curves *a* and *b*. It will be noted that the quantity K is approximately constant within part of the zone of transition. It gradually decreases to a minimum and then, towards the end of the zone, rises rapidly. Fig. 8 further comprises two other curves *c* and *d* both calculated for a value of *a* slightly different from that of curve *a*, viz. the value 0.105. The curves

Table I.

Saliva Kaas A_1, Serum Ulla Anti-A

$\Delta_m = 4 \cdot 6, \quad n' = n'_a = 3, \quad n'_m = 8$

1	2	3	4	5	6	7	8	9	10	11	12	13
Δ	$\frac{\Delta}{4 \cdot 6}$	$\frac{\Delta}{32^{\frac{\Delta}{4 \cdot 6}-1}}$	$M = \frac{\Delta}{1-32^{\frac{\Delta}{\Delta_m}-1}}$	2^{Δ}	$\frac{1}{k_{\Delta}} = \frac{}{M \cdot 2^{\Delta}}$	$\frac{1}{k_{\Delta \cdot 1}}$	$a \cdot \frac{1}{k_{\Delta \cdot 1}}$ $a = 0.111$	$\frac{1-a\frac{1}{k_{\Delta \cdot 1}}}{a\frac{1}{k_{\Delta \cdot 1}}}$	$K = \frac{C_s C_A}{C_{A \cdot f}}$	$a\frac{1}{k_{\Delta \cdot 1}}$ $a = 0.121$	$\frac{1-a\frac{1}{k_{\Delta \cdot 1}}}{a\frac{1}{k_{\Delta \cdot 1}}}$	$K = \frac{C_s C_A}{C_{A \cdot f}}$
0	0	0.0313	0.9687	1.000	0.9687	1.000	0.111	8.01	8.01	0.1210	7.26	7.26
0.5	0.1088	0.0455	0.9545	1.414	1.347	1.391	0.156	5.46	7.94	0.1678	4.96	7.22
1.0	0.2175	0.0667	0.9333	2.000	1.865	1.927	0.2140	3.67	7.84	0.2308	3.33	7.10
1.5	0.3260	0.0970	0.9030	2.83	2.555	2.635	0.2925	2.42	7.50	0.3130	2.195	6.82
2.0	0.4350	0.141	0.859	4.00	3.435	3.545	0.3935	1.544	6.98	0.414	1.415	6.40
2.5	0.5440	0.206	0.794	5.66	4.49	4.64	0.515	0.942	6.21	0.529	0.891	5.88
3.0	0.6525	0.300	0.700	8.00	5.60	5.78	0.642	0.558	5.35	0.630	0.588	5.64
3.5	0.7610	0.437	0.563	11.31	6.36	6.58	0.730	0.370	5.19	0.643	0.556	7.77
4.0	0.8700	0.637	0.363	16.00	5.81	6.00	0.666	0.502	10.22	0.409	1.445	42.9
4.5	0.9780	0.927	0.073	22.60	1.64	1.69	..	..	..	..	..	..

correspond to the two values of the serum concentration $n' = 3$ and 2 respectively. It will be noted that the minimum is more pronounced in the case of the higher serum concentration than in that of the lower.

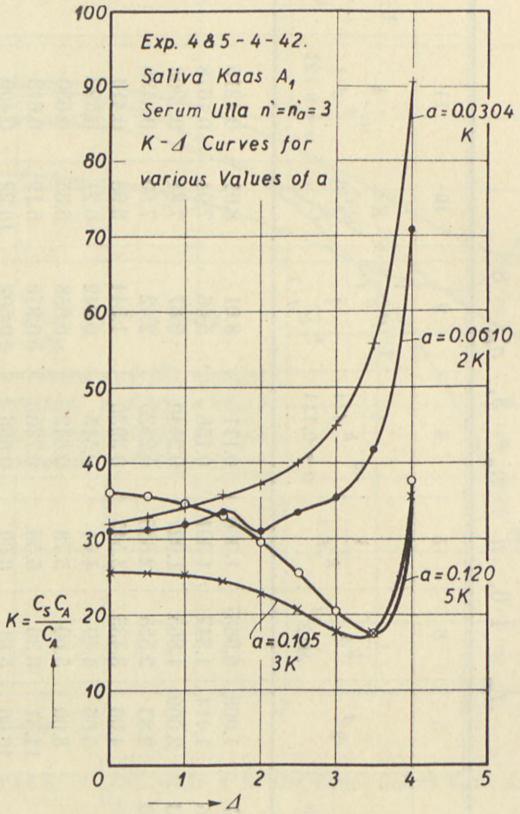

Fig. 9. Comparison of $K-\Delta$-Curves corresponding to various Values of the Factor a: Saliva Kaas A_1, Serum Ulla $n' = n'_a = 3$.

The curves in fig. 8 have been calculated for values of a making $\frac{dK}{d\Delta} = 0$ for $\Delta = 0$ or approximately so. It would seem of interest to compute K-curves for other values of a. Results of such a calculation have been presented in fig. 9. In order to render possible a more direct comparison various multiples of

K have been plotted. It will be noted that all the curves are approximately horizontal within a certain initial part of the zone of transition.

A similar set of curves corresponding to the Pepsine-Ulla Experiment are reproduced in figs. 10, 11 and 12. The $\frac{1}{k_{\Delta \cdot 1}}$—$\Delta$-curves are of the same character as those of fig. 7. The K—Δ-curves

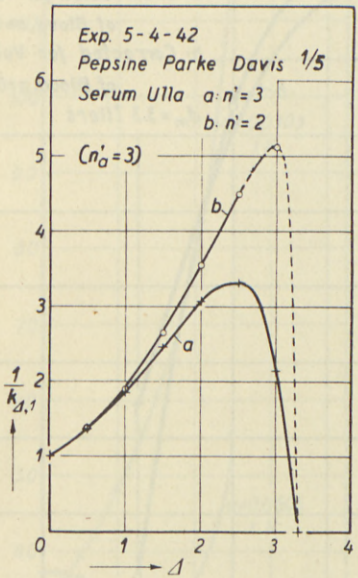

Fig. 10. Variation $\frac{1}{k_{\Delta \cdot 1}}$ with Δ in the Case: Pepsine Parke Davis $\frac{1}{5}$, Serum Ulla, $n = n'_a = 3$, Curve a and $n' = 2$, Curve b.

have also much the same character as the corresponding curves in fig. 8 apart from the minima being much more pronounced in fig. 11 than in fig. 8. They are, in fact, located below $K = 0$. Finally fig. 13, curve c, and fig. 14 correspond to an antigen, Stomach Pt. I. 140, giving with serum Ulla a very wide zone of transition, viz. $\Delta_m = 6$. No complete investigation with the combination of this antigen and serum Ulla was carried out but the existence of a zone of the width $\Delta_m = 6$ was established for the combination. With this width there is no (positive) value of a which will make $\frac{dK}{d\Delta} = 0$ for $\Delta = 0$ (comp. formula

(10) paragraph 4). So the $K-\Delta$-curves in fig. 14 were calculated for a number of small positive values of a.

From the discussion now recorded it would seem that no very simple law of mass-action dominates the fixation between

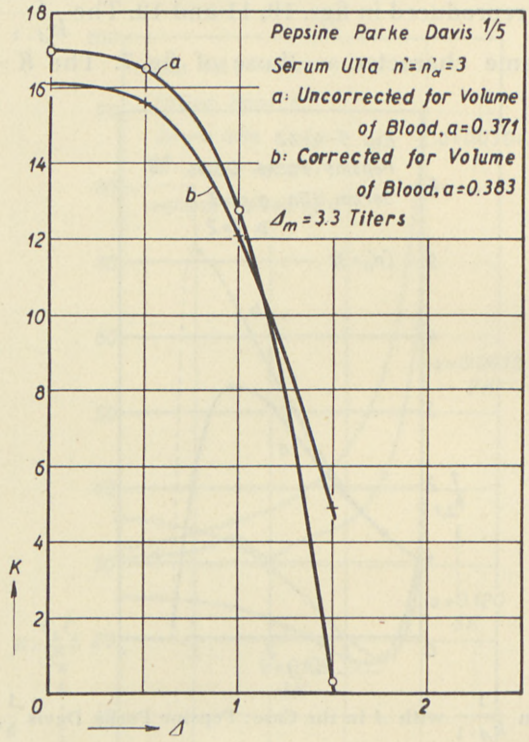

Fig. 11. Variation of $K = \frac{C_s C_A}{C_{A \cdot f}}$ with Δ in the Case: Pepsine Parke Davis $\frac{1}{5}$, Serum Ulla $n' = n'_a = 3$.

the antigen and the serum antibody in the agglutination inhibition test—though there may be an approximation to such a law within certain ranges of concentration of the two components.

6. Corrections for the Volume of the Blood Drop.

In the titer curve fig. 1 for the serum the abscissa characterizes the concentration of the serum—by the quantity n'—as it was before the addition of a drop of blood. Now the volume

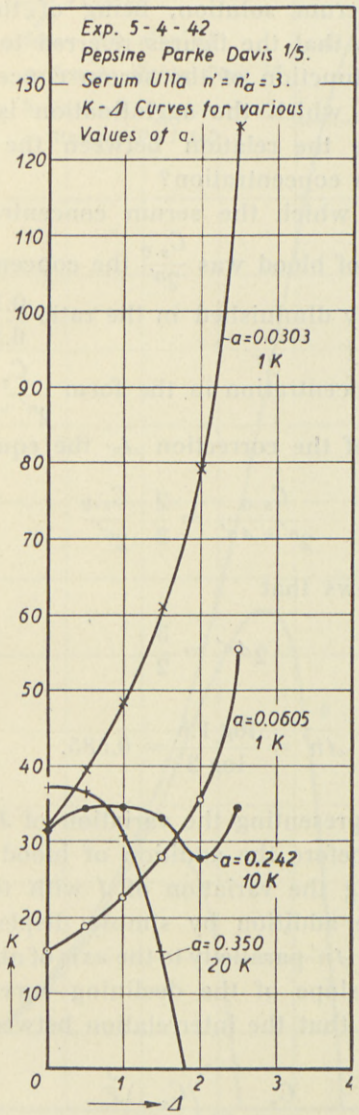

Fig. 12. Comparison of K — Δ-Curves corresponding to various Values of the Factor a: Pepsine Parke Davis $\frac{1}{5}$, Serum Ulla $n' = n'_a = 3$.

of a "drop" is not at all inappreciable compared to the volume 0.1 cm^3, of the serum solution, being of the order of size 0.05 cm^3. It follows that the figures referred to do not give the agglutination as a function of the serum concentration prevailing in the tube in which the agglutination is read. We may ask: what will be the relation between the agglutination J and the true serum concentration?

In the tube in which the serum concentration before the addition of a drop of blood was $\frac{C_{s.0}}{2^{n'}}$ the concentration after the addition is obviously diminished in the ratio $\frac{0.10}{0.15} = \frac{2}{3}$. We may write the actual concentration in the form $\frac{C_{s.0}}{2^{n'+\Delta n'}}$ and have for the determination of the correction $\Delta n'$ the equation

$$\frac{C_{s.0}}{2^{n'+\Delta n'}} = \frac{2}{3} \cdot \frac{C_{s.0}}{2^{n'}} \tag{1}$$

from which it follows that

$$2^{\Delta n'} = \frac{3}{2} \tag{2}$$

or

$$\Delta n' = \frac{\log 1.5}{\log 2} = 0.585. \tag{2a}$$

Hence the curve representing the variation of J with the serum concentration (n') before the addition of blood is changed into a curve representing the variation of J with the concentration $(n'+\Delta n')$ after the addition by simply displacing the former curve by the amount $\Delta n'$ parallelly to the axis of abscissae. Through this operation the slope of the declining curve branch is not altered. This means that the interrelation between C_s and J may still be written

$$\frac{C_s}{C_{s.m}} = \left(\frac{C_{s.a}}{C_{s.m}}\right)^{\frac{J}{J_a}} \tag{3}$$

where $C_{s.a}$ and $C_{s.m}$ on the right-hand side may have the values read on the uncorrected curve or, as well, the corrected values read on the displaced curve, while $C_{s.m}$ on the left-hand side

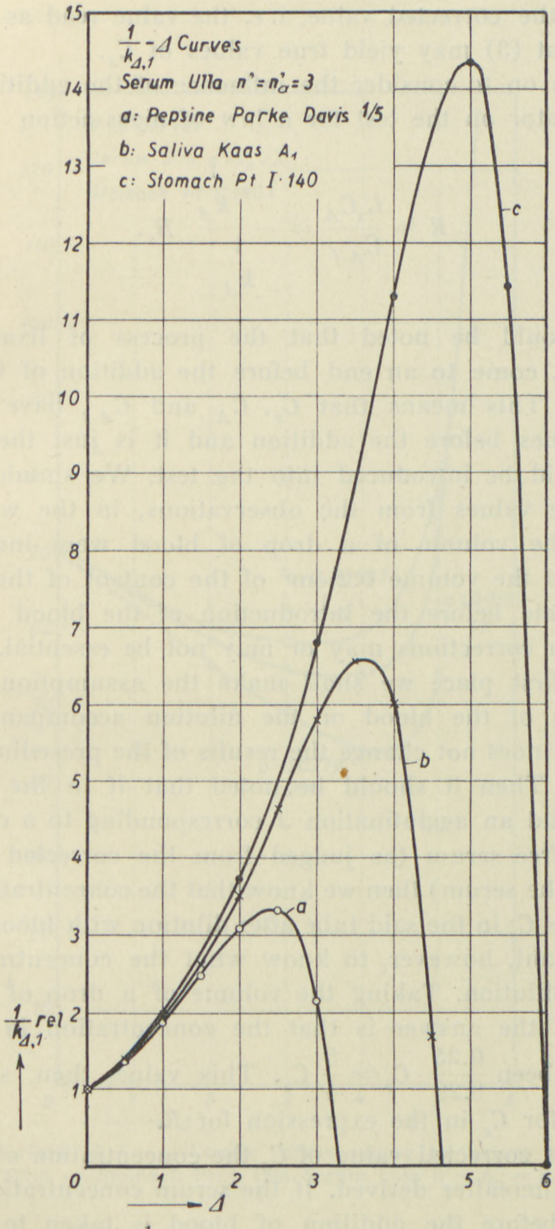

Fig. 13 Comparison of $\frac{1}{k_{\Delta \cdot 1}} - \Delta$-Curves corresponding to various Antigens: *a* Pepsine Parke Davis $\frac{1}{5}$, *b* Saliva Kaas A_1, *c* Stomach Pt. I. 140. Serum Ulla $n' = n'_a = 3$.

should be the corrected value, i. e. the value read as $n'_m + \Delta n'$ in order that (3) may yield true values of C_s.

We pass on to consider the influence of the addition of the blood indicator on the test for a law of mass-action

$$K = \frac{C_s C_A}{C_{A \cdot f}} = \frac{1 - \frac{1}{k_\Delta}}{\frac{1}{k_\Delta}} \cdot B_s .$$

Here it should be noted that the process of fixation has, presumably, come to an end before the addition of the blood suspension. This means that C_s, C_A and $C_{A \cdot f}$ have assumed definite values before the addition and it is just these values which should be introduced into the test. We should directly derive these values from the observations, in the way stated above, if the volume of a drop of blood were insignificant compared to the volume 0.2 cm^3 of the content of the tubes of the titer scale before the introduction of the blood indicator. Now certain corrections may or may not be essential.

In the first place we shall make the assumption that the introduction of the blood or the dilution accompanying this introduction does not change the results of the preceding process of fixation. Then it should be noted that if in the tube no. $n + \Delta$ we read an agglutination J corresponding to a concentration C_s of free serum (as judged from the corrected curve of titration of the serum) then we know that the concentration of the free serum is C_s in the said tube after dilution with blood suspension. We want, however, to know what the concentration was before the dilution. Taking the volume of a drop of blood to be 0.05 cm^3 the answer is that the concentration in question must have been $\frac{0.25}{0.20} \cdot C_s = \frac{5}{4} C_s$. This value, then, should be introduced for C_s in the expression for K.

From the corrected value of C_s the concentration of fixed serum $C_{s \cdot f}$ is hereafter derived. If the serum concentration in the titer scale before the addition of blood is taken to be $C_{s \cdot a}$, compare fig. 6, we have

$$C_{s \cdot f} = C_{s \cdot a} - \frac{5}{4} C_s . \tag{4}$$

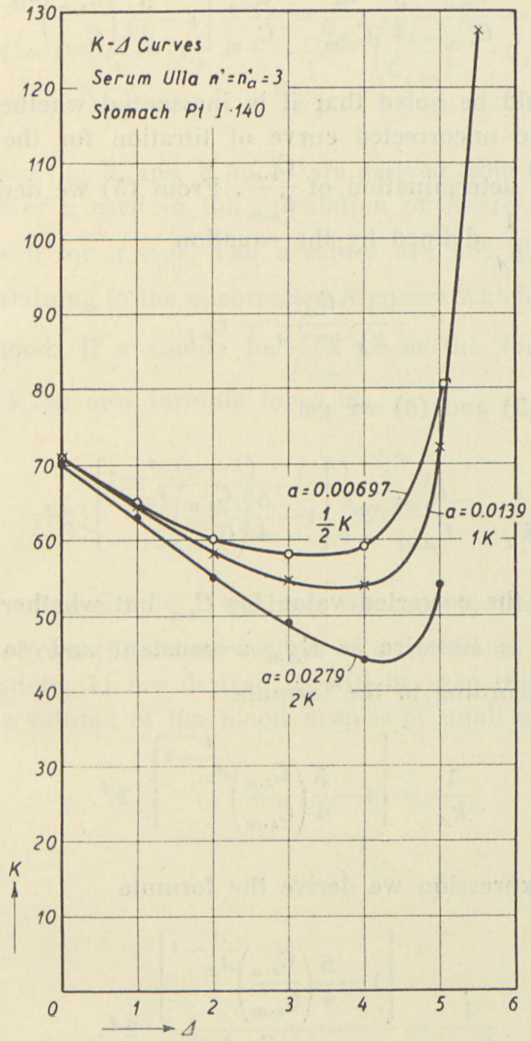

Fig. 14. Comparison of K — Δ-Curves corresponding to various Values of the Factor a: Stomach Pt. I. 140. Serum Ulla $n' = n'_a = 3$.

From (4) we get

$$(5) \qquad \frac{C_{s\cdot f}}{C_{s\cdot m}} = \frac{C_{s\cdot a}}{C_{s\cdot m}} - \frac{5}{4}\cdot\frac{C_s}{C_{s\cdot m}} = \frac{C_{s\cdot a}}{C_{s\cdot m}}\left[1 - \frac{5}{4}\cdot\left(\frac{C_{s\cdot a}}{C_{s\cdot m}}\right)^{\frac{A}{A_m}-1}\right].$$

Here it should be noted that it is inessential whether the corrected or the uncorrected curve of titration for the serum is used for the determination of $\frac{C_{s\cdot a}}{C_{s\cdot m}}$. From (5) we derive an expression for $\frac{1}{k_A}$ defined by the equation

$$(6) \qquad \frac{1}{k_A}\,\frac{C_{A\cdot 0}}{2^{n+A}} = C_{s\cdot f}.$$

Combining (5) and (6) we get

$$(7) \qquad \frac{1}{k_A} = \frac{C_{s\cdot a}}{C_{A\cdot 0}}\cdot 2^n\cdot\left[1 - \frac{5}{4}\left(\frac{C_{s\cdot a}}{C_{s\cdot m}}\right)^{\frac{A}{A_m}-1}\right]\cdot 2^A.$$

Here $C_{s\cdot a}$ is the corrected value for $C_{s\cdot a}$ but whether corrected or not, $C_{s\cdot a}$ is likewise as $C_{A\cdot 0}$ a constant and so $\frac{1}{k_A}$ varies relatively, according to the formula

$$(7\,a) \qquad \frac{1}{k_A} = \left[1 - \frac{5}{4}\left(\frac{C_{s\cdot a}}{C_{s\cdot m}}\right)^{\frac{A}{A_m}-1}\right]\cdot 2^A.$$

From this expression we derive the formula

$$(7\,b) \qquad \frac{1}{k_{A\cdot 1}} = \frac{\left[1 - \frac{5}{4}\left(\frac{C_{s\cdot a}}{C_{s\cdot m}}\right)^{\frac{A}{A_m}-1}\right]}{1 - \frac{5}{4}\left(\frac{C_{s\cdot a}}{C_{s\cdot m}}\right)^{-1}}\cdot 2^A.$$

This formula should be compared to the uncorrected formula (6), paragraph 4 for $\frac{1}{k_{A\cdot 1}}$. The values for $\frac{1}{k_{A\cdot 1}}$ derived from

(7b) are those which should be introduced into the formula for K, viz.

$$(8) \qquad K = \frac{1 - a\frac{1}{k_{\Delta}}}{a\frac{1}{k_{\Delta}}} \cdot C_s = \frac{1 - a\frac{1}{k_{\Delta}}}{a\frac{1}{k_{\Delta}}} \cdot \left(\frac{C_{s.a}}{C_{s.m}}\right)^{\frac{\Delta}{\Delta_m}} \cdot C_{s.m}.$$

The curves b fig. 8 and b fig. 11 are derived from this formula. The values of a used in the calculation of K are such as will make $\frac{dK}{d\Delta} = 0$ for $\Delta = 0$. The a-values are not identical with those appertaining to the uncorrected K-curves which will readily be understood. If z stands for $\frac{C_{s.a}}{C_{s.m}}$ as in the formula 10 of paragraph 4 the new formula for a is

$$(9) \qquad a = \frac{z(z-1)}{\left(z - \frac{5}{4}\right)^2} - \frac{z-1}{z-\frac{5}{4}} \cdot \frac{l_e 2}{l_e z} \Delta_m.$$

If $\frac{5}{4}$ is here replaced by 1 we have returned to the formula 10 referred to. From (9) the two values of a written on the b-curves of fig. 8 and fig. 11 are derived. It will be seen that the correction for the volume of the blood drop is of small moment only.

Part II.

Theory of the $n-n'$-Curve.

1. Shape of the $n-n'$-Curve. The $\frac{1}{k}-n$-Curve.

In fig. 15 the observed $n-n'$-curves from figs. 4 and 5 of the Introduction have been redrawn with the change that n' now corresponds to the concentration of the serum in the titer-scale proper; n' fig. 15 is, thus, greater by 1 than the n' in figs. 4 and and 5 of the Introduction. The observed values of n are marked by crosses. The curves shown have been drawn as smoothed out curves between the crosses. Then it was tried whether the curves might be represented by an expression of the form

$$n^2 = \beta (n' - n'_0) \tag{1}$$

thus by the analytical expression for a parabola with its axis coinciding with the n'-axis in fig. 15 and with its vertex in the point $n' = n'_0$. The values of β and n'_0 may appropriately be determined by means of two sets of points situated on the smoothed out curve. In the case of Saliva Kaas the points with the abscissae $n' = 1$ and $n' = 6$ were used, the corresponding ordinates being read as $n = 3.0$ and $n = 11.7$ respectively. If the two sets of coordinates are introduced for n' and n in (1) two equations are obtained yielding the values $\beta = 25.6$ and $n'_0 = 0.649$, thus the expression

$$n^2 = 25.6\,(n' - 0.649) \qquad \text{(Saliva Kaas).}$$

In a similar manner the formula

$$n^2 = 17.2\,(n' - 0.070) \qquad \text{(Pepsine)}$$

was derived for Pepsine Parke Davis $\frac{1}{5}$. The points marked by

circles in fig. 15 were calculated from these formulae. It appears that the formulae cover the experimental curves with almost astonishing exactness.

It would thus seem that we may with good approximation represent the interrelation between n and n' by the formula (1).

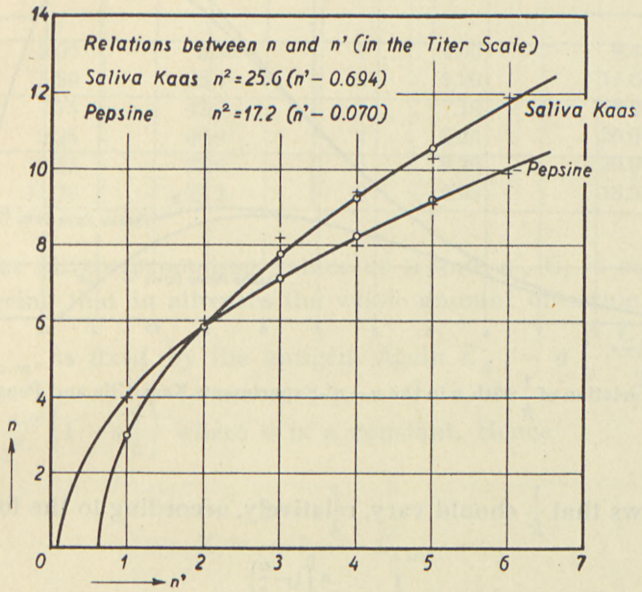

Fig. 15. $n-n'$-Curves from the Kaas-Ulla- and the Pepsine-Ulla Experiments.

Now the fraction $\frac{1}{k}$ of antigen fixed by the serum is defined by

(2) $$\frac{1}{k} \cdot \frac{C_{A\cdot 0}}{2^n} = c \cdot \frac{C_{s\cdot 0}}{2^{n'}}$$

or by

(3) $$\frac{1}{k} = c \cdot \frac{C_{s\cdot 0}}{C_{A\cdot 0}} \cdot 2^{n-n'}$$

where c is so far assumed to be a constant.

Eliminating n' from (3) by means of (1) we get

(5) $$\frac{1}{k} = c \cdot \frac{C_{s\cdot 0}}{C_{A\cdot 0}} 2^{n - \frac{1}{\beta} n^2 - n'_0} = c \cdot \frac{C_{s\cdot 0}}{C_{A\cdot 0}} \cdot 2^{-n'_0} \cdot 2^{n\left(1 - \frac{n}{\beta}\right)}.$$

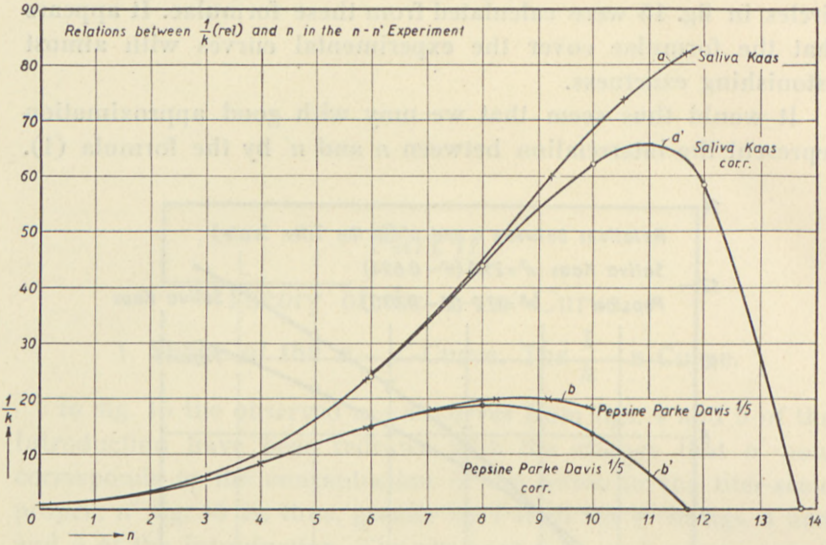

Fig. 16. Variation of $\frac{1}{k}$ with n in the $n-n'$-Experiments Kaas-Ulla and Pepsine-Ulla.

It follows that $\frac{1}{k}$ should vary, relatively, according to the formulae

$$\frac{1}{k} = 2^{n\left(1-\frac{n}{\beta}\right)}. \tag{5}$$

Representations of the variation of $\frac{1}{k}$ with n for the two antigens: Saliva Kaas and Pepsine Parke Davis $\frac{1}{5}$ are given in Table II and in graphical form in fig. 16, curves a and b. It follows from (5) that the curves representing the interrelation between $\frac{1}{k}$ and n are symmetrical with regard to $n = \frac{\beta}{2}$ for which abscissa $\frac{1}{k}$ exhibits a maximum.

One might ask whether free and fixed antigen and serum distribute themselves according to a simple law of mass-action in the $n-n'$-experiment. We may again write down an expression for the quantity

$$K = \frac{C_A \cdot C_s}{C_{A \cdot f}}.$$

Table II.

Saliva Kaas			Pepsine Parke Davis $\frac{1}{5}$		
n'	n	$\frac{1}{k} = 2^{n\left(1-\frac{n}{25.6}\right)}$	n'	n	$\frac{1}{k} = 2^{n\left(1-\frac{n}{17.2}\right)}$
1	3.00	6.30	1	4.00	8.45
2	5.90	23.4	2	5.90	14.6
3	7.75	42.5	3	7.10	18.0
4	9.25	60.0	4	8.25	20.0
5	10.55	73.6	5	9.20	20.0
6	11.70	82.2	6	9.95	18.5

Here, for all corresponding values of n and n', C_s is equal to $C_{s.m}$, seeing that in all cases the whole amount of serum, apart from $C_{s.m}$, is fixed by the antigen. Again $C_{A.f} = a\,\frac{1}{k} \cdot \frac{C_{A.0}}{2^n}$ and $C_A = \frac{C_{A.0}}{2^n}\left(1 - a\frac{1}{k}\right)$ where a is a constant. Hence

$$K = \frac{1 - a\frac{1}{k}}{a\frac{1}{k}} \cdot C_{s.m}.$$

Quite obviously this function cannot assume a constant value if $\frac{1}{k}$ varies with n. In fig. 17 the variation of K with n is represented for the case of Saliva Kaas and for a value of $a = 0.00758$ determined so as to make $a\,\frac{1}{k_{\max}} = 0.5$, where $\frac{1}{k_{\max}}$ is the maximum value of $\frac{1}{k}$ read on the curve a' fig. 16. The meaning of this latter curve will now be explained.

Our formula (5) for $\frac{1}{k}$ is approximate only, seeing that we have in (2) neglected the quantity $C_{s.m}$. Actually (2) should be replaced by the equation

$$\frac{1}{k}\frac{C_{A.0}}{2^n} = \frac{C_{s.0}}{2^{n'}} - C_{s.m} \tag{7}$$

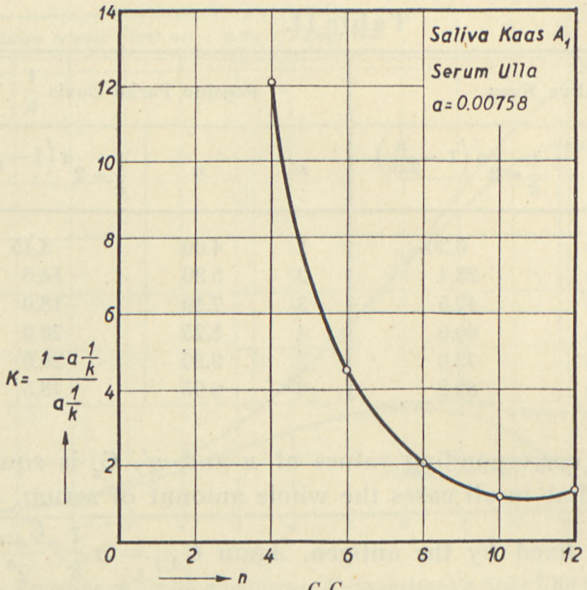

Fig. 17. Relative Variation of $K = \frac{C_s C_A}{C_{A\cdot f}}$ in the $n-n'$- Experiment Kaas-Ulla.

if for the sake of convenience we put $c = 1$. From (7) and (1) we readily derive

$$(8)\quad \left\{\begin{aligned} \frac{1}{k} &= \frac{C_{s\cdot 0}}{C_{A\cdot 0}} \cdot 2^{n-\frac{n^2}{\beta}-n'_0} - \frac{C_{s\cdot m}}{C_{A\cdot 0}} \cdot 2^n \\ &= \frac{C_{s\cdot 0}}{C_{A\cdot 0}} \cdot 2^{-n'_0} \cdot 2^{n\left(1-\frac{n}{\beta}\right)} \left[1 - \frac{C_{s\cdot m}}{C_{s\cdot 0}} \cdot \frac{2^{n'_0}}{2^{-\frac{n^2}{\beta}}}\right], \end{aligned}\right.$$

i. e. relative values of $\frac{1}{k}$ are given by the formula

$$(9)\quad \frac{1}{k} = 2^{n\left(1-\frac{n}{\beta}\right)} \left[1 - \frac{C_{s\cdot m}}{C_{s\cdot 0}} \cdot \frac{2^{n'_0}}{2^{-\frac{n^2}{\beta}}}\right].$$

From this formula it appears that the values derived from (5) are too large by an amount of $\frac{C_{s\cdot m}}{C_{s\cdot 0}} \cdot \frac{2^{n'_0}}{2^{-\frac{n^2}{\beta}}} \cdot 100$ p. c. In the case of Saliva Kaas and Serum Ulla we have:

$\frac{C_{s\cdot m}}{C_{s\cdot 0}} = 2^{-8}$ (comp. fig. 1 Introd.), $\beta = 25.6$, $n'_0 = 0.649$.

From these data we derive $\frac{C_{s \cdot m}}{C_{s \cdot 0}} \cdot 2^{n'_0} = 0.00618$. Introducing the value into (9) and calculating the percentage correction corresponding to a number of values of n we arrive at the curve a', the corrected $\frac{1}{k} - n$-curve, in fig. 16, and in the same way at the curve b' for Pepsine Parke Davis $\frac{1}{5}$. It will be noted that the corrections are pronounced for larger values of n only.

2. Comparison of the $\frac{1}{k}$-Curves derived for the Zone of Transition, $\frac{1}{k_\Delta}$, and from the $n - n'$-Experiment.

It would now seem of interest to compare the two curves: the $\frac{1}{k_\Delta} - \Delta$-curve derived for the zone of transition and the $\frac{1}{k} - n$-curve derived from the $n - n'$-experiment. This comparison has been made in fig. 18. The $\frac{1}{k_\Delta} - \Delta$-curves are those already represented in figs. 7 and 10 of Part I, while the $\frac{1}{k} - n$-curves are reproductions of the curves a and b in fig. 16 or rather of the lower parts of these curves. Again curves representing 2^n are drawn. It will be noted that the $\frac{1}{k}$-curves would seem to coincide, at any rate within a rather wide interval, with the $\frac{1}{k_\Delta}$-curve corresponding to a serum concentration (in the titer scale) between $n' = 2$ and $n' = 3$, say $n' = 2.5$. This is not far from being an average of the values of n' covered by the $n - n'$-experiment. It would thus seem, at any rate at first sight, that a certain relationship has been established between the two quantities $\frac{1}{k_\Delta}$ and $\frac{1}{k}$, derived from the two sources, viz. the zone of transition- and the $n - n'$-experiment.

Before this relationship is further discussed attention should, however, be drawn to the following experience. The parabolic $n - n'$-curves may within limited intervals be regarded practically as straight lines. In a preliminary investigation the $n - n'$-curves were actually interpreted as straight lines. Now the

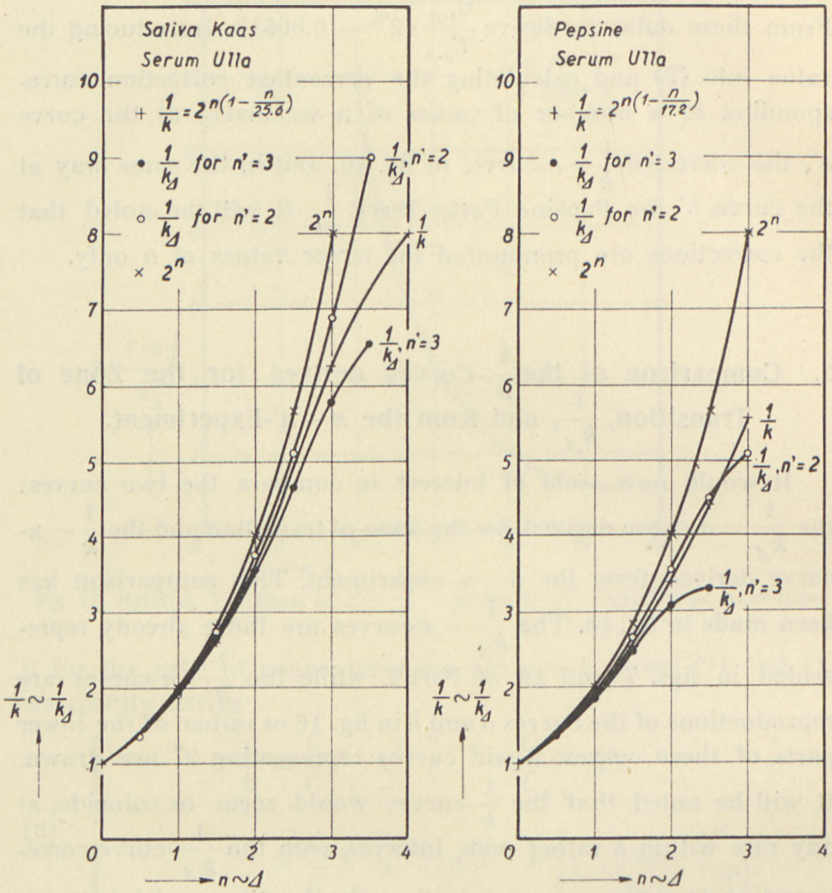

Fig. 18. Comparison of the $\frac{1}{k_\Delta}-\Delta$-Curve with the $\frac{1}{k}-n$-Curve.

experience referred to is as follows. It would seem that $n-n'$-curves of greater steepness A, i. e. with a greater (average) value of $\frac{dn}{dn'}$, also mean a greater width Δ_m of the zone of transition. The correlation is illustrated in Table III. Apparently Δ_m is approximately proportional to $\frac{dn}{dn'}$. True, the value of $\Delta_m \Big/ \frac{dn}{dn'}$ is, in the case of saliva AL, comparatively low. This, however, most likely finds its explanation in the circumstance that $\frac{dn}{dn'}$ is estimated too high. The experiment with saliva AL is just

Table III.

Correlation between Δ_m and $\frac{dn}{dn'}$. Serum Ulla, Anti *A*.

Antigen	$\frac{dn}{dn'}$	Δ_m	$\Delta_m / \frac{dn}{dn'}$
Saliva Kaas A_1	1.30	4.6	3.54
Pepsine Parke Davis $\frac{1}{5}$	1.00	3.3	3.30
Saliva *AL*	2.2	6.0	2.7

one of those preliminary tests in which the $n - n'$-curve or rather the lowest part of it was interpreted as a straight line. This obviously means that the result of this experiment is bound to yield a relatively high value of $\frac{dn}{dn'}$ as compared to the two other experiments of Table III, seeing that the values of $\frac{dn}{dn'}$ in these latter cases were derived from the upper, less steep part of the parabola.

An attempt will now be made to show that the interrelation between Δ_m and $\frac{dn}{dn'}$ may actually be predicted from what has been stated above—or at any rate an interrelation approximately to that effect. The expressions for $\frac{1}{k_\Delta}$ and $\frac{1}{k}$ may be written in the shapes.

$$(1) \qquad \frac{1}{k_\Delta} = \frac{1 - \left(\frac{C_{s.a}}{C_{s.m}}\right)^{\frac{\Delta}{\Delta_m} - 1}}{1 - \frac{C_{s.m}}{C_{s.a}}} \cdot 2^{\Delta}$$

$$(2) \qquad \frac{1}{k} = 2^{n} \cdot 2^{-\frac{n^2}{\beta}}$$

it being assumed in the expression (1) that the serum concentration in the tubes of the titer scale is $C_{s.a}$ or that $n' = n'_a$. In

the case of serum Ulla $\frac{C_{s\cdot a}}{C_{s\cdot m}} = 2^5$, and so the equation (1) may be rewritten as

$$\frac{1}{k_\Delta} = \frac{1-2^{5\left(\frac{\Delta}{\Delta_m}-1\right)}}{1-2^{-5}} \cdot 2^\Delta. \tag{1a}$$

Now it should here be remarked that $2^x = e^{l_e 2 \cdot x}$ where $l_e 2$ is the natural logarithm of 2. Hence (1a) may further be changed into

$$\frac{1}{k_\Delta} = \frac{1-e^{5\cdot l_e 2\cdot\frac{\Delta}{\Delta_m}}\cdot 2^{-5}}{1-2^{-5}} \cdot 2^\Delta = \left(1-e^{5\cdot l_e 2\cdot\frac{\Delta}{\Delta_m}}\cdot 2^{-5}+2^{-5}\right)2^\Delta \tag{1b}$$

seeing that 2^{-5} is small compared to 1. Confining ourselves to small values of Δ we may replace $e^{5\cdot l_e 2\frac{\Delta}{\Delta_m}}$ by the three first terms of its series development. Hence

$$\left\{\begin{aligned} \frac{1}{k_\Delta} &= \left[1-\left(1+5\cdot l_e 2\cdot\frac{\Delta}{\Delta_m}+\frac{1}{2}\left(5\cdot l_e 2\cdot\frac{\Delta}{\Delta_m}\right)^2\right)2^{-5}+2^{-5}\right]\cdot 2^\Delta \\ &= \left[1-\frac{5\cdot l_e 2}{2^5}\cdot\frac{\Delta}{\Delta_m}-\frac{(5\cdot l_e 2)^2}{2^6}\cdot\left(\frac{\Delta}{\Delta_m}\right)^2\right]\cdot 2^\Delta \\ &= \left[1-0.108\,\frac{\Delta}{\Delta_m}-0.220\left(\frac{\Delta}{\Delta_m}\right)^2\right]\cdot 2^\Delta. \end{aligned}\right. \tag{3}$$

In much the same way we may rewrite (2) in the shape

$$\frac{1}{k} = 2^n \cdot e^{-l_e 2\cdot\frac{n^2}{\beta}}. \tag{4}$$

Developing in series we get

$$\frac{1}{k} = \left[1-l_e 2\cdot\frac{n^2}{\beta}+\frac{1}{2}(l_e 2)^2\cdot\frac{n^4}{\beta^2}\right]\cdot 2^n. \tag{5}$$

Now from the equation of the parabola, viz.

$$n^2 = \beta\,(n'-n'_0) \tag{6}$$

it follows that

$$\frac{dn}{dn'} = \frac{\beta}{2n} \tag{7}$$

thus proportional to the quantity $\frac{\beta}{n}$. Let A be an average value of $\frac{dn}{dn'}$ or of $\frac{\beta}{2n}$ within a certain minor interval of the $n-n'$-curve covering small values of n only, then we may with a certain degree of approximation rewrite (5) as

$$\frac{1}{k} = \left[1 - \frac{l_e 2}{2} \cdot \frac{n}{A} + \frac{(l_e 2)^2}{8} \cdot \frac{n^2}{A^2}\right] \cdot 2^n. \tag{8}$$

A comparison of (3) and (8) now shows that, for such small values of n and Δ that the terms of the second degree in n and Δ may be neglected, the two expressions will yield identically the same values for $\frac{1}{k_\Delta}$ and $\frac{1}{k}$, with $n = \Delta$, if

$$\frac{l_e 2}{2} \cdot \frac{1}{A} = \frac{5 \cdot l_e 2}{2^5 \cdot \Delta_m},$$

i. e. if

$$\Delta_m = \frac{5}{2^4} \cdot A.$$

Hence the condition for coincidence of the two characteristics, viz. the $\frac{1}{k_\Delta} - \Delta$-and the $\frac{1}{k} - n$-characteristics, would, with small values of Δ and n, seem to be that of $A = \frac{dn}{dn'}$ being proportional to Δ_m or conversely. Seeing that the two characteristics actually coincide the conclusion may presumably be drawn that they do so because the interrelation referred to is substantially a reality, though it is hardly one of exact proportionality.

3. The Power of Fixation of the Antigen.

Above we have defined the quantities $\frac{1}{k}$ and $\frac{1}{k_\Delta}$ as the fractions of the antigen uniting with the serum antibody. Another way of visualizing the said quantities is to consider them as

measures of the ability of the antigen to fix the serum antibody. For this reason $\frac{1}{k_A}$ or $\frac{1}{k}$ may appropriately be termed the power of fixation of the antigen. A most conspicuous result of the preceding discussion is that $\frac{1}{k}$ in the $n-n'$-experiment is approximately the same function of n as $\frac{1}{k_A}$ is of A in the zone of transition experiment. (Comp. figs. 18). It is rather obscure why it should be so and the author is unable to offer any explanation. *A priori* one might guess at a constant value of the power of fixation of the antigen. It will, however, readily be seen from the experimental results that $\frac{1}{k_A}$ and $\frac{1}{k}$ cannot be constant but that they must increase with decreasing values of the antigen concentration. This follows immediately from the two experimental facts: 1° that the zone of transition has generally a greater width than should be expected if the power of fixation had a constant value independent of the antigen concentration, 2° that the (average) steepness of the $n-n'$-curve is greater than 1, i. e. the constant value it would exhibit in the case of a constant power of fixation. We shall terminate our discussion by showing this.

Let the titer reading for a given antigen on a given titer scale be n. Then, if the power of fixation were constant, there would in tube no. $n+1$ of the scale be an amount of free serum equal to $\frac{C_{s\cdot a}}{2}$, in tube no. $n+2$ the amount $\frac{3}{4}\,C_{s\cdot a}$ and in tube no. $n+3$ the amount $\frac{7}{8}\,C_{s\cdot a}$, $C_{s\cdot a}$ being the serum concentration in the tubes of the titer scale. For in tube no. $n+1$ the amount of antigen is half that of tube no. n and so half of the serum should be fixed if $\frac{1}{k_A}$ were constant, in tube no. $n+2$ the amount of antigen is $\frac{1}{4}$ of that of tube n and consequently with constant power of fixation $\frac{1}{4}$ of the serum should be fixed, leaving $\frac{3}{4}$ of the serum free etc.

It may be noted that we have here neglected the amount of serum $C_{s\cdot m}$, i. e. the amount of free serum corresponding to the

degree of agglutination 0 in the titer curve of the serum.—It is seen that in the case of a constant value of $\frac{1}{k_A}$ a practically complete agglutination should be anticipated within a zone of transition covering 3 titers only. Now actually the zone may have a width considerably in excess of this, say a width of up to 6 titers. This clearly suggests the idea of a power of fixation increasing with decreasing antigen concentration. For if in tube no. $n+1$ more than half of the serum is fixed, in tube no. $n+2$ more than $\frac{1}{4}$ is fixed and so on, then obviously the zone of transition must be wider and the more so the greater the increase of the power of fixation. Again, if the power of fixation of the antigen $\frac{1}{k}$ were constant, i. e. independent of the antigen concentration, the titer reading n should in the $n-n'$-experiment rise by one unit for each unit's increase of n' or, we should find $\frac{dn}{dn'} = 1$. Now, as a general rule $\frac{dn}{dn'}$ is found to assume higher (average) values. This experience too shows clearly that the power of fixation $\frac{1}{k}$ must increase with decreasing values of the antigen concentration. For it means that if the serum concentration is reduced to half its former value, i. e. n' increased by 1, then the antigen concentration must be diminished not to $\frac{1}{2}$ but to a value smaller than that, say to $\frac{1}{4}$ of its former value, in order to just fix the serum (apart from $C_{s.m}$). But this can only be so, if the power of the antigen to fix serum rises considerably when the antigen concentration is reduced.

On the other hand it is clearly seen that there must be a limit to the increase of the power of fixation with decreasing antigen concentration. Such a limit is obviously given by $\frac{1}{k_A} = 2^A$ $\left(\text{or } \frac{1}{k} = 2^n\right)$. The latter relation would mean that with half the concentration of antigen the power of fixation should be doubled, i. e. the amount of serum antibody which could be fixed would remain unaltered. The consequence hereof would be a zone of transition of infinite width and an $n-n'$-curve of infinite steep-

ness. This is directly seen but it follows also from the results of our discussion. For it may be noticed that the two expressions (3) and (8) of the preceding paragraph show that $\frac{1}{k_A}$ and $\frac{1}{k}$ vary approximately as 2^A and 2^n respectively and that a variation exactly as 2^A and 2^n must imply $A_m = \infty$ and $A = \infty$. So the deviations from the latter variations expressed by the factors in brackets just account for the finite values of the zone of transition and of the steepness of the $n - n'$-curve. Geometrically these deviations have been illustrated in figs. 18, where curves for 2^n (2^A) have been drawn.

Acknowledgment.

The present paper has been greatly influenced, as to its form and content, by Dr. med. MARTIN KRISTENSEN who with great patience has discussed various fundamental questions with me. In this place I desire to express my sincere gratitude to Dr. KRISTENSEN. I also wish to thank the Trustees of the Carlsberg Foundation for enabling me to find the necessary spare time for the completion of the work in question.

The Laboratory of Technical Physics.
The Royal Technical College.

Copenhagen May 1943.

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BIOLOGISKE MEDDELELSER, BIND XIX, NR. 6

SOME MARINE ALGAE FROM MAURITIUS

III. RHODOPHYCEAE

PART 3

RHODYMENIALES

BY

F. BØRGESEN

KØBENHAVN
I KOMMISSION HOS EJNAR MUNKSGAARD
1944

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This part of "Some Marine Algae from Mauritius" contains the species of the order *Rhodymeniales* which I have been able to identify in the present collections. That some few specimens surely belonging to this order are still left in the collections cannot be denied, but this material is poor and in a rather bad condition, so that it is impossible to determine it. This applies especially to material collected by the native DARUTY which often bears the stamp of having been picked up along the shore far from always in a fresh condition.

It has also been a great drawback that because of the war I have not been able to consult the herbarium of J. AGARDH in Lund, and incidentally the same applies to some of the former parts.

In the following list are mentioned only 10 species of this order from Mauritius, a not particularly large number which no doubt will be much augmented by later examinations. Several of the species are dredged in deep water and are therefore of special interest, just as several of these have proved to be species not yet described.

Besides upon Dr. JADIN's collection, in which DARUTY's gatherings are included and which belongs to the Muséum National d'Histoire Naturelle, Paris, the present part is based upon the collections made by Dr. TH. MORTENSEN and Dr. R. E. VAUGHAN.

V. Rhodymeniales.

Fam. 1. ***Rhodymeniaceae.***

Subfam. 1. Faucheae.

Gloioderma J. Agardh.

1. **Gloioderma Robillardii** nov. spec.

Frons caespitosa, irregulariter globosa, usque ad 8 cm. ambitu expansa, tenuis, membranaceo-papyracea in sicco, in vivo verisimiliter mollis et lubricosa, irregulariter divisa, ex laciniis e basi repetite-dissectis, inferne attenuatis, ca. 3 mm. latis, ad apicem versus latioribus 8—9 mm. latis, mutualiter superpositis et verisimiliter anastomosantibus, marginibus sinuosis et integris, summis laciniarum emarginatis et acutis composita.

Sporangia cruciatim divisa in superficie thalli dispersa.

Mauritius: Without locality in the collection of V. de Robillard, Herb. Thuret in Muséum National d'Histoire Naturelle, Paris.

Dr. Hamel, Muséum National d'Histoire Naturelle, Paris has most kindly sent me an undetermined specimen of a *Florideae* from Mauritius collected by V. de Robillard and kept in Herb. Thuret. Upon the label of the specimen Bornet has written: "Genus, ut videtur, novum ad *Rhodymeniaceas* (*Gloiocladieas*) forte referendum. Ex Schmitz in litt. 1894". Furthermore Reinbold has added upon the label: "Vielleicht der Gattung *Gloioderma* (*Horea*) identisch, jedenfalls sehr ähnlich".

After an examination of the specimen (Fig. 1) in question it seems to me, as to Reinbold, that it is referable to the genus *Gloioderma*. The anatomy agrees with this genus and the sporangia being cruciately divided and scattered over the surface of the thallus likewise agree with it; but it is of course a drawback that the cystocarps are unknown.

Fig. 2 shows a small piece of a transverse section of the thallus with sporangia. It consists in the middle of a layer of large cells the largest of these in the figure being 120 μ long and 100 μ broad. On both sides of these cells there is a layer of much smaller cells oval in transverse section; the innermost are the largest, of variable size up to about 60 μ long and 25 μ broad,

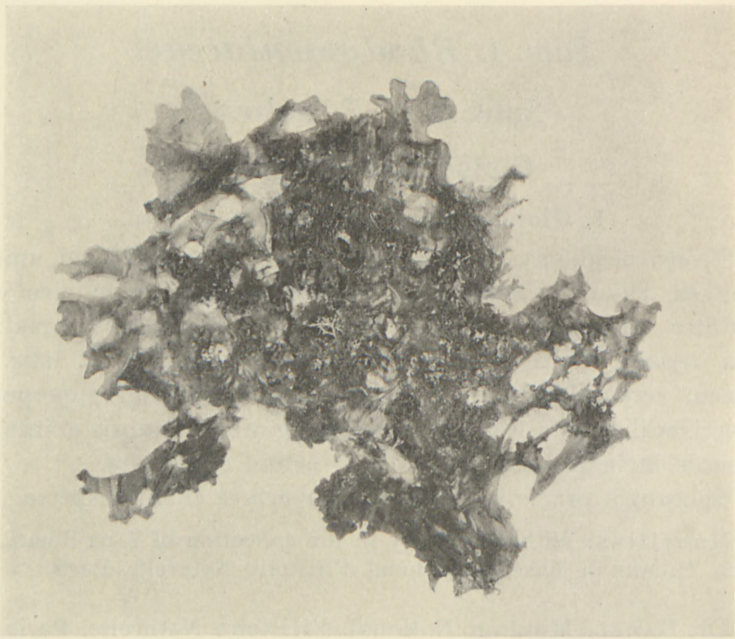

Fig. 1. *Gloioderma Robillardii* Børgs. The original specimen in Herb. THURET. Natural size.

decreasing in size towards the cortical layer which is formed by thin ramified filaments composed of small oval cells with rather a long distance between the cells. These are about 1—2 μ broad.

Where the sporangia are developed in the cortical layer the filaments become longer, more straight and less ramified.

The consistence of the thallus in the dried condition is papyraceous-cartilaginous but when saturated with water it quickly swells and becomes very soft and lubricous.

As to the habit of the plant, when living it most probably forms roundish clumps, the lobes of the thallus radiating to all sides, separated by smaller or larger openings. The lobes

are about 3—8 mm. broad, sometimes broader sometimes narrower, irregularly divided or subfurcated and often anastomosing. The terminal lobes are more or less emarginate and broadly rounded above.

The habit of this alga seems to agree very well with a plant from the Malayan Archipelago which Mme WEBER (1928, p. 458, fig. 195) has determined as *Fauchea*(?) *mollis* Howe var. *intermedia*. The plant of Mme WEBER is cystocarpic, the cystocarps

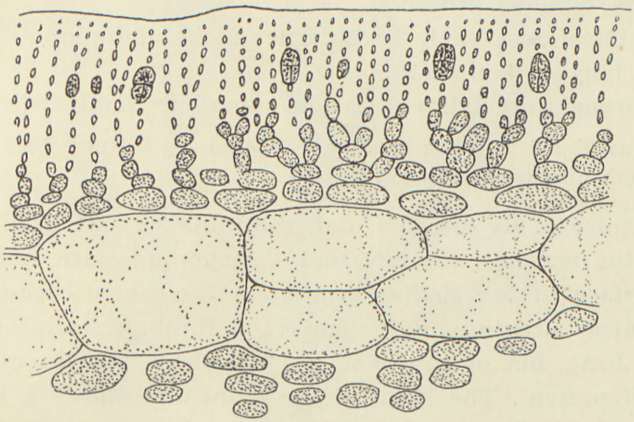

Fig. 2. *Gloioderma Robillardii* Børgs. Transverse section of the thallus with tetrasporangia. (× c. 300).

not being provided with hornlike processes this feature places it in the genus *Fauchea*, while hornlike processes are present in *Gloioderma*. On the other hand, the specimen I have had for examination is, as stated above, tetrasporic and as the tetrasporangia are scattered over the surface of the thallus, as is the case in *Gloioderma*, it is referred to this genus, since in *Fauchea* the sporangia are placed in the sori.

Regarding *Fauchea*(?) *mollis* Howe, Phycological Studies, V, 1911, p. 507, pl. 32 and pl. 33, fig. 6, then this species is described upon sterile material only and, as hinted by HOWE who placed a ? after the generic name, the determination is therefore doubtful. Moreover the plant from Mauritius is also rather different from that from Mexico, the thallus of the Mexican plant being larger with broader segments, and the anatomy being also different, so it seems most correct to regard it as a separate species.

The plant from Mauritius must also be compared with *Gloioderma*(?) *expansa* Weber (1914, p. 283, pl. 18, figs. 28—29) but this species is in all respects larger with much broader lobes; also its anatomical structure is different; thus the cortical layer has not a filamentous character.

2. **Gloioderma mauritiana** nov. spec.

Frons plana, furcata, ca. 1 cm. lata ad apicem vertens angustior, ex margine prolifera.

Prolificationes valde irregulariter dichotomo-pinnatifidae, ca. $^3/_4$ cm. longae et ultra(?).

Sporangia cruciatim divisa in superficie thalli dispersa.

Mauritius: Without locality, collected by DARUTY, 1892 in Herb. JADIN sub nomine *Suhria vittata.*

Because of its furcated, flat, solid thallus, the prolifications along the margins, the fact that the sporangia are spread over the surface of the thallus and upon the whole because of its anatomical structure, this plant is, I think, referable to *Gloioderma* J. Ag. but of course it is a drawback that the cystocarps are not present. The habit of the plant also shows a fair likeness to Australian species, for instance *Gloioderma fruticulosum* (Harv.) De Toni and *G. halymenioides* (Harv.) De Toni according to the illustrations in HARVEY, Phycologia Australica. The plant may also as to its habit be very like *Meristotheca tasmanica* J. Ag. according to KYLIN's figure of the original specimen in Herb. J. AGARDH; but according to KYLIN (*Gigartinales*, 1932, p. 29, pl. 12, fig. 29) this plant has a structure like that of *Faucheopsis.*

Figure 3 shows a photographic representation of the specimen which is a fragment only, and it cannot therefore be said how large the plant may grow. The fragment is 13 cm. long and below the thallus is about 1 cm. broad. The flat thallus is repeatedly furcated; along the margins numerous proliferations (Figs. 3, 4), very irregular in shape, are given out, the largest ones in the specimen reaching a length of about $^3/_4$ cm.; upon the flat side of the thallus no proliferations are found.

As to the anatomical structure (Fig. 5), the middle of the thallus is occupied by large cells, oblong in transverse section

Fig. 3. *Gloioderma mauritiana* Børgs. Habit of the original specimen. Natural size.

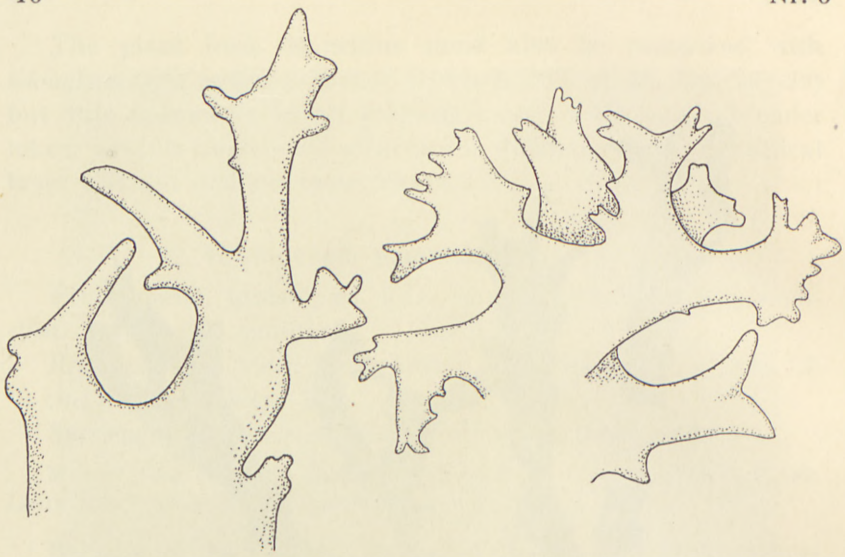

Fig. 4. *Gloioderma mauritiana* Børgs. Some of the marginal proliferations. (about × 15).

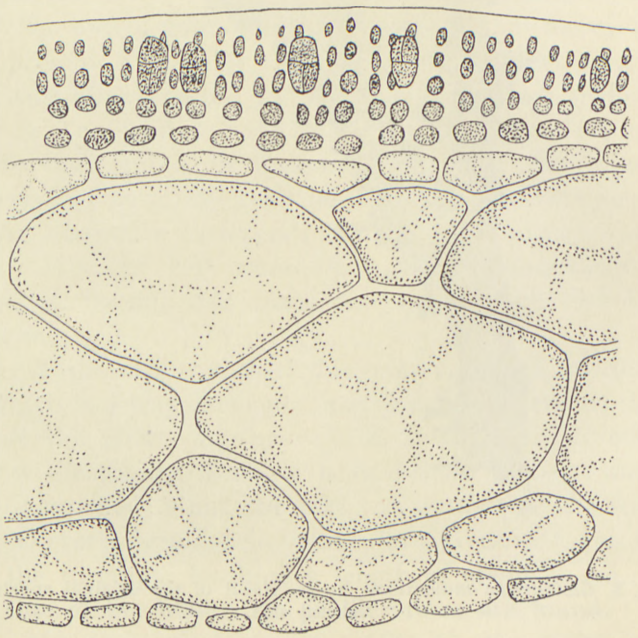

Fig. 5. *Gloioderma mauritiana* Børgs. Transverse section of the thallus. (about × 300).

and covered on both sides by smaller ones decreasing in size towards the periphery; from the outmost of these cells issue anticlinal assimilating filaments forming the cortical layer.

The tetrasporangia are formed in the cortical layer, being scattered over the surface of the thallus, and likewise found in the proliferations. They are cruciately divided.

The plant of which only a single specimen is found is mentioned in JADIN's list p. 163 as *Suhria vittata*. No exact locality is given. JADIN writes about it: "Cette plante paraît rare, je ne l'ai pas trouvée et n'ai reçu qu'un exemplaire receuilli sur la plage par DARUTY".

Fauchea Mont.

1. **Fauchea profunda** nov. spec.

Frons pygmæa, ca. 2—3 cm. alta, subcaespitosa aut flabellatim expansa, irregulariter lacinulata et lacerata, laciniis inferne angustioribus ad apicem vertens latioribus, cornubus damae persimilibus, per sinus rotundatos separatis, marginibus saepe proliferis et hic illic cum lobis vicinis anastomosantibus.

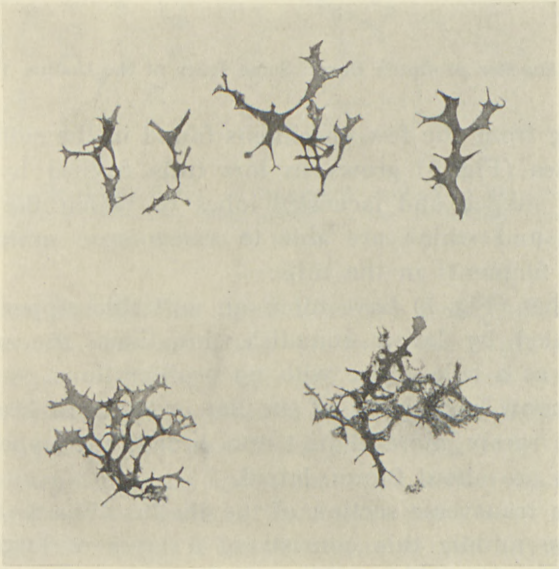

Fig. 6. *Fauchea profunda* Børgs. Habit of the original specimens. Natural size.

Substantia in sicco cartilagineo-membranacea.
Color plantae pulchre roseus-sanguineus.
Tetrasporangia in soros aggregata, ca. 35 μ longa et 24 μ lata.
Cystocarpia non praesentia.

Mauritius: Between Gunner's Quoin and Flat Island, dredged at a depth of 25 fathoms, 15. Oct. 1929, Th. Mortensen.

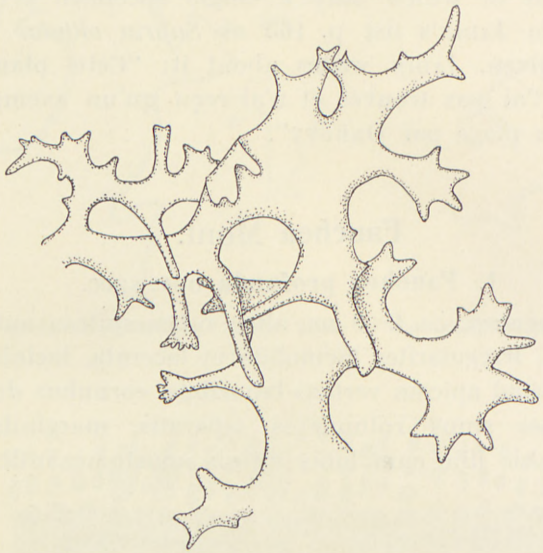

Fig. 7. *Fauchea profunda* Børgs. Some lobes of the thallus. (× c. 5).

Judging from the few specimens found in the collection this little species (Fig. 6) grows in low tufts formed by the very irregularly shaped and lacerated lobes of which the thallus is composed, and which are able to anastomose mutually with neighbour filaments in the tufts.

The lobes (Fig. 7) have often an antlerlike appearance and are separated by larger roundish sinuations, the margins of which are as a rule entire with no proliferations.

The narrow basal parts of the flat or more or less sinuated thallus are rarely more than 1 mm. broad, the upper lobes of the thallus are about 2 mm. broad.

From a transverse section of the thallus (Fig. 8 *a*) it is seen that in the middle this consists of a layer of large subrectangular cells about 90 μ thick and often more than 200 μ long;

in places also several layers of smaller cells are found; when observed from above the large cells are elongated-polygonal (Fig. 8 *c*). On both sides of the large cells some smaller ones are found from which issue the short assimilating filaments a few times divided. While the innermost cells in the assimilating

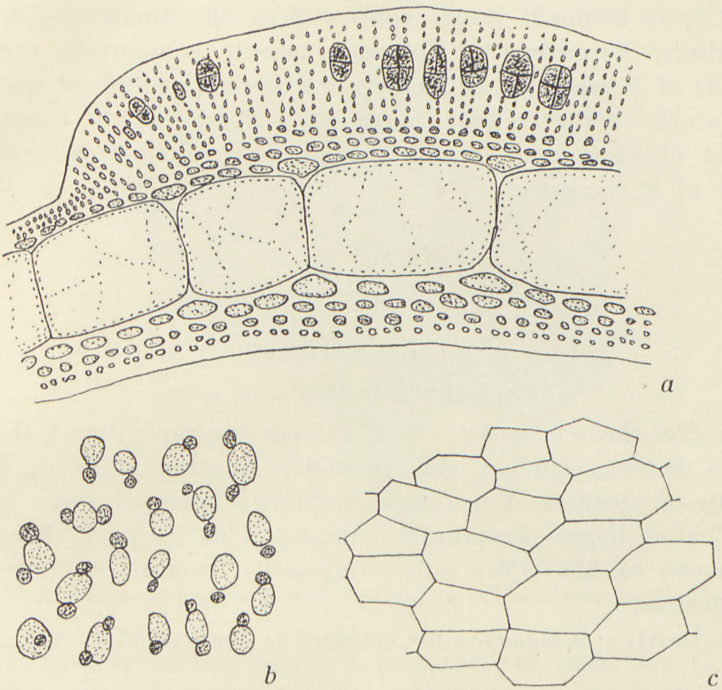

Fig. 8. *Fauchea profunda* Børgs. *a*, transverse section of the thallus with a sorus. *b*, peripheral cells seen from above. *c*, the medullary layer seen from above. (*a*, about × 400; *b*, about × 700; *c*. about × 75).

filaments are oblong, the uppermost are nearly globular. The small peripheric cells are given out from the larger ones below to a number of 1—2 (Fig. 8 *b*); they are scattered over the surface of the thallus with rather a long distance between them.

Only in one of the specimens have I succeeded in finding a single tetrasporic sorus and since most unfortunately, before I had discovered the sorus, I had made transverse sections of this part of the thallus I am not able to say anything about the shape of it. A transverse section (Fig. 8 *a*) of the sorus

shows that it has a well-marked edge, vaulted up above the surface of the thallus; the sorus is about 110 μ thick, having nearly the same thickness as that of the vegetative thallus.

Fauchea microspora Bornet (1890, p. 139), collected by Rodriguez in deep sea near the Balearics, which Mme Weber has found in a collection of algae from the Indian Ocean (1914, p. 282), is a much larger species than the small one from Mauritius. According to Bornet's minute comparison (1890, p. 142) of *F. repens* and *F. microspora* the last-mentioned species has much smaller tetrasporangia than *F. repens*. The tetrasporangia of *F. profunda* are somewhat shorter and broader than those of *F. microspora*.

Subfam. 2. Rhodymenieae.

Coelothrix Børgs.

1. **Coelothrix indica** nov. spec.

A *Coelothrice irregulari* (Harv.) Børgs. imprimis differt, thallo magis dense caespitoso, sine rhizoideis anastomosantibus, filamentis tenuioribus et structura anatomica etiam diversa.

Thallus dense caespitosus, suberectus, ca. 5—6 cm. altus, ex filamentis ca. 250—450 μ latis, irregulariter ramosis et contextis compositus.

Mauritius: Without locality, collected by Daruty 1893 (Herb. Jadin).

Of the monotypic genus *Coelothrix* hitherto only known from the West Indies I have found a specimen in Dr. Jadin's collection which even if it is nearly related to the West Indian plant (*Coelothrix irregularis* (Harv.) Børgs., 1920, p. 389, figs. 373—4) nevertheless seems to be the representative of another species. The specimen forms a dense tuft in shape and also in size very like a shaving brush (Fig. 9). The rather slender filaments are densely crowded and stick together, and this though I searched in vain for the groups of rhizoids which are so common in the West Indian plant and by means of which the filaments in this very irregularly shaped plant are connected. The filaments in the plant from Mauritius have a diameter of 250—400 μ rarely up to 450 μ, while those of the type-specimen

of *Coel. irregularis* (collected by PALLE BANG) are about 300—500 μ; but in the specimen of this species published in Phycotheca Bor.-Amer. no. 18 from Key West the breadth of the filaments is up to 900 μ.

The anatomical structure too presents differences. Upon a transverse section (Fig. 10) of the thallus it is seen that the epidermal cells are oblong-lanceolate, about 23 μ long and 8—9 μ

Fig. 9. *Coelothrix indica* Børgs. Habit of the typical specimen. Natural size.

broad. The medullary tissue is composed of some few layers of cells, the largest ones having a diameter of about 23—38 μ. The innermost cells upon which the glands are placed, facing the cavity in the interior of the thallus, are smaller. When compared with a transverse section of the West Indian plant the epidermal cells are proportionally shorter and broader, about 20 μ long and 12 μ broad. The medulla is thicker and composed of larger cells up to 50 μ broad. This statement is taken from the type specimen of *C. irregularis*; in the stouter plant from Key West the epidermal cells are about 23 μ long and 12—16 μ broad, the medullary tissue is very thick and the diameter of the larger cells is up to 60 μ long. When the peripheric cells of the cortical layer of the plant from Mauritius are viewed (Fig. 11) from above, the shape of the cells is irregularly elong-

ated angular of variable size, the larger cells attaining a length of about 45 μ and a breadth of 16—18 μ. In the specimen from St. Croix these cells are proportionally shorter and broader but a comparison is always difficult as the shape of the peripheric cells varies a good deal in different parts of the thallus.

Taking into consideration the differences mentioned above between the West Indian plant and that from Mauritius it seems

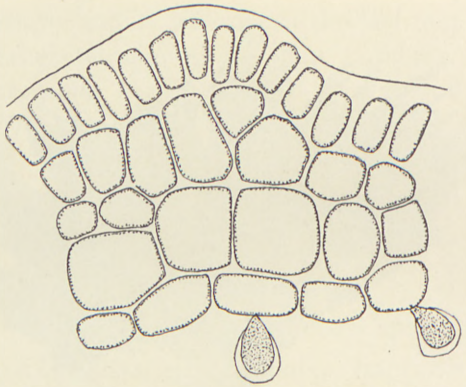

Fig. 10. *Coelothrix indica* Børgs. Transverse section of the thallus. (about × 300).

to me justifiable to keep the two forms, even if nearly related, specifically separated.

Most regrettably the specimen from Mauritius is sterile.

About the fruiting of *Coelothrix* I have mentioned in my paper (1920, p. 391) the rather few and incomplete details which were known when I described the genus. It is somewhat strange that in none of the papers it is said directly that the sporangia are cruciately divided, but as the plant was referred to the genus *Cordylecladia*, which has cruciately divided sporangia, it is of course justifiable to presume that in *C. irregularis* also the sporangia were cruciately divided.

Taylor also in his later published work: The Marine Algae of Florida, 1928, p. 160 gives no more exact information about its fructification. To be sure Taylor was the first to publish a habit figure of the thallus with stichidia but about the fructification he only says: "reproduction by tetraspores carried in short swollen ovoid pedicellate branches". Meanwhile there

seems to be no doubt that the sporangia of *Coelothrix* are cruciately divided (like those of *Cordylecladia*).

When therefore KYLIN, 1931, p. 31, in some critical remarks about *Coelothrix* and its systematic position pointed out concerning the tetrasporangia that they are tetrahedrally divided and this fact made its systematic position uncertain, I again made a thorough search in the literature for information about

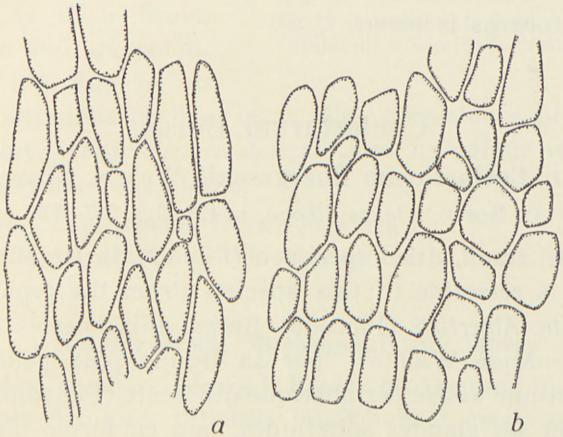

Fig. 11. Cortical cells of *Coelothrix* seem from above. *a*, from the specimen of *C. indica* Børgs. *b*, from the typical specimen of *C. irregularis* from St. Croix. (about 300).

the division of the tetrasporangia, and being unable to find anything else than that mentioned above I asked Professor KYLIN whether his statement was based upon his own investigations not yet published or, if this was not the case, where it was to be found. Professor KYLIN has now in a letter dated 25. Febr. 43 most kindly informed me that he himself has not seen the sporangia and that at present he cannot remember whence his information is derived. Finally KYLIN writes (translated into English):

"The information that the sporangia of *Coelothrix* are tetrahedrally divided I presume to be wrong, wheresoever it may have come from".

According to this declaration I think we can leave out of consideration the question of tetrahedrally divided sporangia in *Coelothrix*.

When I described the plant in Mar. Alg. D. W. R., vol. II, p. 389 I placed the new genus in the Subfam. *Rhodymenieae* of the Fam. *Rhodymeniaceae* just before the genus *Chrysymenia* at that time still comprehensive, to which genus and some of its sister genera it is surely most nearly related; and there it has also been placed in the present paper.

Meanwhile, for the final conclusion as to the systematic position of *Coelothrix* we must wait until an exact description of the cystocarps is given.

Coelarthrum Børgs.

1. Coelarthrum Boergesenii Web. v. Bosse.

Weber van Bosse, *Algues Siboga*, p. 473, figs. 207–208.

A single, small, dried specimen (Fig. 12), in Dr. Mortensen's collection is referable to this species. From the typical species *Coelarthrum Albertisii* (Piccone) Børgs. this species differs according to Mme. Weber: "par sa fronde plus petite, par sa paroi ayant une assise périphérique différente (Fig. 208), ses anastomoses et ses glandes sphériques, non en forme d'étoile."

Of these characters it is especially the cortical layer which makes the difference from that in *Coelarthrum Albertisii*, as it is coherent and formed by 1—2 layers of small cells. As to the anastomoses of the thallus I have not been able to ascertain this in the small dried specimen from Mauritius but such anastomoses are surely also present in *Coelarthrum Albertisii*. And as for the small irregularly stellate cells upon which the spherical gland-cells are placed in *Coelarthrum Albertisii* (comp. my figures 390—391 in Mar. Alg. D. W. I., vol. II, pp. 404—6) I have not, to be sure, been able to find them in the dried material from Mauritius, and thus convince myself that they are like those in *Coel. Albertisii*, but when Mme. Weber says that the gland cells are spherical, not stellate in *Coel. Boergesenii*, according to which statement it must be supposed that the gland cells in *Coel. Albertisii* should be stellate, this supposition must be said to be due to a misunderstanding. It is the mother-cells carrying the gland-cells which are stellate, while the glands themselves are spherical; compare my figures in Mar. Alg.

D. W. I., p. 405, figs. A and E and pag. 406, fig. 391.

After a comparison with West Indian material the habits and shapes of the plants from both areas seem to be rather alike, but the thallus of the plant from Mauritius is of a firmer consistence and its colour a darker red due to its thicker, continuous cortical layer. The consistence of the West Indian plant is much more delicate and its colour is rosy-red.

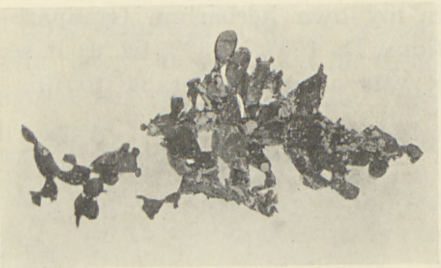

Fig. 12. *Coelarthrum Boergesenii* Weber. Habit of a specimen. Natural size.

The specimen was sterile.

Mauritius: Flat Island, 17. Oct. 1929, Th. M.
Geogr. Distr.: Malayan Archipelago.

1. **Coelarthrum Mortensenii** nov. spec.

Coelarthro opuntiae (J. Ag.) Børgs. proximum, quod tamen thallo magis firmiore, articulis latioribus et magis ovalibus et structura anatomica diversa a nostra specie praecipue distinguitur.

Frons rosea, subcylindrica vel subcomplanata, circiter 10 cm. alta et ultra, identidem furcata et articulata.

Articuli elongati-oblongi in parte basali ca. $2^1/_2$ cm. longa et 4 mm. lata ad apicem vertens minores et magis obovales.

Substantia thalli molliuscula.

Tetrasporangia cruciatim divisa in superficie thalli dispersa.

Cystocarpia singula hic illic praesentia.

Mauritius: Between Gunner's Quoin and Flat Island, dredged at a depth of 25 fathoms, 15. Oct. 1929, Th. Mortensen.

Dr. Mortensen's collection contains some specimens of a *Coelarthrum* which I at first presumed to be the same as the Indian *Coelarthrum Opuntia* (J. Ag.) Børgs., but after a comparison with the figure of the type specimen in J. Agardh's herbarium (compare Kylin, Rhodymeniales, p. 33, pl. 20, fig. 46) and with Mme. Weber's good figure in Algues Siboga, p. 408, pl. XVI, fig. 7 as also with a specimen from South India found

in my own herbarium (compare the figure of it in Contributions, II, 1937, p. 333, fig. 9) it seemed to me doubtful whether it was actually referable to this species. Consequently I have

Fig. 13. *Coelarthrum Mortensenii* Børgs. Habit of the original specimen. Natural size.

made, too, some comparison of the anatomical structure of the two plants and as I have also found some differences here I have no hesitation in considering the plant from Mauritius as a distinct species.

If the habit of the plant from Mauritius (Fig. 13) is compared with that of *Coel. Opuntia*, it will be seen that the joints of which the former is composed are much slenderer, more elongated oblong with cuneate base than the much broader joints obovate in shape found in *Coel. Opuntia*.

In the habit figure of the large specimen (Fig. 13) the joint to the left above the long basal joint in the figure, is $1^1/_2$ cm.

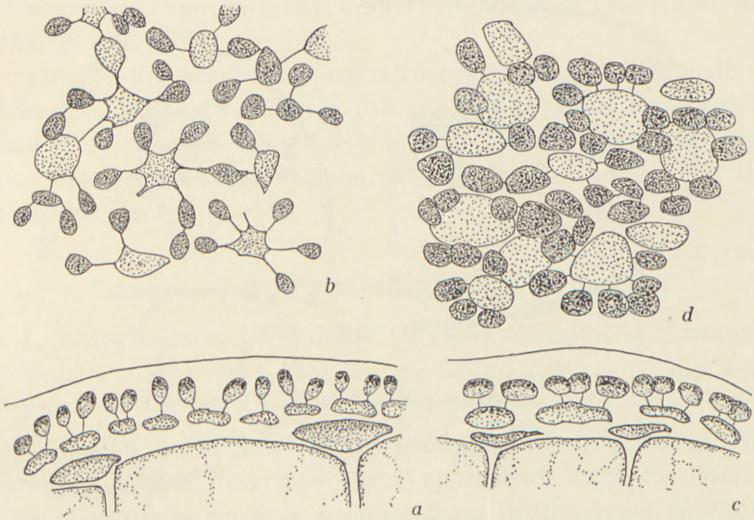

Fig. 14. Transverse sections and surface view of *Coelarthrum Mortensenii* Børgs. (*a, b*) and of *Coelarthrum Opuntia* (J. Ag.) Børgs. (*c, d*) respectively. (× about 500).

long, above 4 mm., decreasing slowly to the base; and the joint to the right is at the top 3 mm. broad, increasing downwards to 4 mm. and then tapering to the cuneate base where it is 1 mm. broad below. Upwards in the thallus the joints decrease in size, especially in length, becoming more oblong. From the upper ends of each joint two, in more rare cases, three joints are given out. The colour of the thallus is rosy red and its consistency is rather delicate; this may perhaps have some connection with the fact that the plant was dredged in deep water.

As to the anatomical structure of the plant from Mauritius the cortical layer in transverse section (Fig. 14 *a*) is composed of about two layers of cells, some larger cells below, from which two to four or more obovate cells are given out towards the

periphery. When the peripheric tissue is observed from above (Fig. 14 *b*) the larger cells below are found to be very irregularly shaped, often even stellate and from the corners of these cells the more coloured obovate, peripheric, assimilating cells are given out. As the figures show, these cells are rather distantly placed. For the sake of comparison I have made some similar drawings of the cortical layer of *Coel. Opuntia* (Fig. 14 *c*, *d*).

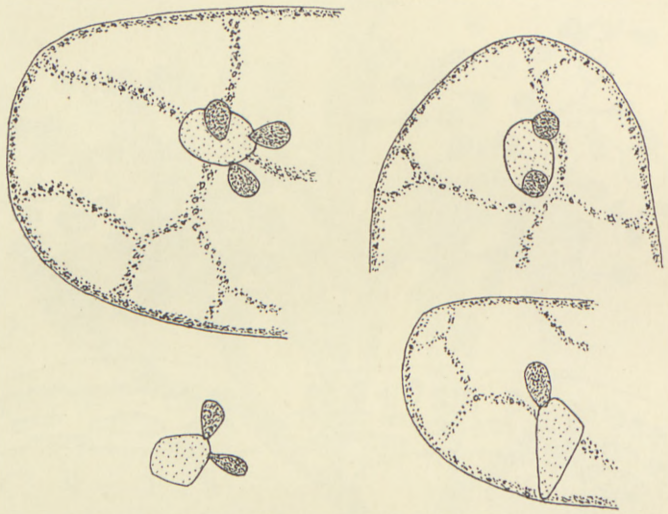

Fig. 15. *Coelarthrum Mortensenii* Børgs. Fragments of the large cells of the wall facing the cavity carrying small cells with glands. (× about 200).

A glance at the figures of both plants is all that is needed to see the differences prevailing, the cortical layer in *Coel. Opuntia* being much more firmly built than that of *Coel. Mortensenii* owing to the fact that the peripheric cells as well as those below are larger and much more densely placed than those of *C. Mortensenii*. Also the shape and size of the cells is different, as those of *Coel. Opuntia* are more roundish and the subepidermal cells not stellate.

The diameter of the peripheric cells of *Coelarthrum Opuntia* is 5—12 μ long while in *Coel. Mortensenii* by far the greater number of them are about 5 μ broad, only some few larger ones up to 8 μ.

Below the cortical layer there is a single layer of large clear cells which in *Coel. Mortensenii* are up to about 500 μ long and

nearly half as broad. Upon those facing the cavity in the interior of the thallus small cells are found here and there, provided with 1—3 glands (Fig. 15), the shape of these small cells is mostly oblong, also more irregularly polygonal, but real stellately shaped ones, as these usually are in *Coelarthrum*, I have not found. The large cells in *Coel. Opuntia* are roundish-polygonal and much smaller, having a diameter of only 110—150 μ, and the cells carrying the glands are more irregularly shaped, often stellate; compare my figure 10 *a* and *b* in Contributions II, 1937, p. 334.

Two of the specimens are tetrasporic; the cruciately divided sporangia are scattered over the surface; a small fragment of a plant is cystocarpic. Like those in *Coel. Opuntia* (comp. my figure mentioned above) the cystocarps are developed here and there upon the thallus.

Botryocladia Kylin.

1. **Botryocladia Kuckuckii** (Weber) Yamada et Tanaka.

Yamada and Tanaka, Mar. Alg. Yonakuni, 1938, p. 77. figs. 8–9. — *Chrysymenia Kuckuckii* Weber, Alg. Siboga, 1928, p. 466, fig. 199.

It seems to me rather questionable whether *Chrysymenia Kuckuckii* Weber described upon specimens from the Malayan Archipelago is to be kept separate from *Chrysymenia Skottsbergii* Børgs. from Easter Island established by me in the year 1920 in my paper on the algae of this island. In spite of both plants being so very nearly related, Mme. Weber when describing her species has evidently not taken into consideration the plant from Easter Island.

As I have in the material of algae from Mauritius some very good specimens of a plant (Fig. 16) of the same type as Mme. Weber's species mentioned above, I have made a comprehensive comparison of this plant with that from Easter Island and have arrived at the result that they are very much alike not only as to their habit but also in their anatomical structure, both plants having a continuous firm cortical layer and the gland-cells placed in groups up to a number of 10 upon the mother cell. To be brief, the difference between the two species is that *Chrysymenia Kuckuckii* seems to be in all respects some-

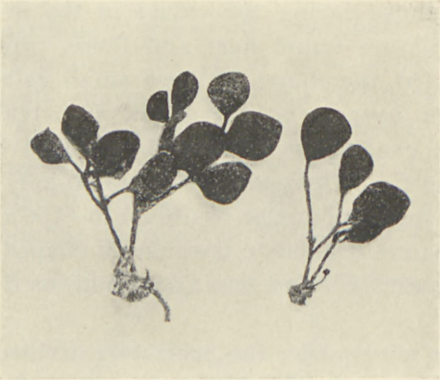

Fig. 16. *Botryocladia Kuckuckii* (Weber) Yamada et Tanaka. Habit of specimens. Natural size.

what larger than *Chrysymenia Skottsbergii* and to this must be added as perhaps the most essential difference that the wall of the vesicles of *Chrysymenia Kuckuckii* in transverse section proves to be composed in most cases of two layers of large cells reaching a thickness of about 150 μ, while that of *Chrysymenia Skottsbergii* has mostly one layer only; here and there, however, two layers are found and the thickness of the wall is about 100 μ. Compare as to this feature Mme. WEBER's fig. 199 and YAMADA and TANAKA's fig. 9 with my figure 50 *c*.

Concerning the relative sizes of the two species, the vesicles of *Chrysymenia Skottsbergii* are 5—6 mm. long and 4—5 mm. broad, while those of *Chrysymenia Kuckuckii* are about 6—8 mm. long and 6 mm. broad. And similarly, in accordance with this the innermost large cells in the wall of *Chrysymenia Skottsbergii* are up to about 100 μ broad, while those in *Chrysymenia Kuckuckii* may reach a breadth of 120—150 μ.

Fig. 17 shows some of the large cells of both plants drawn to the same scale of magnification.

The cortical cells of the plant from Mauritius are roundish when seen from above, about 7 μ broad; in transverse section they are oblong.

Occasionally groups of gland cells are found, but not in great number. The gland cells (Fig. 18) issue from small somewhat polygonal cells protruding somewhat above the surface of the surrounding large cells of the wall facing the cavity in the interior of the vesicles. These cells in some cases carry only a single or a few glands, in some they are densely covered by glands up to a number of 10 or more; the glands vary a good deal as to size, the larger ones having a diameter of about 35 μ. The arrangement and shape of the gland cells of the plant from

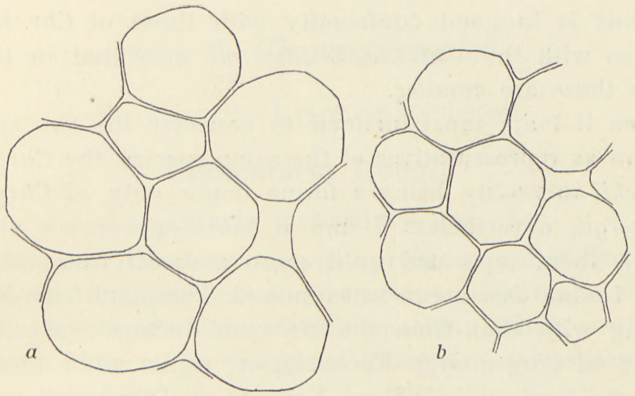

Fig. 17. Some of the large cells of the wall. *a*, from the plant from Mauritius; *b*, from that of Easter Island. (× about 125).

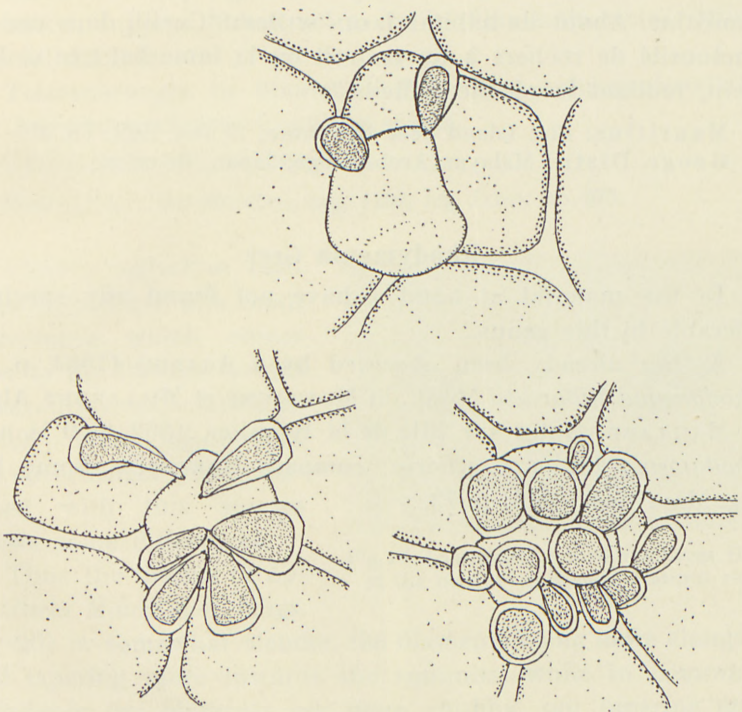

Fig. 18. *Botryocladia Kuckuckii* (Weber) Yamada et Tanaka. Groups of gland cells. (× about 200).

Mauritius is in good conformity with those of *Chr. Kuckuckii* and also with those of *Chr. Skottsbergii*, only that in the latter species these are smaller.

Even if I am most inclined to consider the two species in question as representatives of the same species, the *Chrysymenia Kuckuckii* in reality being a forma *major* only of *Chrysymenia Skottsbergii*, nevertheless I find it more appropriate at present to keep them separated until more material from other parts of the Indian Ocean can be examined. The plant from Mauritius agreeing with that from the Malayan Archipelago is therefore referred to *Chrysymenia Kuckuckii* or, as its name now is, *Botryocladia Kuckuckii* (Weber) Yamada et Tanaka.

JADIN in his list p. 166 mentions *Chrysymenia obovata* Sonder from Réunion. Dr. JEAN FELDMANN has most kindly sent me a small piece of JADIN's specimen from the examination of which it is obvious that it is in reality the same species as that from Mauritius. About its habitat JADIN writes: "Cueilli dans une anfractuosité de rochers à un endroit où la lame bat très violamment, rendant la récolte difficile."

Mauritius: Flat Island near the shore, 17. Oct. 1929, TH. M.
Geogr. Distr.: Malayan Archipelago, Japan, Réunion.

Rhodymenia Grev.

In the material at hand I have not found any specimen referable to this genus.

As has already been observed by J. AGARDH (1884, p. 64), *Rhodymenia Millardetii* Mont., in MONTAGNE et MILLARDET, Algues in MAILLARD, Notes sur l'Ile de la Réunion, 1862, p. 9 is not a *Rhodymenia* but a *Gracilaria*; compare BØRGESEN, Some Mar. Alg. Mauritius, 1943, p. 72.

Fam. 2. Champiaceae.

Subfam. 1. Lomentarieae.

Lomentaria Lyngb.

1. Lomentaria mauritiana nov. spec.

Lomentariae corallicolae Børgs. proxima sed differt thallo paulo graciliore, filamentis ad apicem vertentibus magis elongatis et gracilioribus, ramis interdum suboppositis; cellulis corticalibus a superficie visis magis elongatis. Filamentis tetrasporangiferis etiam gracilioribus.

Frons usque ad 1 cm. alta, caespitosa, teres aut subcomplanata (?), tubulosa, filamenta crassiora ca. 500—600 μ lata, in superiore parte tenuiora ca. 200 μ lata.

Rami hic illic irregulariter evoluti, in parte basali plus minus constricti, superne obtusi vel saepe in filamentis vicinis rhizoideis adhaerentes.

Tetrasporangia in filamentis latioribus, subcomplanatis (?) per totam superficiem frondis dispersa.

Cystocarpia non praesentia.

Mauritius: Ilot Barclay, Aug. 1890, leg. JADIN no. 373.

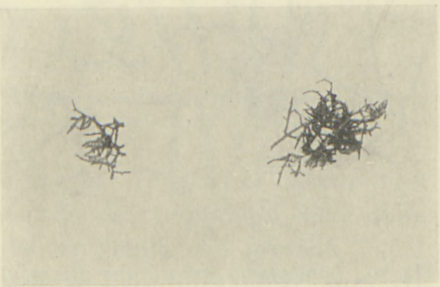

Fig. 19. *Lomentaria mauritiana* Børgs. Habit of the original specimen. Natural size.

JADIN's collection contains a specimen of a small *Lomentaria* which comes near to the Iranian species *Lomentaria corallicola* Børgs. (1939, p. 113—6, figs. 30—32), but nevertheless when compared with this species shows some differences.

Thus the thallus of *Lomentaria Mauritiana* (Figs. 19—20) is somewhat slender, the filaments being more elongated and tapering more towards the summits, while in *Lomentaria corallicola* the filaments are more chubby, not tapering much towards the summits which are more broadly rounded.

And while the filaments in the plant from Mauritius are in

most cases provided with some few branches which are often subopposite ly placed (Fig. 20) those in *L. corallicola* had a few scattered branchlets or none at all.

As is the case in *L. corallicola*, so also in *L. mauritiana* rhi-

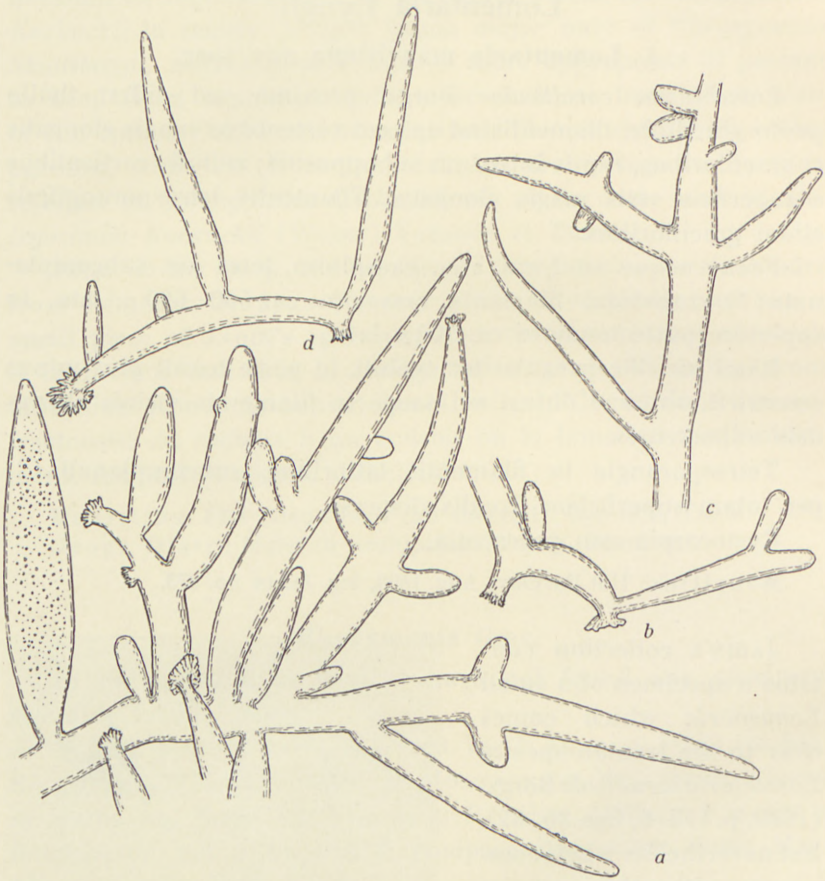

Fig. 20. *Lomentaria mauritiana* Børgs. Fragments of the thallus; in *a*, the erect branch to the left with tetrasporangia. × about 10 natural size.

zoids are often developed from the apices of the filaments (Fig. 20) by means of which the filaments of the tuft become firmly connected, thus strengthening the tuft; decumbent filaments likewise are able to fix themselves to the substratum.

Fig. 21 *a* shows a small part of the peripheric cortical cells seen from above; these are oblong, elongated in the direction of

the thallus with oblique walls above and below, and not arranged in rows. The larger cells are about 40 μ long and half as broad, but several of the cells are smaller. Compared with the cortical cells in *L. corallicola* those in this species are more roundish-polygonal, proportionally broader and shorter, having a diameter of up to 27 μ in the larger cells.

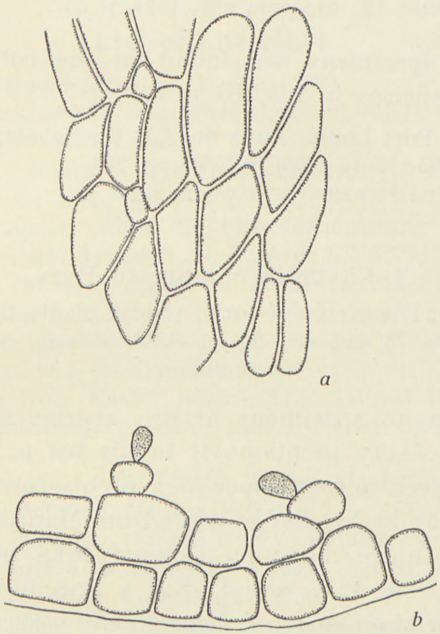

Fig. 21. *Lomentaria mauritiana* Børgs. *a*, cortical cells seen from above; *b*, transverse section of a fragment of the thallus. (× c. 250).

A transverse section of the thallus (Fig. 21 *b*) shows that this consists of a few layers of cells only; here and there upon the innermost cells facing the cavity glands are present.

The tetrasporangia occur scattered over the thallus; they are cruciately divided and are about 42 μ broad.

According to the number of the specimen (no. 373) JADIN in his list calls this plant *Champia Kotschyana*. As to its habitat he writes: "Croissant sur des coquilles rejetées à la plage."

Subfam. 2. Champieae.

Champia Desv.

1. Champia parvula (Ag.) Harv.

Harvey, W. H., Nereis Bor.-Am. vol. II, p. 76. J. Agardh, Epicrisis, p. 303. Bliding, C., Studien über ... Rhodymeniales, 1928, pp. 5—22. — *Chondria parvula* Ag., Systema alg., 1824, p. 207.

Some few specimens are found in the collections. Jadin mentions this species in his list p. 167.

Mauritius: Port Louis, Jadin no. 351. Rochebois, Aug. 1890, Jadin no. 377. Flic-en-Flac, July 1939, R. E. V., no. 279.
Geogr. Distr.: Expansive in warm seas.

2. Champia compressa Harv.

Harvey, W. H., The genera of South African plants, 1838, p. 402; Nereis Australis, 1847, p. 78, tab. 30, figs. 1—6. J. Agardh, Spec. alg., p. 370; Epicr., p. 305.

I have seen no specimens of this species from Mauritius. From Réunion Jadin mentions it in his list p. 166. From this island I have seen a small specimen of his (no. 143).

Dickie (1875) in his list of algae from Mauritius based upon a collection of algae made by Colonel Pike mentions p. 193 *Ch. compressa* from this island. But a specimen collected by Colonel Pike at Mauritius and determined by Dickie as *Ch. compressa* is in reality *Ch. parvula*. The specimen belongs to the Riksmuseum, Stockholm.

As *Ch. compressa* occurs at Réunion there is every reason to believe that it also occurs at Mauritius, for which reason it is inserted in the list.

Réunion: Sainte-Gilles, Jadin, April 1890, no. 143.
Geogr. Distr.: Ceylon, Cape, Borneo, Friendly Islands etc.

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with some few synonyms, the latter printed in italics.

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Færdig fra Trykkeriet den 4. Marts 1944.

DET KGL. DANSKE VIDENSKABERNES SELSKAB
BIOLOGISKE MEDDELELSER, BIND XIX, NR. 7

ON THE SPICULE-FORMATION OF *SPONGILLA LACUSTRIS* (L.)

1. THE DEPENDENCE OF THE SPICULE-FORMATION ON THE CONTENT OF DISSOLVED AND SOLID SILICIC ACID OF THE MILIEU

BY

C. BARKER JØRGENSEN

KØBENHAVN
I KOMMISSION HOS EJNAR MUNKSGAARD
1944

CONTENTS

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INTRODUCTION[1]

In the fresh-water sponge, *Spongilla lacustris*, the spicules belong to two classes, viz. flesh-spicules, microscleres, which seem to be scattered about loosely in the sponge tissue, and skeletal spicules, megascleres, which go to form the main skeleton of the sponge.

The formation of spicules in the siliceous sponges has been the subject of many investigations. MINCHIN (1909) has given an excellent review of older works. Recent investigations on fresh-water sponges have been carried out by SCHULZE (1923), WIERZEJSKI (1935) and SCHRÖDER (1936). The accounts by different authors of the origin of the spicules contain considerable contradictions; among the views expressed with regard to *Spongilla* the following is the best-founded and agrees with my own observations. The spicules are laid down in a mother-cell as an organic axial thread, the nature of which is rather unknown. The axial thread is stated to be formed by quite small granules forming a line and fusing into a fine thread (SCHRÖDER 1936). Upon this thread silica secreted by the mother-cell is deposited. There is still some disagreement as to whether the mother-cell alone is able to complete the formation of microscleres while, on the other hand, the megascleres evidently need the aid of several secondary silicoblasts for the completion of their formation.

Microscleres and megascleres are said to be laid down in cells of different types. The skeletal spicules arise in "undifferentiated" archaeocytes with nucleolus containing nucleus,

[1] The present paper forms part of a series of investigations into the developmental physiology of Spongillidae, planned by the laboratory. Work already published is: H. V. BRØNDSTED: Formbildungsprozesse bei einem sehr primitiven Metazoon, dem Süsswasserschwamm *Spongilla lacustris* (L). I. Die Entstehung der äusseren Form. "Protoplasma" 1943, Bd. XXXVII, Heft 2.

while the microscleres originate from "specialized" cells with nuclei rich in granulae and without nucleous. (EVANS 1899). WIERZEJSKI states, however, that he has observed the formation of microscleres in cells of a type which is generally ascribed to megascleroblasts.

As regards the chemical compositions the spicules of the siliceous sponges consist mainly of SiO_2 (ca. 92 %). The greater portion of the remainder is water (ca. 7 %), but some MgO, K_2O and Na_2O also occurs. C and N do not appear in such quantities as to be demonstrated (BÜTSCHLI 1901). SOLLAS (1888) and other authors have carried out investigations into the quantity of water contained in dried spicules. Excepting the fact that THOULET (1884) found the water percentage of the spicules to be 13, all other analyses have shown a water percentage varying from 6 to 7 and but rarely a little more. It is difficult to say to what extent water is chemically bound, but from the fact that the presence of submicroscopic watery vacuities has been proved in the inner layers of relatively larger spicules (SCHMIDT 1926), it can at any rate be inferred that water may occur also in other ways.

Below special questions regarding the formation of spicules will be discussed, particularly the formation of microscleres, which has been investigated on gemmula sponges kept in water with varying contents of silica. It has been my special endeavour to clear up a possible state of inter-dependence between the silicic acid content of the surrounding medium and the content of spicules, their form and size, in the gemmula sponges. Finally the question has been examined, whether the sponges themselves are able to dissolve solid silicates, or whether their formation of spicules depends exclusively on the presence of dissolved silicic acid. Before mentioning the experimental investigations a brief account will be given of the first development of the gemmula sponge from germination until differentiation of the sponge tissue has commenced.

Germination, Spreading and First Differentiation.

The content of the gemmulae of *Spongilla lacustris* consists only of one single type of cells, viz. the undifferentiated two-nucleused archaeocytes (WIERZEJSKI 1935). After germination

the latter are modified into all the various cell-components of fully developed sponge. The cell material leaves the gemmulae through the preformed porus, which in the intact gemmula is covered with a thin lid. When leaving the gemmula the cell material is normally quite undifferentiated, but an initiatory differentiation of the cell-components in the ungerminated gemmula has, however, been recorded, which can proceed so far that the formation of canals, flagellated chambers and spicules can be observed (WIERZEJSKI 1935). I never noticed this myself. In the experiments described below the cells which have crept out of the gemmulae have, at any rate, always been undifferentiated and sharply limited.

While passing out of the gemmula, the cell material will gradually cover the shell of the gemmula and spread on the underlayer. The size of the area covered by a young gemmula sponge depends upon both the number of cells and the nature of the underlayer (BRØNDSTED 1943). Germination and spreading of the content of a single gemmula will last about 24 hours at room temperature, but by the end of that time the shell of the gemmula is not necessarily empty; some of the content may still be left.

In the course of the first 24 hours I did not observe any development of specific tissues, but an initiatory formation of syncyties does take place, especially at the borders of the young gemmula sponge spread on the underlayer. Smooth transitions can be noted between the isolated, centrally lying archaeocytes, which have just broken out from the gemmula, and the strongly distended syncytieforming cells in the peripheric parts of the sponge.

About 48 hours after the cells have broken out the formation of the spicules of the young gemmula sponges usually commences, it being possible to see quite thin spicules, less than 1 μ thick, scattered about in the sponge tissue. The megasclerformation seems to be more vigorous than that of the microscleres at the outset, but the former is soon out-distanced by the latter. At first the formation of spicules shows a distinct tendency to localization since the sponge hat many patches with numerous developing spicules and great intermediate areas of tissue without spicules or with a few developing ones only.

In the course of the second 24 hours the formation of the dermal epithelium will also begin; after three days it can be fully developed, covering the strongly developed subdermal room. At the same time the epithelium covered canals are laid down in the sponge tissue and the differentiation of the flagellated chambers and their functional union takes place. Simultaneously the epidermis is perforated for the formation

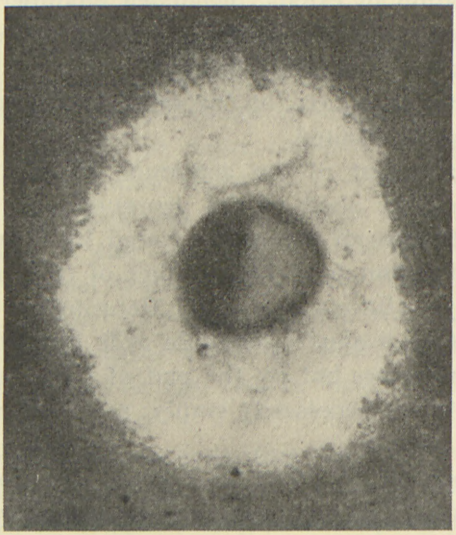

Fig. 1 a. Gemmula completely spread.

of osties, one or a few in each sponge, and also the oscular tubes are developed, likewise one or a few in each gemmula sponge, which thus passes into the functional stage (fig. 1 b). The diameters of the osties vary rather considerably. Three measurements showed the following lengths: 6, 20 and 40 μ. In spite of the fact that carmin granules have been observed to flow vigorously through the osties after carmin suspension has been added to the sponge cultures, I have never seen openings in the oscula on my own sponges. The easiest way of discovering the osties, which may otherwise be difficult to detect, is to add such suspensions. Outlets on such functional sponges have never been found in spite of numerous attempts.

The carmin granules are accumulated in the phagocytic cells of the nearest flagellate chambers, whereto they are carried

through a system of canals connected with the subdermal room. Carmin granules, however, are not found in the peripherical, flattened out portions of the sponge, where the flagellated chambers are primarily absent, and neither are they in the epidermis proper. Thus the cells of the epidermis do not, apparently, take up formed constituents although they are able to form pseudopodies; in other words, there seems to be no phagocytic action from the surface of the sponge.

Primarily, at any rate, the formation of microscleres takes place in the interior of the sponge, and during the first days

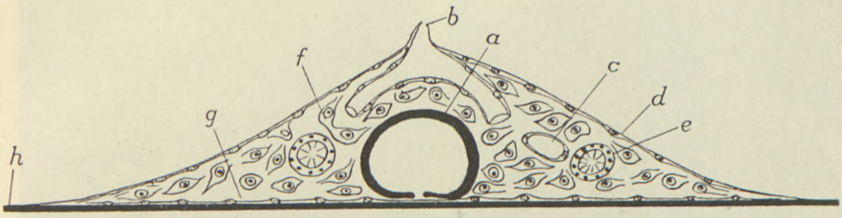

Fig. 1 b. Diagrammatic vertical section of a germinated gemmula.

after their appearance the microscleres hardly ever occur in the epidermis. But as early as two or three days after the first ones have been laid down the microscleres are carried to the surface of the sponge, where they are gradually placed in great numbers in the epidermis proper. Many are also placed in the epithelium covered walls of the subdermal room and canals. It is not only the fully formed spicules, which move peripherically, but spicules at every stage of development are met with in the epidermis at this juncture, all enclosed in their respective spiculiblasts. The larger spicules, however, prevail, while quite young ones are rare.

The development of the megascleres is not exactly known. They arise in the same places as do the microscleres. At their first appearance no definite arrangement or orientation in the sponge tissue can be detected; but gradually, often in the course of a week, they can be seen lying separately or in bundles of 5 to 6 spicules, placed more or less at a right angle to the surface of the sponge, their free ends projecting. The microscleres of the epidermis are, on the contrary, always placed on a level with it (fig. 2).

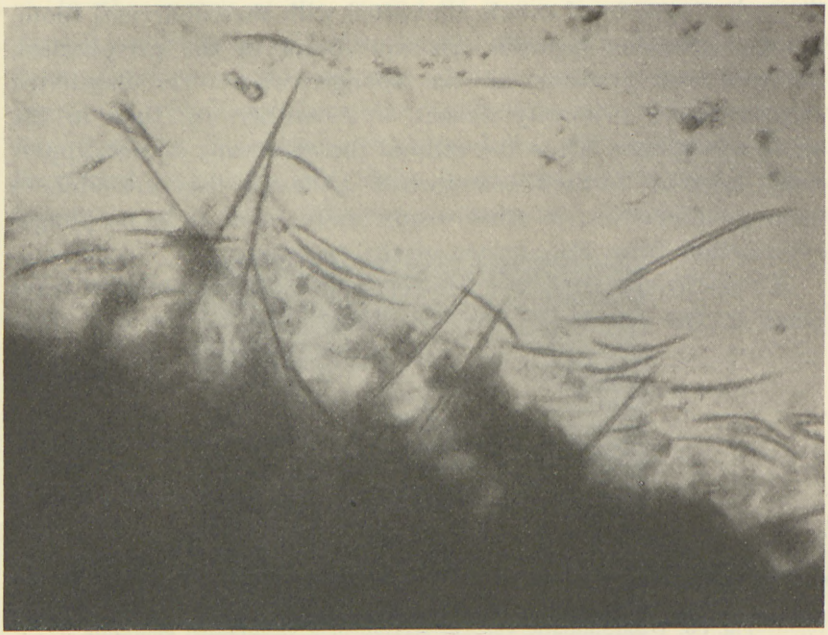

Fig. 2. The edge of a gemmula sponge 11 days old from a culture with 0.32 mM SiO_2. The megascleres are placed at a right angle to the surface of the sponge, while the microscleres lie in the epidermis proper. The subdermal room is easily distinguished above the deeper lying more compact sponge tissue. (× 190).

As regards the rate of growth no systematic experiments have been undertaken, but it may to some extent be estimated. In gemmula sponges kept in tap water, containing sufficient silicic acid as to permit a strong production of spicules, the first scattered microscleres, less than 1 μ thick, were discovered about 24 hours after germination and spreading. On examination 40 hours later the sponges showed the presence of numerous microscleres of 3 to 4 μ thick. 24 hours later a still greater number of microscleres had attained these dimensions, but none exceeded them. That is to say that the spicules can attain their full size, in this case 3—4 μ, in less than 40 hours at the temperature, silica concentration and in the experimental conditions used. Since it must be supposed that the rate of growth depends on many various factors e. g. the silicic acid content of the medium, a thorough investigation on this problem is strongly required.

Material and Technique.

The gemmula which were isolated for the experiments were as far as possible cleansed of adhering skeletal remains and loose spicules. The germination and development took place in paraffined *petri* vessels with 9 cm.'s openings containing about 50 ml double-destilled water, to which was added the necessary salts together with silica in the form of Na_2SiO_3, 8 H_2O in varying quantities. The second destillation was carried out in a glass-retort, and the water destilled was collected in a paraffined flask. The composition of the salt solution without silica is given in table I.

Table I.

	Cl^-	SO_4^{--}	Na^+	K^+	Ca^{++}	Mg^{++}
Milliequivalents	0.86	0.43	0.36—0.52 (2.56)	0.04	0.68	0.16

Each culture contained about 50 gemmulae, which during the germination partly gave rise to separate sponges, and partly constituted colonies containing varying numbers of gemmulae. The p_H values of the cultures were about 7 to 7.5 during the experiment excluding only the glass vessel with the greatest content of SiO_2 (1.28 mM) where p_H was nearer 8.

The formation of spicules commenced immediately after germination and spreading had finished. When the young sponges were 4 days old—that means about 4 days after germination and spreading, which lasted about 24 hours in the case of all gemmulae—the experiment was interrupted, and the sponges were fixed in 70 % alcohol.

The formation of spicules was estimated by counting and measuring the formed spicules, which were cleansed in the following manner. The young sponges were placed in a hollow-ground slide and covered with a few drops of 70 % lactic acid, which was carefully heated over a small gas-flame until it started boiling. In the course of a few minutes the soft bodies had entirely dissolved. Thereupon the solution was diluted with water and counts and measurements were carried out on the preparation treated in this manner, where the loss of spicules

was completely avoided. Lengths and thicknesses of the spicules were measured by means of a measuring ocular. In the following the measurements of the spicules are given in units of measurements at the degree of magnification used, unless otherwise started. 1 unit = 0.206 μ.

The method of measurement was quite simply as follows. All the spicules, which entered the field of vision as the preparation was carried before the objective, were measured. A cross-table served to conduct the object. In this way any special selection of spicules was avoided. As a rule, the thicknesses of the spicules have only been measured on the regularly formed spicules without middlethickenings and outgrowths, and the curves for the variation of the thickness of the spicules were based exclusively on slender regular spicules.

As regards the counting of the total number of spicules, which was made by means of a net ocular, all spicules were counted in one single case, but this method was found to be too laborious. In all other cases the spicules were only counted on a rectangular area, the breadth of which was equal to the side of the biggest square of the net ocular, which could be seen in the field of vision without the corners cut off and the length of which was equal to the diameter of the preparation (about 20 times the breadth). The counting was most frequently carried out along two diametres at right angles to each other, and in such cases about $^1/_8$ of the whole area containing spicules has been counted. Owing to the hollow grinding of the underlayer the spicules are inclined to concentrate towards the middle of the preparation, and the total number of spicules has therefore been calculated from the formula $2\pi \sum r \cdot n$. r is the distance from the centre of the preparation, using the side of the net ocular as a unit of measurement, and n is the number of spicules in the square at such a distance. In this way two or four values for the total number of spicules were obtained, according as the countings had been carried out along one or two diameters respectively. On the basis of the greatest and smallest number of spicules found and the volume of gemmulae, which gave rise to the spicules, the number of spicules per volume unit of gemmulae was calculated. The numbers of spicules thus obtained may directly be compared.

Formation of Spicules at Various Silicic Acid Concentrations.

At the outset the silicic acid content may be considered to influence the following factors during the formation of spicules, viz. the number, length and thickness of the spicules. In order to examine if such a relation does actually exist gemmulae were kept in cultures with increasing silica content. The composition of the culture medium without silicic acid has been referred to on pag. 9. To each of the six paraffined *petri* vessels containing 50 ml of this weak salt solution sodium silicate was added in such quantities that the following concentrations were obtained (table II).

Table II.

Culture	Quantity added of Na_2SiO_3, 84_20 in mg	SiO_2 in mM
Si 0	0	0
Si 2	0.53	0.02
Si 4	1.06	0.04
Si 8	2.12	0.08
Si 16	4.25	0.16
Si 128	34.00	1.28

Thus the various cultures differed both as to the content of silica and sodium. It is, however, supposed that the variations in the sodium content are without influence on the sponges and thereby also on the formation of spicules. As matter of fact only about 2.5 mM Na^+ is found in culture Si 128, while *S. lacustris* has been found in nature in localities, which can, at any rate have a salt promille of 1.6 = ca. 27.5 mM Na.

Just after the silicate had been added, the p_H values of the cultures with a fairly great silicate content were rather strongly displaced in the basic direction, but in the course of a few days the values were 7.0 to 7.5 in all the vessels, excluding that with 1.28 mM SiO_2, where the p_H value was nearer 8.

When the reaction proved stable, 50 to 60 newly gathered gemmulae were placed in each vessel (17th April 1942). As early as the next day the germination of some gemmulae had started in most of the vessels. On the 21st all gemmulae had germinated and spread, and there was a strong formation of

spicules. On the 23rd the experiment was interrupted, and the sponges were fixed in 70 % alcohol.

a) Number of Spicules.

The counting of the number of spicules was carried out exclusively on sponges, where the gemmulae as far as possible had been quite emptied, and with empty gemmula shells, which had still kept their globular shape, thus making it easy to calculate their volume. Each culture contained only a few sponges complying with these demands. The result is given in tables III and IV.

The results of the counts of the number of microscleres are shown in table III. The table also comprises the number of spicules in sponges from experiments, which will later on be referred to (pag. 39). In the latter the gemmulae were kept in cultures of various substratum, as e.g. the spicules of the siliceous sponge Jophon sp. and pulverized ortoclas. These series of experiment swere carried out simultaneously, the gemmulae used in both series originating from the same sponge. The figures can accordingly be directly compared. The lines 1—2 give the smallest and greatest total number calculated on the basis of the counts made along a radius. 3 denotes the number of radia along which the counting has been undertaken. 4 shows the volume of the gemmulae giving rise to the sponge, from which the spicules have been counted. 5 denotes the number of gemmulae composing the sponge, and 6—7 give the number of spicules formed per 0.01 mm^3 gemmulae, calculated on the basis of the total numbers from line 1 (6) and from line 2 (7).

The number of megascleres for the same sponges, whose microscleres were counted, are shown in table IV.

As the two types of spicules in question behave somewhat differently under the influence of silicic acid content of the water, each type will be separately considered, and the conditions of the microscleres will first be dealt with.

It may be seen from the table that the counting of the number of spicules is most uncertain, owing to the difficulties in distributing the spicules evenly in the preparation. It appears, however, from the calculated number spicules formed per volume unit of gemmula in various cultures, that the number of spicules

Table III.

Nr.	Si O	Si 2	Si 4	Si 8	Si 16	Si 128_1	Si 128_2	Iophon par a	Iophon par b	Ortoclas par a	Ortoclas par b	
1	<10	3100	4300	2300	1600	2600	1700	3300	2900	6200	3500	Total number of spicules (lowest and highest number calculated).
2		6200	7200	2500	2800	4600		5500	3800	8800	4700	
3		2	4	2	4	4	Directly counted	4	4	4	4	Number of radies counted.
4		0.277	0.250	0.155	0.125	0.212	0.125	0.176	0.209	0.414	0.212	Volumen of gemmulae in mm³.
5		2	2	2	1	2	1	2	3	4	1	Number of gemmulae.
6		140	170	150	130	120	140	190	140	150	160	Lowest and highest number of spicules per 0.01 mm³ gemmulae.
7		270	290	160	220	220		310	180	210	220	

Table IV.

Nr.	Si 2	Si 4	Si 8	Si 16	Si 128_1	Si 128_2	Iophon par a	Iophon par b	Ortoclas par a	Ortoclas par b	
1	640	800	130	410	280	Directly counted 80	400	320	760	320	Total number of spicules (lowest and highest number calculated).
2	1020	1320		630	390		950	480	1300	580	
3	28	32	8	33	13	6	23	15	18	15	Lowest and highest number of spicules per 0.01 mm³ gemmulae.
4	45	53		50	18		54	23	31	17	

for sponges from all the cultures fall within nearly the same interval of spreading—that is to say, are of the same size. Apparently, the number of spicules do not in the least tend to increase at higher concentrations of silicic acid. Sponges from the culture Si 2 have shown the same intensity of spicule-formation within the variations occurring in the counts as have the sponges from the culture Si 128. This applies also to the sponges from the culture with ortoclas compared, for instance, with those from the Jophon cultures, in spite of the fact that the silicic acid content of the latter, as will appear later (pag. 42) has been considerably higher than the concentration of the culture where ortoclas is the source of the silicic acid. On the basis of the results given in table III we are, however, certainly justified in saying that if the size of production of spicules increases somewhat with the increasing silica content of the culture medium, viz. from 0.02 to 1.28 mM SiO_2, this effect must be considered rather slight, at any rate, when compared whit the rise in intensity of production due to a change in the silica content from 0 to about 0.02 mM SiO_2. As shown in the table hardly any microscleres are formed in the culture, which is (approximately) without silicic acid. The quite few spicules per gemmula were also very thin (hardly 1 μ thick).

The above mentioned facts lead to the conclusion that the formation of microscleres apparently reaches its maximum rate already at rather low silicic acid concentrations, that is to say, the laying down of microscleres does not increase, even when greater quantities of silica are placed at the disposal of the sponge.

A microscopic investigation of the gemmula sponges from the culture Si 2 showed that 0.02 mM SiO_2 evidently comes near to the lowest limit at which the formation of microscleres may take place to its full extent at the temperature used (room temperature) and in the period that the experiment lasted. The fact is that in some of the sponges the formation of microscleres was found to be defective, varying from hardly any production through medium stages to a normal number of spicules, as represented in the culture Si 2 recorded in table III. (A defective formation of microscleres has not been observed in healthy sponges belonging to any of the other cultures with greater silica content.)

Unfortunately the number of spicules has not been counted on sponges with a reduced production of spicules in the culture Si 2, but the fact that the number of microscleres may be reduced appears indirectly in one case from an estimation of the relation between the numbers of microscleres and megascleres. As mentioned in the following, the megascleres make about 10

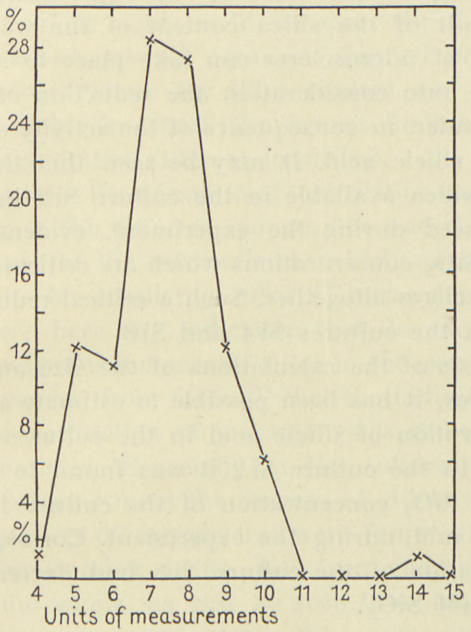

Fig. 3.

to 25 % of the total number of spicules formed, when the content of silica is sufficient to allow a formation of microscleres to its full extent. At silica concentrations, which are too low for the development of the highest number of microsleres during the period of the experiment, the megascler-formation has, apparently, not yet been appreciably retarded. Even with the extremely small quantities of silicic acid, which may have been contained in the culture Si 0, the production of megascleres is rather considerable. As, therefore, it was found that the megascleres made 46 % of the total number of spicules in the preparation $Si\,2_5$ (table V), and that all microscleres were rather thin (curve $Si\,2_5$ fig. 3), this must mean that the formation of

microscleres has been reduced in this sponge on account of too low silica concentrations. As a matter of fact it must be considered extremely unlikely that the number of megascleres should absolutely have been far higher in this preparation than is otherwise the case; neither is this indicated by microscopic observations.

In estimating on the basis of the experiments in question the lowest limit of the silica content of the water, at which the formation of microscleres can take place to its full extent, we must take into consideration the reduction of silica in the surrounding water, in consequence of the activity of the sponges in taking up silicic acid. It may be seen that the quite small quantities of silica available in the culture Si 2 have been considerably reduced during the experiment, evidently they have decreased to SiO_2 concentrations which are critical to the formation of microscleres altogether. Such a critical reduction has not taken place in the cultures Si 4 and Si 8.

On the basis of the calculations of the size and number of the microscleres, it has been possible to estimate approximately the total absorption of silicic acid in the cultures Si 2 and Si 8 (see pag. 26). In the culture Si 2 it was found to be about 20 γ SiO_2, i. e. the SiO_2 concentration of the culture has decreased to about 0.01 mM during the experiment. Correspondingly the SiO_2 concentration of the culture Si 8 had decreased to about 0.06 to 0.05 mM SiO_2.

Germination and spreading did not occur quite simultaneously in all gemmulae, but the processes on the whole lasted about 24 hours that is to say that, at the time when the sponges first germinated have actually started the spicule-formation, the last ones are still germinating. Thus the formation of spicules of the latter will, as a rule, begin at a slightly lower SiO_2 concentration than that of the sponges first germinated. It is most probable, therefore, that it is just these gemmulae last germinated which exihibit the reduced numbers of microscleres. We may, therefore, conclude that the lowest limit for obtaining the full number of spicules is a little below 0.02 mM SiO_2. This low limit is, however, doubtless subject to individual and racial variations, and depends upon other factors in its surroundings.

This fact is evident from JEWELL's most interesting experi-

ments on the ecology of fresh-water sponges (JEWELL 1935). She has among other things examined the SiO_2 content of the water in a long series of localities in which *S. lacustris* was present, and she found that the typical form containing well-developed spicules only rarely occurred at a silicic acid content as low as 0.005 mM SiO_2. In a single case the sponge containing well-developed spicules were found in a locality, where only traces of silicic acid could be seen. On the other hand, the species had on several occasions formed no microscleres at all in localities where the silica content was between 0.005 and 0.01 mM SiO_2. In short, the lowest limit of silica is not constant as regards the different localities[1].

With regard to the counts and calculations of the number of megascleres in the different sponges, the uncertainty is greater than in the case of the microscleres, which might be expected, considering the far smaller number of megascleres formed; but neither is there here any indication that the number of megascleres rises with the increase of silica in the surrounding water. It appears, however, from table IV that the individual variations in the rate of production of megascleres is greater than in the case of microscleres. Especially in the cultures where *Jophon* spicules and ortoclas were the sources of silicic acid, comparatively low numbers of megascleres were frequently found, without these low number being, however, characteristic of the cultures on the whole, as can be seen from table V. Here the numbers of megascleres are expressed in a percentage of the total numbers of spicules, which have been directly counted, i. e. all the spicules of the preparation, which have entered the field of vision have been counted. In the tables the figures Si 2, Si 4, Si 16, Si 128_1 and 128_2, *Jophon* par a and b, ortoclas par a and b originate from counts, on the basis of which the absolute numbers of the table III and IV have been calculated. All the other megascler percentages originate from the counts which have been undertaken simultanously with the measurements of thick-

[1] In this connection another factor must likewise doubtless be taken into consideration viz. the state of the silicic acid in the surrounding water—that is, whether it appears in an ionized, molecular or more or less polymerized form, as it is reasonable to suppose that the absorption of silicic acid by the sponges depends on these conditions. Investigations dealing with this problem are being carried on.

Table V.

Culture- and preparation-marks	Total number of spicules	Number of megascleres	Megascleres expressed in % of total number
Si O	83	79	95
Si 2_5	156	72	46
Si 2	441	83	19
Si 4	1302	346	25
(Si 8	138	8	6)
Si 16	521	124	24
Si 128_1	1117	78	7
Si 128_2	1780	80	4
Si 128_3	466	24	5
Si 128_4	1660	115	7
Iophon par a	586	79	14
» » 3	255	55	22
» » b	515	57	11
» needles 2	254	44	17
Ortoclas par a	1185	162	14
» » b	647	80	12

ness and length of the spicules. Table V shows that although the percentage of megascleres undergoes rather considerable variations, it has, however, only once exceeded 25 % at silica concentrations of between 0.02 and 0.16 mM SiO_2, namely in the culture Si 2_5, as has been explained above, and the percentage does not drop lower than 10. As mentioned above, the fluctuations, if not altogether due to the uncertainty of the counts, are most likely due to individual variations of the number of megascleres.

If we descend to quite low values of silica content—at 0.02 mM SiO_2 we were alredy near the limit during our investigations —it was seen that the formation of megascleres was far less retarded than was the formation of microscleres. Even sponges from SiO contained a fairly large number of megascleres, all of which were, however, quite thin, about 1 μ thick. As the silicic acid content of this culture can only have been extremely low, we are almost forced to suppose that the spicules were

formed from silica which was present in the gemmulae before these were placed in the water free of silicic acid. This view is also strengthened by records from other investigators (see WIERZEJSKI 1935) regarding the fact that the formation of spicules may arise in the gemmula before germination has commenced. As spicules do not enter the gemmula at its formation, they have consequently been formed either from the stores of silicic acid in the gemmula, which were present at its formation, or—and this is perhaps most likely—they have been formed from silica absorbed by the gemmula from the surrounding water.

Another possibility is that the quite thin spicules which were found in the sponges of the culture SiO consist mainly or completely of organic substance—in fact, we are dealing with the axial thread.

The fact that the formation of megascleres can take place with lower quantities of silicic acid in the water than that of the microscleres, indicates that there is a physiological difference between the respective spicule-forming cells, and thus supports EWAN's statements that both microscleroblasts and megascleroblasts are present. Observations on the formation of megascleres at the highest silica concentration in our experiments likewise confirm this theory.

The number of megascleres is comparatively small in the sponges of the culture Si 128. It appears from table V that the percentage of megascleres is below 10 in all cases examined, and if we compare the tables III and IV, it is seen that the drop is absolute. There is no sign to indicate an increased production of microscleres, while the number of megascleres is, apparently, lower than that of the sponges examined from the other cultures. (Exluding only the sponge examined from Si 8, which is, however, no true representative of the formation of spicules of this culture. As the number of microscleres seems to have been very small, the sponge is considered to have been defective. Unfortunately there are no other counts available of the number of spicules of this culture). Accordingly it appears that the megascler- and microscler-forming cells have reacted differently in the culture with a high silica content.

It is difficult to say if the drop in the number of megascleres indicates an injury to the spicule-forming cells, due directly

to the high silicic acid concentration. It must, however, be mentioned that the outgrowths on the spicules occurred far more frequently in this culture than in those with lower SiO_2 contents (table VII). As the outgrowths are considered an abnormity, this phenomenon suggests that the large silica content has an injurious effect on the sponge tissue, and that the megasclerforming cells are affected much more by the high silica content of the water than are the microscler-forming cells. As previously

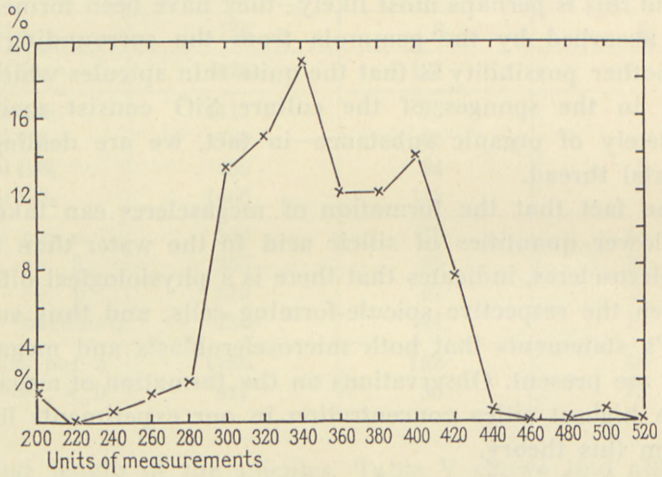

Fig. 4.

mentioned it is unlikely that a higher Na^+ content should bring about any reaction on the part of the sponge, nor should it be responsible for the formation of outgrowths. Also the somewhat higher p_H value might be left out of consideration. JEWELL (1935) has found normal *Spongilla lacustris* in water-courses with a p_H value of 8.6.

b) The Length of the Spicules.

The length of the microscleres of the young gemmula sponges varies considerably. Values ranging right from about 40 μ to about 110 μ have been found, but by far the most are between 60 and 80 μ. A typical distribution of the spicules according to length is given in fig. 4, which shows the measurements from $Si\,8_5$. Almost all the short spicules under 50 μ will prove to be quite young and newly laid down, but among the quite short

Table VI.

Si 0		Si 2		Si 4		Si 8		Si 128		Adult sponge	
Average length in μ	Number	Average length in μ	Number	Average length in μ	Number	Average length in μ	Number	Average length in μ	Number	Average length in μ	Number
290	4	330	100	350	200	350	175	355	100	340	100
..	..	330	100	..	..	365	200	..	..	..	..
..	..	340	300	..	..	350	286	..	..	..	..

spicules we may also find thicker ones with a more finished appearance. On the whole, there is only a slight correlation between the length of the spicules and their thickness, i. e. their age, as can be seen from fig. 5. The spicules are here laid down in a co-ordinate system, where the abscissae are the thicknesses of the spicules, and the ordinates are the length of the same. Only this single example has been recorded, as all the other cases are quite similar. It will be noted that the final length of a spicule is usually far longer than that of the newly laid down axial thread and spicule, but the increase in length taking place during the growth of the spicules, varies considerably, and no length seems to be especially preferred within the interval of 60 to 80 μ. This also applies to all the sponge preparations examined, no matter to what culture they belonged. If the silicic acid content of its surroundings has any influence on the length of the spicule, it must be very slight.

The average length of the microscleres from the various cultures are shown in table VI. The somewhat lower average lengths of Si 0 and Si 2 are due to the higher percentage of quite young spicules present in these cultures. The table also includes the average length of the spicules from an adult *Spongilla*, which was taken from the same locality as the gemmulae used in the experiments.

On the basis of the available material, nothing could be decided with certainty concerning the relation between the lengths of the megascleres and the silicic acid content of the water, because of the smaller number and greater variations in length of the spicules, but there is no sign to indicate that the megascleres and microscleres behave differently in this respect.

c) The Thickness and Volume of Spicules.

While it could not be demonstrated that the intensity in formation, the length of the spicules and the silicic acid content depended on each other, the average thickness of the spicule was found to increase with the increasing silica content of the liquid of the culture. Typical microscleres from two different

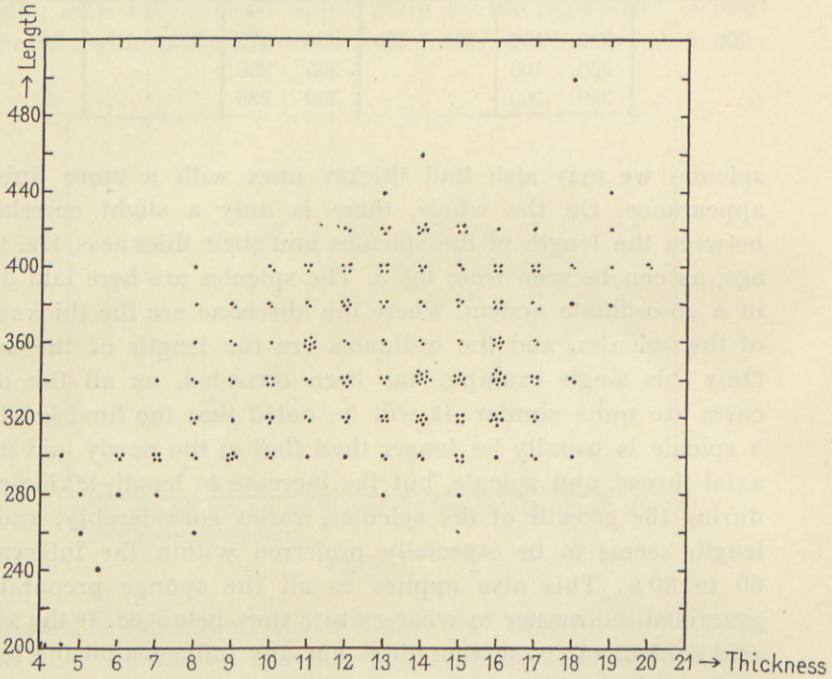

Fig. 5.

silica concentrations are shown in figs. 6 and 7. Figs. 8 and 9 show the distribution of the spicules according to the various thicknesses expressed in a percentage of the total number of microscleres measured from Si 2, Si 4, Si 8 and Si 128. Each of the curves represents the average of several gemmula sponges, each originating from one or more gemmulae. It is striking to note, how the number of microscleres, grouped round thicknesses of 8 to 10 measuring units decreases with the increase of SiO_2 content of the culture medium, while the number of microscleres, which reach the greatest thicknesses, is correspondingly increased. Of the 571 spicules measured, on the basis of which the curves

for Si 2 have been drawn, only about 20 percent exceeded 11 units, i. e. were c. 2 μ thick; of 569 spicules from the culture Si 4 about 35 % exceeded 11 units, of 557 spicules from the culture Si 8 about 65 %, and of 344 spicules from the culture Si 128 about 75 %. For comparison a curve is given (fig. 10) for the distribution of the thickness of the microscleres of an adult *Spongilla*. The individual, from which the spicules are derived, has been taken in the same locality as the gemmulae. The spicules show a distribution similar to a curve of normal distribution. We note, however, that at the same time a certain number of young thin spicules are present, which must be supposed to originate from the zone of growth in the sponge tissue. The curve is based on 400 measurements of thickness.

It is difficult to say, what the above mentioned curves for the distribution of the thickness of the microscleres signify, as their courses are due to an interplay between rather an unknown number of factors, the quantitative effect of which are practically unknown, Some of these factors may be mentioned: continuous but possibly varying new-formation of the spicules, decreasing silicic acid concentration in the culture medium during the experiment, the effect of which is presumably stronger, the lower the original concentration has been, rate of growth of the spicules, which is considered to rise with increasing supply of silica, individual variations and so on.

Hence it appears that we must deal with the curves most carefully, and not draw too many conclusions from their special courses, until we have a clear understanding of the importance which may be ascribed to the different factors, influencing the courses of the curves. But in any case, the thicknesses of the spicules and their distribution may even now lead to certain conclusions.

When the production of spicules has lasted 3 to 4 days, we may certainly conclude that the largest microscleres found in each of the different cultures have nearly attained those values for thickness which can on the whole be reached at the silica concentrations used, with the material employed and under the conditions of experiment in question[1]. As may be seen, these values

[1] This is doubtful only in a single case viz. the culture Si 2. As early as the day before the fixation, however, the thicknesses of some spicules in this culture were observed to be of the same size as the largest ones from the fixed sponges.

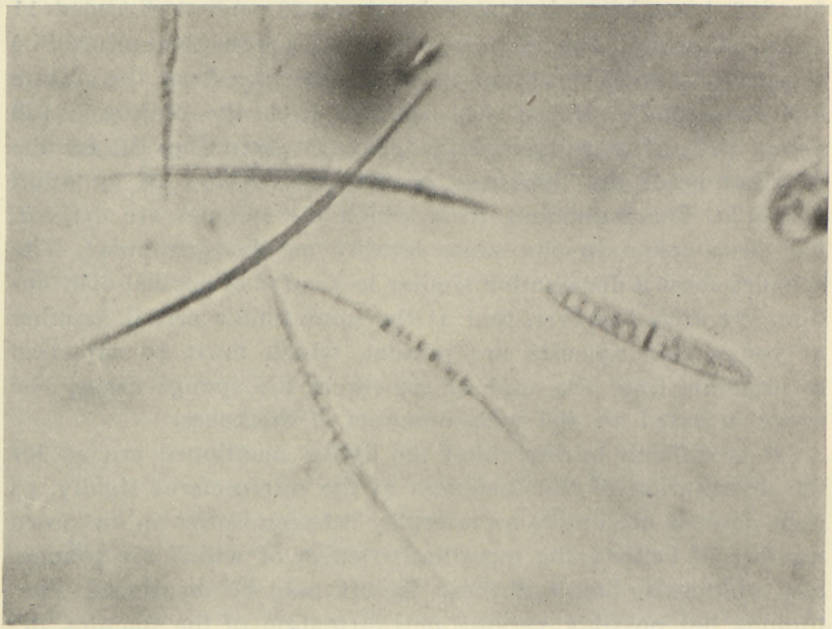

Fig. 6. Microscleres from a culture with 0.02 mM SiO_2. The spicules are slender and without middlethickenings. (The formation showing a dark cross line on the middle is a fungus germ.) (× 740).

for the thicknesses of the spicules lie higher, the higher the silica content of the culture is. It appears furthermore, as mentioned above, that the number of spicules, which attain the greatest thicknesses characteristic of each culture, are larger, the higher the silica content of the culture has been. Quite analogous conditions are also met with in the cultures with various solid silicates as substrata (see fig. 13, 14 and 15). The experiences obtained can be summarized in the following view on the action of the spicule-forming cells:

The easiest way to explain the fact that at the end of the experiment, the average maximal thicknesses were found to be the largest in the sponges from the cultures with the greatest silica content, is, to assume that the functional stage of the microscler-forming cells is limited, and that the spicule-forming faculties of each cell are exhausted, wholly or partly, within a certain space of time, in our experiments probably in the course of a few days. After the elapse of this time, further growth is

Fig. 7. Microscleres from a culture with 0.64 mM SiO_2. Three out of four spicules with more or less distinct thickenings. (× 740).

only minimal. The thickness, which can be attained by a spicule, thus depends on the quantity of silicic acid supplied to the scleroblast, while still in possession of its spicule-forming ability. Hence it follows that the rate of growth of the spicules increases with an increasing silica content of the culture medium. This view is further strengthened by the fact that with an increasing silica content, an increasing percentage accumulation of spicules takes place in the final thicknesses of spicules, characteristic of each of the silica concentrations. This may, however, also be due partly to the decreasing silica concentration of the various cultures.

Partly to estimate the absolute drop in the silica content during the experiment, but mainly to find out how the distribution of the spicules was according to volume, approximate calcutations of the volumes of the spicules from the cultures Si 2 and Si 8 were made, these being the only cultures from which a sufficient number of simultaneous measurements of the lengths and thicknesses of the microscleres were available.

The volumes of the spicules were calculated according to the formula for a spheroid (see pag. 32). The distribution according to volume is shown in the curves on fig. 11, where the numbers falling within the different size-groups are expressed in percentage of the total number of spicules. The curve "Si 2" has been drawn on the basis of 489 measurements; "Si 8" is based on 558 measurements.

From the measurements of the volumes, it is possible to

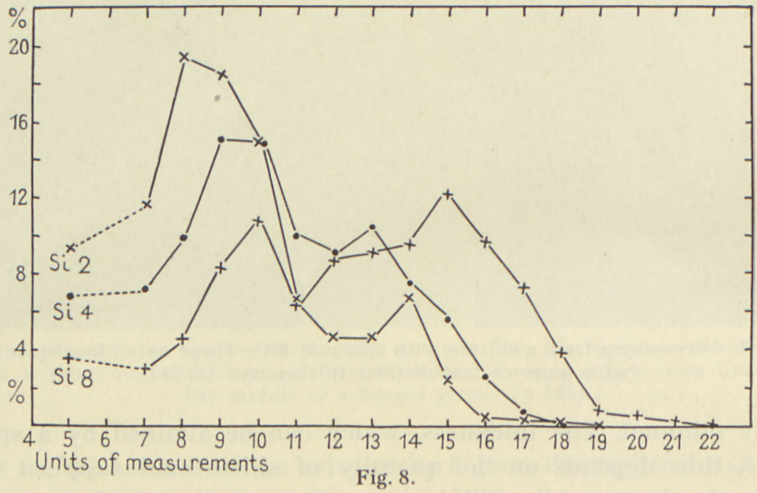

Fig. 8.

estimate the quantity of SiO_2, which has been accumulated in the spicules, although the estimation is most uncertain, especially owing to our incomplete knowledge of the total volume of the megascleres and of the actual number of spicules in the whole culture.

In the Si 2 culture the average volume of microscleres is 127 μ^3. If the volume of the axial thread is reckoned at 23 μ^3, the specific gravity at 1.96 and the water content at 15 % (see pag. 35) it results in the average silica content of each spicule being 0.000173 γ SiO_2. In the culture Si 2 about 50 developed gemmula sponges were found, of which the average number of spicules must be considered as having been at most 1500. The total content of the microscleres has thus been about 13 γ SiO_2. The average volume of the megascleres has roughly been calculated at about 250 μ^3, corresponding to the length of a spicule

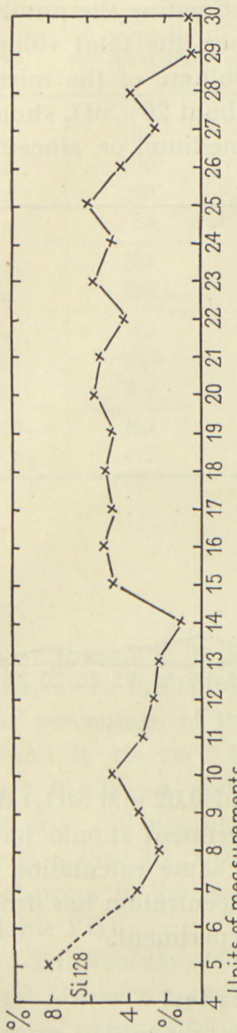
%
8
4
%
Si 128
4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
Units of measurements

Fig. 9.

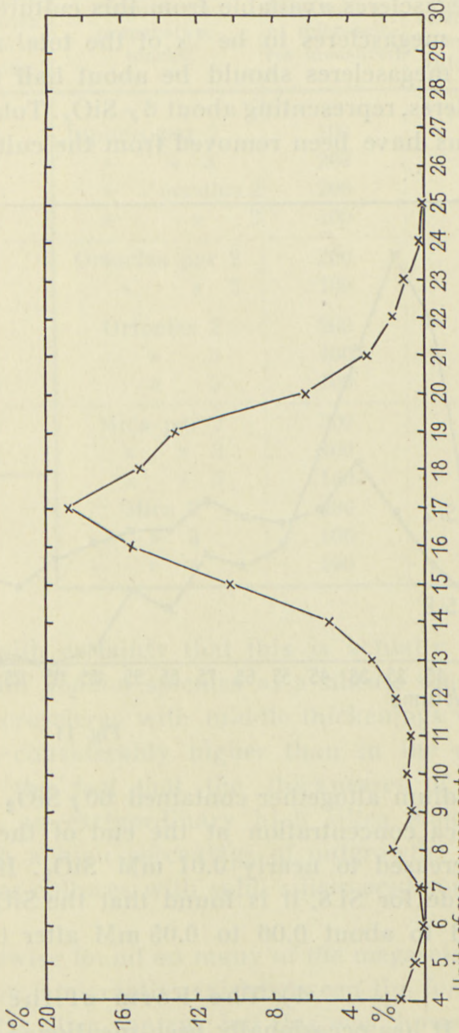
%
20
16
12
8
4
%
4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
Units of measurements

Fig. 10.

of about 120 μ and a thickness of about 2 μ. (This represent the average of the only 10 incidental measurements of the megascleres available from this culture.) Estimating the number of megascleres to be $^1/_5$ of the total number, the total volume of megascleres should be about half the volume of the microscleres, representing about 6 γ SiO_2. Totally about 20 γ SiO_2 should thus have been removed from the culture medium, or, since the

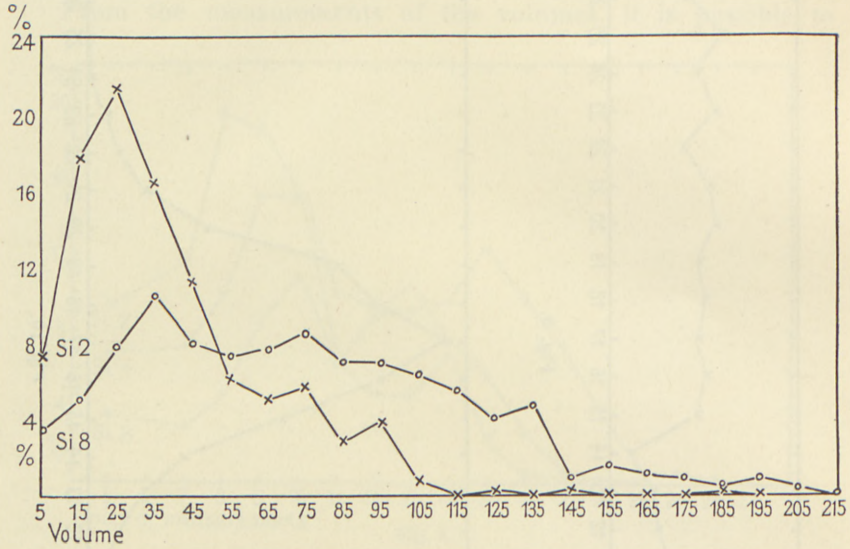

Fig. 11.

medium altogether contained 60 γ SiO_2 (50 ml 0.02 mM SiO_2) the silica concentration at the end of the experiment should have decreased to nearly 0.01 mM SiO_2. If the same calculation is made for Si 8, it is found that the SiO_2 concentration has dropped to about 0.06 to 0.05 mM after the experiment.

d) The Form of the spicules.

It has occasionally been mentioned in the above that microscleres with middlethickening sometimes occur to the sponges and their frequency increases with increasing silica concentrations. This appears clearly from table VII (Compare also figs. 6 and 7). It is tempting to suppose that there is a direct causal relation between the percentage of the microscleres with outgrowths and the silica content of the surrounding water. How-

Table VII.

Culture- and preparation-marks	Total number of microscleres measured	% of microscleres with middle thickening	Culture- and preparation-marks	Total number of microscleres measured	% of microscleres with middle thickening
Si 2_2	102	2.0	Jophon par 2	100	85
Si 2_3	100	2.0	» » 3	200	88
Si 2_4	299	2.3	» needles 2	200	74
Si 2_5	83	2.4	» » 3	100	90
Si 4_2	208	2.9	Ortoclas par 2	200	1
Si 4_3	408	10.0	» » 3	100	2
Si 8_3	178	14.0	Ortoclas 2	200	5
Si 8_4	205	16.1	» 3	100	6
Si 8_5	285	18.6	» 5	100	3
Si 128_2	444	56.8	Mica par 2	300	4
Si 128_3	442	65.8	» » 3	100	6
			» » 5	140	4
			Mica 2	200	4
			» 3	100	5
			» 4	100	4

ever, it cannot be said with certainty that this is actually the case, as in the culture with *Jophon* spicules as a silicate source, the percentage of the microscleres with middle thickenings was found to be very high—considerably higher than in the culture Si 128—in spite of the fact that the thicknesses of the spicules did not indicate an extraordinary high silica concentration (see fig. 15). Such a high percentage of outgrowths did not occur in the two other cultures with solid silicic acid source (table VII).

Thickenings were likewise found on many of the megascleres, and alsohere a correlation, apparently, exist between the formation of outgrowths and the silica content; but this was, however, not statistically ascertained.

Mechanism of the Formation of Spicules.

Statements dealing particularly with the mechanism of the formation of spicules are rather rare in literature. Such speculations are, however, always based primarily on the view advo-

cated by BÜTSCHLI that the spicules are congealed silica gels. SCHRÖDER (1936) is the only author who has tried to lay down a theory for the mechanism of the growth of spicules. The safe suppositions for his theory are as follows: The spicules are of intracellular origin, arising in the form of the organic axial thread, the full length of which has, however, not yet been attained. The further growth of the spicules takes place by apposition of a siliceous substance in such a way that the spicule grows in thickness primarily accompanied by a simultaneous growth in length, corresponding to the lengthening of the axial thread. As a rule, however, the final length seems to have been attained before the spicules has reached its full thickness. This is a brief outline of the observations made by many investigators since the days of CARTER about the middle of last century.

SCHRÖDER is now of opinion that he has observed in his preparations of the spicule-forming gemmula sponges of *Ephydatia mülleri* the presence of vacuoles with silicic acid gels, which would place themselves close to the developing spicule, covering it and thus involving its growth. SCHRÖDER states, moreover, that he has found vacuoles on fixed material, farther removed from the spicule and nearer to the surface of the cell, which he believes to contain silicic acid sol.[1] He therefore supposes that the sol travels from the periphery of the spicule-forming cell into the centre, becoming more and more concentrated and ultimately gelatined. Finally when the gel has completely reached the spicule it spreads and covers it and is congealed. For various reasons, which will be referred to below, I doubt the correctness of theories of this kind.

1) When observing living spicule-forming gemmula sponges, one of the most conspicuous features met with is the presence of lively and continuous currents of protoplasma, which occur in all the scleroblasts. The movements of the protoplasma are revealed by observing the movements of the numerous granulae, which characterize the spicule-forming cells.

Cells with young and quite thin spicules especially contain great quantities of such granulae, all of which show rather a

[1] SCHRÖDER do not mention the word "sol". He only speaks about "im Dunkelfeld ... grauen Vakuolen" containing silicic acid.

uniform size, about $^1/_2\ \mu$ in diameter. In older spiculiblasts there are fewer granulae, and they may almost have disappeared in cells with fully formed spicules. We have no information regarding the nature of these cell-elements. Granulae move at rates up to about $2\ \mu$ per sec., i. e. about 7 mm. per hour.

The currents of protoplasma in the spiculiblasts do not follow any fixed courses, but outside the spicule a strong mixture of all cell components takes place. It may be seen how the granulae rarely proceed more than about $5\ \mu$ at a time and often far less; then they stop, and when starting to move again, they often follow quite another course than before. Movements in both directions along the longitudinal axe of the spicule most frequently occur. Granulae situated in a layer of protoplasma near the surface of the spicule, will thus, as a rule, flow in one direction, while the layers of protoplasma in the periphery flow in the opposite direction. In the course of a short time, the current changes and goes in opposite directions. The granulae may, however, also take courses forming all kinds of angles to the longitudinal axe of the spicule, and by such courses are often led right away from the periphery of the cell to the surface of the spicule, and conversely. Thus we actually get the idea that the protoplasma elements of the spicule-forming cells are effectively mixed. It is understood without further explanation that it is difficult to imagine how vacuoles containing silicic acid should be able to migrate, independently of these confused currents of plasma, from the periphery of the cells to the surface of the spicule, while increasingly changing from the sol to the gel stage.

The above observations do not of course mean a rejection of the gel-theory in the main, but serve only to give us an idea of the difficulties in maintaining the theory in SCHRÖDER's particular form. There are, moreover, cases, which entirely contradict the gel-theories as a whole.

2) A gel must be very hydrous indeed to be able at all to spread over a surface, in this case over the developing spicule. From v. BEMMELEN's classic investigations from about the year 1900, we know the consistency of the silicic acid gel at various water contents. A gel is solid enough to be cut, when it contains

30 to 40 mol H_2O per mol SiO_2, and containing 20 mol H_2O per mol SiO_2 it is already rather stiff and quite without liquid abilities. A maximally shrunk silicic acid gel contains 1.5 to 3 mol H_2O per mol SiO_2. Such a gel is very solid. As early as at 6 mol H_2O it may be ground in a mortar to an apparently dry powder.

We may, therefore, safely conclude that the required liquid abilities of the gel are only present when it is newly formed and containing at least 25 mol H_2O per mol SiO_2 and probably more. The older gel soon looses its thixotrophy. 25 mol water per mol SiO_2 corresponds to about 88 % water or 12 % SiO_2.

In other words, it is most likely that the silicic acid gels which, according to SCHRÖDER's view, should occur in the protoplasma of the spicule-forming cells, may contain at the most about 12 % SiO_2, or rather less. In the case of *S. lacustris* the consequences of these facts will be seen from the following rough calculations.

The form of the microscleroblasts resembles somewhat that of a spheroid where the short radius equals $^1/_5$ of the long one. The volume of the cell may thus be calculated from the formula $^4/_3 \pi \cdot a \cdot b^2$, where a is the long radius and b the short one. If a length of a scleroblast is estimated to be equal to the length of a spicule, which is almost correct, and if a calculation is made for the average length of a spicule, viz. about 70 μ, a volume of the cell of about 72.000 μ^3 results. The thickness of a spicule is estimated to be 3 μ, and it has also approximately the form of a spheroid. Its volume is then about 330 μ^3. The total volume of the fully developed microscler is thus about $^1/_{20}$ of that of the spiculiblast. These figures are only recorded in order to show how the relations in regard to size may be between the cell and the spicule.

As previously mentioned a greater number of analyses have shown that the spicules of the siliceous sponges contain about 6 to 7 % water. All specific gravities determined hitherto lie between 2.096 and 2.018. (see BÜTSCHLI 1908). The specific gravities have been determined on spicules of many different sizes, both large and small, but all the spicules could easily be discerned by the naked eye. We dare not, therefore, absolutely reckon with the specific gravities and water percentages also

being applicable to the microscopically small spicules of the fresh water sponges. Consequently I have carried out some calculations of the specific gravities for megascleres and microscleres of *S. lacustris.*

The spicules were freed from the sponge tissue by boiling in 70 % lactic acid, and completely cleansed in diluted KOH (their cleanliness was controlled under the microscope) for finally to be rinsed several times in destilled water in the centrifuge. They were dried at 120° C. for half an hour and then kept about 24 hours in the atmospheric air at room temperature before the calculations of the specific gravities were undertaken[1].

The specific gravity was determined by observations on the motions of the spicules in liquid mixtures with known specific gravities. For the mixtures aethylen bromide (s. g. 2.17) and brom benzene (s. g. 1.49) were used. The calculated specific gravities of the mixtures were controlled on the Westfals scales.

Owing to the minuteness of the spicules (the megascleres were about 250 μ and the microscleres about 70 μ long), the calculations of the specific gravities had to be made in a thermostat with a constant temperature. Fluctuations of the temperature of the surroundings of a few one-hundredth degrees, involved convection currents in the liquid, which carried the spicules with them, making calculations of the specific gravities impossible. The calculations were carried out at the cyto-chemic department of the Carlsberg Laboratory. (Concerning the construction of the thermostat see H. Holter: C. R. lab. Carlsberg sér. chim. 24 nr. 18, 1943.)[2]

The liquid, containing a great number of spicules, was placed in glass tubes just inside the glass wall of the thermostat and these could be observed by the aid of a microscope standing horizontal outside the thermostat. The measurements of the motions of the spicules were made, when the system was in termical equilibrium (temperature 23.1° C.). Only the rates of motion of the vertically standing spicules were measured. It was then

[1] Being heated to 120° C. the spicules may loose some water. This amount of water is, however, soon absorbed again from the humidity of the air, when the temperature drops again (Vosmaer and Vijsman 1905).

[2] I wish to express my sincere thanks to Dr. H. Holter, the leader of the department and his assistant Mr. E. Zeuthen, M. Sc. for allowing me to make these calculations of the specific gravities in the Carlsberg Laboratory, and for their kindness and willingness to help.

found that the spicules, at a specific gravity of 1.90 of the liquid mixture, floated towards the bottom of the glass at a rate of about 8 μ per sec. (see fig. 12). At the specific gravity of 1.96 by far the greater number of the spicules also moved towards the bottom, but the speed was far slower, less than 2 μ per sec. and the rates of motion of a great number of spicules came very near to 0, while also a smaller number of spicules rose in the liquid at very small rates. At 2.00 the spicules showed a marked

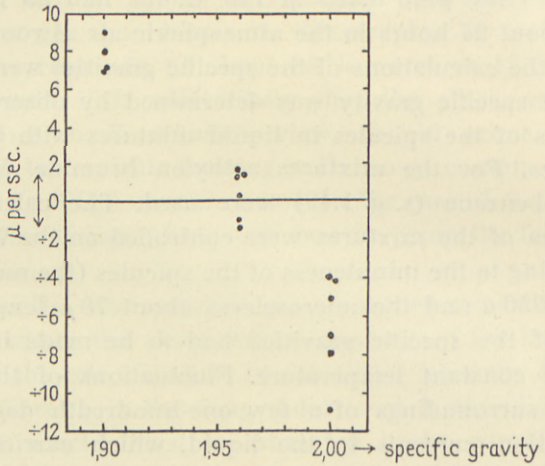

Fig. 12.

tendency to rise at rates up to 11—12 μ per sec. Quite a few spicules, however, likewise moved towards the bottom at this specific gravity of the surrounding liquid. Since the latter, on all sides and at a distance of less than 100 μ, were surrounded by spicules that were lighter than the liquid, the downward motion cannot be due to termical currents but must indicate that these spicules were actually heavier than the surrounding liquid.

It may be seen that the specific gravity is not quite the same in the case of all the spicules, but by far the majority showed specific gravities very near to 1.96. There was no assignable difference between the megascleres and microscleres. The specific gravity of the small spicules of *S. lacustris* is, therefore, somewhat lower than that of the larger ones examined earlier, which is probably due to a somewhat greater water content in

the latter. It is difficult to say how great the water content actually is in the spicules of *Spongilla*, as there is no agreement between the statements in literature of the specific gravity and of the water content of the silica spicules; thus a determination of the water content of the *Spongilla* spicules by extrapolation cannot be made.

The substance of the siliceous spicules is very similar to the opals. The chemical compositions, specific gravities, refractive index, etc. both of the substance of the spicules and that of the opal, particularly hyalith, are very closely related. As the specific gravities of the opals nearly always lie between 1.9 and 2.2, I tried to compare the *Spongilla* spicules with some of the lighter opals. Neither in the case of the opals is there complete correlation between the specific gravity and the water percentage. For instance, it may be mentioned that the water contents of two opals with low specific gravities, viz. kachelong (s. g. 1.884) and michalith (s. g. 1.886) are 7.74 % and 1.35 % respectively. Two siliceous sinters with specific gravities of 2.046 and 2.031 contained 12.86 % and 3.06 % water respectively (see Bütschli 1908). As a rule, the specific gravity for transparent opals, with a SiO_2 content of about 90 % and a water content of about 7.5 %, lies between 1.9 to 2.03. As to non-transparent opals with about 87 % SiO_2 and 9 % water the specific gravities are determined at 1.94—1.97. (The statements are recorded from Gmelin-Kraut, Handb. anorg. Chemie III, 1. 1912.)

Since the water percentage, even at specific gravities under 1.9, did not rise higher than to about 17, we may surely conclude that neither do the water percentages of the *Spongilla* spicules exceed values of this size. If the water content of the spicules is considered to be about 15 %, this is certainly a high estimate; the water percentage surely comes nearer 10.

In the following calculations the SiO_2 percentage is reckoned at 85 and the water percentage at 15.

The volume of a normal microsclere is, as mentioned, 330 μ^3. If a correction is made for the organic axial thread, which, at a high estimate, is 0.8 μ thick with a volume of about 23 μ^3, the spicule is found to contain about 0.00051 γ SiO_2. This quantity of silica distributed on a 12 % gel gives a gel weight of 0.0042 γ. If the specific gravity of pure SiO_2 is reckoned at 2.3, the spe-

cific gravity of this gel becomes about 1.16. The volume will thus be 3600 μ^3,

If the volume of the hypothetic silica gel, before it is deposited on the spicule, is supposed to have a volume equal to one half of the fully developed spicule, i. e. about 165 μ^3,[1] this will mean that about 22 of such gels are required to form a single spicule.

Our knowledge of the rate of the spicule-formation is rather uncertain, but 2 days must be considered to be ample time for the formation of a spicule 3 μ thick and 70 μ long. In many cases only half the time may be required. But supposing that the duration of formation equals 50 hours, this must mean that a gel containing 12 % SiO_2 must be ready for deposition, every second hour, at least, and probably far more frequently. Since in the course of comparatively few minutes many hundreds of developing spicules could be observed in a culture with young gemmula sponges, it might be expected that, if the gel theory was correct, the formation of gels and the deposition upon the spicules would be a phenomenon frequently met with in such cultures. As a matter of fact, however, the deposition of the gel has never been directly observed, in spite of the fact that numerous trained observers have closely studied and watched the spicule-formation in many live fresh-water sponges and other siliceous sponges. SCHRÖDER, however, believes he has witnessed the formation of a siliceous globe on a spicule in one single case. He saw, how an amoebocyt, moving along a megascler which was so far developed that it was no more enclosed by the scleroblast, stopped for a few seconds, then it suddenly contracted, whereby a siliceous globe appeared on the spicule. The globe did not spread on the spicule. The amoebocyt passed on. Being the only support in favour of the gel-theory this observation must be considered to be insufficient evidence. One cannot completely free oneself of the suspicion that the thickening of the spicule may have been present beforehand.

At any rate, the latter is the only record of the growth of a spicule taking place discontinuously. Otherwise the growth of spicules is stated as being invisible to the eye, which also corresponds with my own experience obtained after lengthy micro-

[1] Such a gel will, if of globular shape, have a diametre of about 7 μ and this corresponds rather closely to the size of the "silica" vacuoles in SCHRÖDER's fig. 6.

scopic observations of young gemmula sponges with lively formation and growth of spicules.

3) Finally still another fact should be mentioned which seems to contradict the gel-theory. If the formation of gels of silicic acid were to take place before the silica was deposited upon the spicule itself, it would be expected that these gels could be demonstrated by staining with basic vital dyes, as for instance methylen blue, which especially is strongly adsorbed to silicic acid gels *in vitro*. One might, therefore, expect that bluish vacuoles of the spicule-forming cells would fairly often be demonstrated in the young gemmula sponges in cultures with e. g. methylen blue. This was, however, not found to be the case, as will appear from the following.

The gemmulae were placed in petri vessels with staining-solutions in tap water with a power of about 1/100.000—50.000, 50 to 100 in each vessel[1]. The vital dyes used were methylen blue, neutral red, Bismarck brown, brilliant cresyl blue and methylen green.

Furthermore a silicic acid gel of about 10 % was made in the usual manner i. e. by neutralizing a 10 % sodium silicate solution with HCl. After the neutralization the gelatination took place in the course of a few minutes. The gel thus formed was broken up and washed for several days in tap water, which was often changed. Very small fragments of the washed silicic acid gel were placed in petri vessels with the same vital staining-solutions as used in the cultures with gemmulae. In the course of a few minutes the gel fragments had been intensively coloured in the methylen blue culture, and in the course of some hours a strong absorption of methylen green and brilliant cresyl blue had also taken place. Not until 24 hours later did the staining with neulral red and Bismarck brown also occur.

The staining gels were observed under the microscope with the water immersion objective, magnified in such a way as used

[1] The gemmulae were procured on March 3rd 1943; they were kept for some hours at room temperature before they were placed in the refrigerator, where they were left until the 9th April. At this juncture they were all frozen up. In spite of this their germination was almost complete in the experiments (more than 90 %) and spreading and differentiation had a normal course. Germination took place at room temperature on the 12th and 13th April 1943. They had been thawed on the 9th April, immediately before they were placed in the experimental cultures.

for the gemmula sponges. Also when thus magnified the staining was intensive and homogeneous even of the smallest particles of the silicic acid gel.

During and after the germination and spreading the gemmula sponges took up abundant dye in all cultures, excluding that with methylen green. (After being made up the methylen green solution soon became completely colourless). The dyes were mainly accumulated in some of the intracellular green algae but also in some of the granulae not further identified and of which the most were about $^1/_2\,\mu$ in diametre. There was no indication that the cell components of the scleroblasts were more frequently coloured than the other cell-types, on the contrary, they contained fewer coloured elements than did the dominating cell-type viz. the amoebocyte or cell-type II (BRØNDSTED 1936), since the scleroblasts do not contain algae cells, as is the case with the latter. The remaining coloured granulae also occurred rather less numerously in the scleroblasts than in the other cells of the sponge. Most frequently the spicule-forming cells contained no coloured constituents at all, and but rarely more than one coloured concretion was found at a time, which, apart from the colour, could not be distinguished from the other granulae, which are so numerous in the young scleroblasts.

Is there, however, not a possibility that these few formations in the spicule-cells which actually take up basic vital dyes may be silicic acid gels? In any case, being far too small, they do not correspond to SCHRÖDER's siliceous vacuoles, and besides it is difficult to prove or disprove such an assumption. A quite simple quantitative estimation, however, shows that this supposition is improbable, and at any rate it may be seen that if they are silicic acid gels, these few coloured granulae are quite insufficient in number to be solely responsible for the formation of the spicules.

The diametre of the granules is about $^1/_2\,\mu$ i. e. their volume is about $0.07\,\mu^3$. Supposing that they contain about 12 % SiO_2, as mentioned above (pag. 32), about 50.000 such granulae would be required to form an average-sized microscler ($70\,\mu$ long and $3\,\mu$ thick, corresponding to a gel volume of about $3600\,\mu^3$ and with 12 % SiO_2). In other words, reckoning, as before mentioned,

with a period of formation of 50 hours, this must mean that the deposition upon the spicule of 20 such coloured granulae per minute might be observed. This is, however, not at all the case. On the contrary the microscopic observations show that the coloured granulae, as would be expected, behave in the same way as the numerous non-coloured granules. They are conducted round in the cell by the protoplasma currents, now along the spicule, now in directions more or less at an acute angle to the longitudinal axe of the spicule.

Judging from the facts stated above, it must be considered improbable that the silicic acid would occur in the form of a gel in the protoplasma before it is deposited on the axial thread or on the part of the spicule already formed. The most probable theory is that the silicic acid, whether it is absorbed in an ionised, molecular or more or less polymerised form, is deposited directly on the spicule in whatever form or manner this process may take place. Investigations dealing with this question are under preparation, and I hope later to get an opportunity to return to this problem.[1]

Is *Spongilla* able to dissolve and utilize solid silicic acid?

Although it must be regarded as most likely that the silica required by the sponges is mainly supplied through the content of dissolved silicic acid in the surrounding water, one cannot beforehand exclude the possibility that the sponges may be able to dissolve solid silicates and thus procure silica from an underlayer of encrusted particles containing it, as presupposed by SCHULZE (1923). In order to decide this question the following experiments were made.

In three paraffined petri vessels, each containing about 50 ml artificial fresh water without silica (As to composition see pag. 9) spicules of *Jophon piceus*, pulverized ortoclas and pulverized

[1] In order to explain the lamellar structure occurring in the larger siliceous spicules, but lacking in the homogeneous microscleres, P. SCHULZE (1925) assumes that each lamel is laid down in the form of a silicic acid sol, derived from the scleroblast or scleroblasts and covering that part of the spicule already formed. Not until the sol has passed into the gel stage and has congealed, is silicic acid sol again deposited upon the spicule, thus forming a new lamel. As will appear from the above statements, all the observations on the formation of spicules in *Spongilla lacustris*, however, contradict such a mechanism of formation of its spicule-types.

dark mica were placed, covering the bottom with a thin layer. Finally a control vessel without substratum was arranged. In each of the vessels 20 gemmulae were placed which germinated in the course of a few days. Three or four days later a rather strong development of spicules could be noted in all the cultures with substratum, especially in the culture with pulverized mica, whereas no spicule-formation could be observed in the control

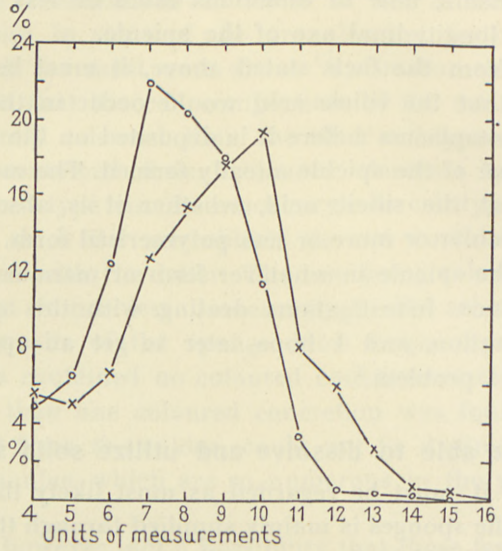

Fig. 13. Distribution of spicules from sponges which have grown on paraffin —×—×— and directly on ortoclas —o—o—.

culture. In order to decide to what degree the formation of spicules is due to the silicic acid dissolved in the water, the young sponges were removed from the petri vessel, and about 4 weeks after the various substrata had been put into the culture vessels without silica, these were again filled with gemmulae, which were placed, partly directly on the substratum and, partly, on a great paraffined cover-glass placed over the mineral constituents at the bottom. In each vessel about 100 gemmulae were placed, one half of which was put directly on the paraffined cover-glass, and one half on the ortoclas, mica or *Jophon* spicules respectively. In the culture vessel without silica about 60 gemmulae were placed. The gemmulae were newly gathered. The experiment started on the 17. 4. 43. at normal room temperature.

18. 4. 43. the germination had started. 23. 4. 43. the experiment was suspended and the young sponges originating partly from a single gemmulae, partly from several fused together, were fixed in 70 % alcohol. The counting of numbers and measuring of thickness of the spicules was made as recorded on pag. 9.

The thicknesses of the spicules from the ortoclas and mica cultures cannot directly be compared with those of the microscleres from sponges which have grown in the culture with *Jophon*

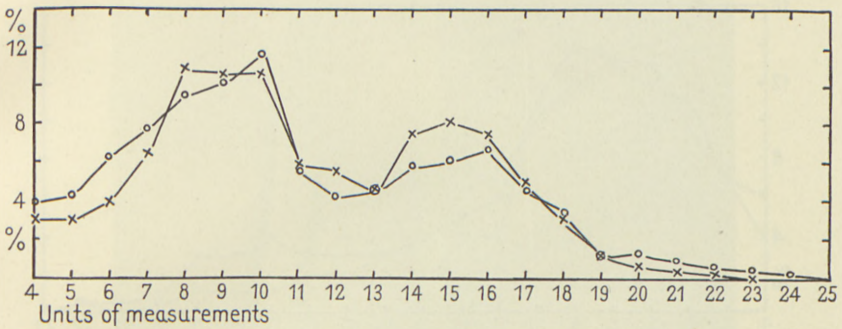

Fig. 14. Distribution of spicules from sponges which have grown on paraffin —o—o— and directly on mica —×—×—.

spicules, as the latter have almost all of them outgrowths on the middle (about 80 % of all the microscleres). The thicknesses of the latter were therefore measured just below the point where the middle outgrowths made the spicules extremely thick. The existence of this formation of outgrowths is the more striking because the 20 gemmulae in the first experiment in the same culture did not show any middlethickenings. Almost all the microscleres from the cultures containing ortoclas and mica had no middlethickenings (table VII).

The results of the measurements are given in the curves in figs. 13, 14 and 15 and in tables III and IV. It clearly appears that neither as regards the thickness of the spicules, nor their number (the microscleres alone have been considered) is there any difference between the sponges, which have grown on paraffined cover-glasses and those, which have directly been in contact with the substance containing solid silica. A microscopic observation showed that in the latter case the contact between

the sponge tissue and the substratum has actually existed. The gemmula sponges had completely embodied large quantities of quite small ortoclas and mica particles, and they had stretched themselves between the *Jophon* spicules which seemed to be completely incorporated in the sponge tissue (fig. 16). In spite of this, there is nothing to indicate that these sponges might have had more silica at their disposal for the formation of spicules than those which have only been in contact with

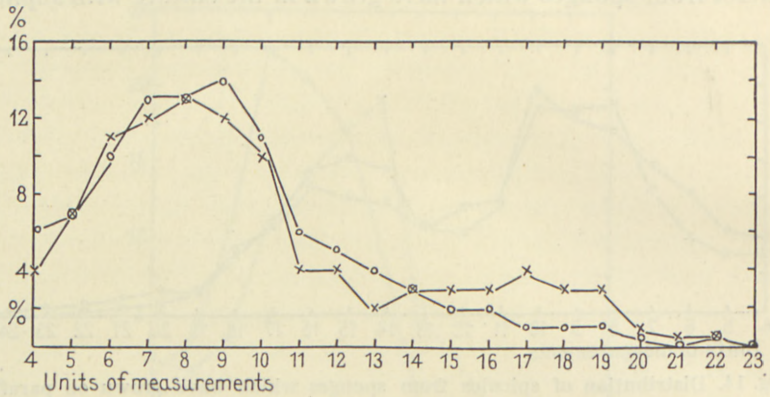

Fig. 15 Distribution of spicules from sponges which have grown on paraffin —o—o— and directly on spicules of Jophon —x—x—.

the paraffined underlayer. However, there is a marked difference in the size of spicules of the various cultures. On an average the spicules are stronger in the cultures with mica and *Jophon* spicules as substratum than in the case of the ortoclas.

In the control culture only extremely few thin spicules were developed, as mentioned before. We may, therefore, conclude that of the silicic acid used for the formation of spicules in the experiments with ortoclas, mica and *Jophon* spicules as substratum, by far the greater part is, at any rate, derived from silica dissolved in the water without the aid of the sponge. The greater silica acid concentration, which one would expect to be present in the immediate vicinity with the sponges growing directly on the substance containing silica, has apparently been too small to involve an increased growth of the spicules, which could make itself felt against the individual variations in the microscler production of the gemmula sponges.

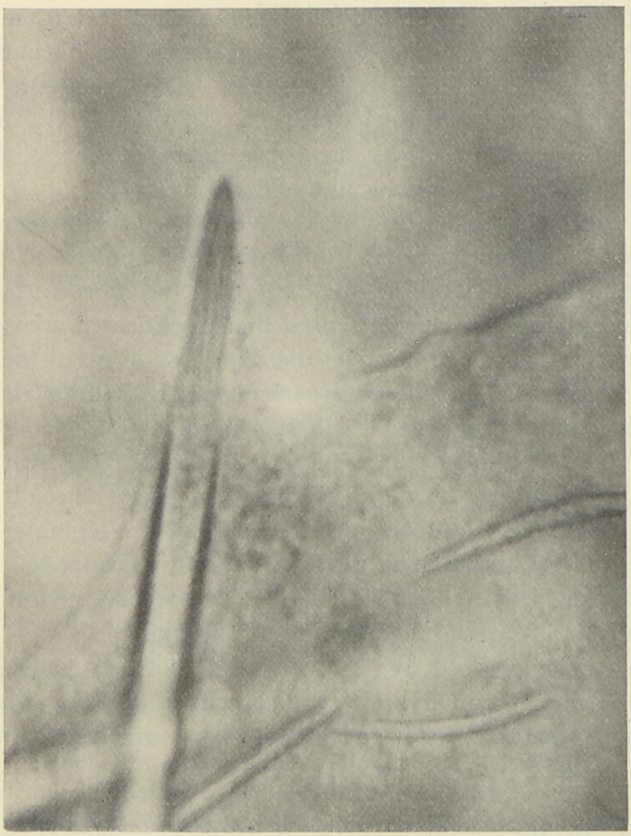

Fig. 16. *Halichondria* spicule completely embodied in the spongilla tissue (× ca. 400).

Summary.

The formation of spicules, mainly the formation of microscleres of the gemmula sponges in *Spongilla lacustris* has been examined with special regard to its dependence on the silicic acid content of the surrounding medium. The gemmula sponges were kept in artificial salt solutions, partly without silica and partly containing known silica contents varying from between 0.02 to 1.28 mM SiO_2.

It was found that the number of spicules formed per unity of volume of gemmula during the experiment was independent on the silica concentration, when this was over a certain minimum value. Under the conditions of experiment in question

the limit for full spicule production came close to 0.02 mM SiO_2 in the case of production of microscleres. The formation of megascleres is less sensitive to a low silica content. At the highest silica concentrations used, i. e. 1.28 mM SiO_2, the production of megascleres was, however, retarded, while the production of microscleres was not effected.

The length—unlike the thickness—of the microscleres was not affected by variations in the silicic acid content in the surroundings. The maximum thickness of the spicules and the percentage of microsleres attaining this thickness increased with the increasing silica content in the liquid of the culture.

The percentage of the microscleres with middlethickenings rises with increasing silica content of the water.

The theory put forward by SCHRÖDER that the spicule-formation takes place by deposition on the axial thread of preformed silicic acid gels, or on the part of the spicule already formed, has proved untenable. The growth of spicules must be considered to be brought about by the protoplasma continuously depositing silica upon the surface of the spicules.

The view expressed by SCHULZE that the fresh-water sponges should be able to meet their requirement of silica by dissolving silicic acid from solid substances containing silica, does not seem to hold good.

I am greatly indebted to Dr. H. V. BRØNDSTED, for working facilities and for his unfailing readiness to help and interest in my work. I wish to record my thanks to the Carlsberg Foundation, whose grants placed at my disposal by the laboratory, made this work possible.

From the Laboratory
for experimental morphology and cytology, Birkerød,
Leader: H. V. Brøndsted, Ph. D.

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ON SPECIFIC CONSTANCY AND SEGREGATION INTO RACES IN SEA-FISHES

BY

AD. S. JENSEN

KØBENHAVN
I KOMMISSION HOS EJNAR MUNKSGAARD
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CONTENTS

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I.

Race-forming species.

In 1901 the present author showed that the species *Lycodes vahlii* Reinhardt belonging to the subfamily *Lycodinae* (family *Zoarcidae*) falls into three races each of them inhabiting a distinct geographical area, viz. South Scandinavia, Iceland and Greenland. These races are very much alike inter se, only differing from each other by the number of vertebrae and rays in the median fins, as will appear from the table below quoted from the above paper:[1]

	Scandinavia	Iceland	Greenland
Number of vertebrae.....	98—101	105	112—116
— - rays in dorsal fin.. .	95—96	103—105	113—117
— - - - anal fin.....	85—86	90	90— 98

It will be seen that the number of vertebrae and rays in the median fins increases from warmer to colder regions. In harmony with this variation there is an increase in the body size; measurements of a great number of individuals showed that no specimen larger than 196 mm was known from the Kattegat, the Skagerrak and southern Norway, while the largest specimen from Iceland was 355 mm long and the largest specimen from Greenland 520 mm.

These races had earlier been regarded and described as distinct species, the Scandinavian as *Lycodes gracilis* M. Sars (1866), the Icelandic as *L. lugubris* Lütken (1880) and the Greenland race as *L. vahlii* Reinhardt (1831), but were by me referred as races to one and the same species and called *L. vahlii gracilis*, *L. vahlii lugubris* and *L. vahlii typica*.

In my monograph: "The North-European and Greenland

[1] The notes will be found in the appendix to the paper.

Lycodinae"[2] the question has been resumed and treated in more detail on pages 13—21, and the correctness of my preliminary notice has been confirmed.—In the appendix on page 21 it is stated that I had the opportunity to examine a specimen from northernmost Norway (Baas Fjord in East Finmark) which differed from *L. vahlii gracilis* by having 101 rays in the dorsal fin, 89 in the anal fin, and by its considerable size, 268 mm, and which approached the Icelandic *L. vahlii lugubris*; this was explained by the fact that in the fjords of East Finmark "the conditions are half arctic."

In the common Gunnel, *Pholis gunellus* L. (syn. *Centronotus gunellus*) I found similar conditions as in *Lycodes vahlii*, the number of vertebrae and rays in the dorsal and anal fins being somewhat greater in the Greenland than in the European specimens, while the Icelandic specimens form a transition. This will be seen from the table below which is based on 12 specimens from Denmark, 19 from Iceland and 12 from Greenland.

	Number of vertebrae	Mean	Number of dorsal fin rays	Mean	Number of anal fin rays	Mean
Denmark .	84—87	85.2	77—80	78.8	42—46	43.7
Iceland . . .	85—88	86.5	78—81	80.1	43—46	44.3
Greenland	85—89	87.5	79—82	81.1	44—48	45.1

As regards this fish also we are confronted with the phenomenon that colder conditions—from Denmark via Iceland to Greenland—are correlated with a higher number of vertebrae and fin rays[3].

As a third example of the fact that the sea area from the Kattegat towards the northwest across the Faroes and Iceland to Greenland, with its gradually decreasing temperature of the water, caused the segregation of a species into races may be mentioned the species *Triglops pingelii* Reinhardt belonging to the Sculpins (*Cottidae*). An investigation of 240 specimens for number of vertebrae and rays in the 2nd dorsal fin and the anal fin recently published by me[4] shows that within this area four races can be distinguished, the average numerical values of which will appear from the table below:

	Vertebrae	2nd dorsal fin	anal fin
1. *T. pingelii murrayi:* Kattegat, Skagerrak, North Sea, Scotland, Shetland and Faroes	42.7	20.4	20.0
2. *T. pingelii islandicus:* Iceland	43.6	21.4	21.0
3. *T. pingelii pietschmanni:* Davis Strait and central West Greenland	45.0	22.3	22.2
4. *T. pingelii pingelii:* Southern and northern West Greenland and East Greenland	48.5	24.8	24.8

Moreover, in a number of specimens which had to be spared, only the fin rays were counted and there was no change in the result.

Considered as a whole the number of vertebrae in *Triglops pingelii* may vary from 42 to 51, the number of rays in D_2 from 18 to 28 and in A from 18 to 28, which is a considerable range of variation.

It will be seen from the table that the average number of vertebrae and fin rays in D_2 and A increases gradually as we proceed from warmer to colder seas. There is a distinct correlation between the temperature of the water and the average values both for vertebrae and rays in D_2 and A.

It also appeared that the body size increases in arctic areas, the largest individuals available from the area of the Kattegat-Faroes and Iceland being 125 mm, while individuals from Greenland attain a length of 200 mm.

The fact that the race with the highest number of vertebrae and fin rays (*T. pingelii pingelii*) occurs in southernmost West Greenland, while the race with the somewhat lower number of vertebrae and fin rays (*T. pingelii pietschmanni*) is found in central West Greenland might seem to be an exception to the rule that these numerical values increase from south to north. The contrast is only apparent; this agrees precisely with the hydrographic conditions since the cold polar current coming from East Greenland round Cape Farewell has a stronger influence on southernmost West Greenland than on central West Greenland where the polar current is much weakened.

Still another example can be given. The writer has advanced the view[5] that *Ammodytes lancea* Cuv. (*A. tobianus* autt., non Linné), *A. marinus* Raitt and *A. dubius* Reinhardt do not represent separate species, but are only subspecies of one species, characterized by an increasing number of vertebrae and fin rays:

	Number of vertebrae	Mean	Number of dorsal fin rays	Mean	Number of anal fin rays	Mean
A. lancea lancea..	60—66	62.6—64.5	51—57	52.9 —54.7	26—31	27.1—28.4
A. lancea marinus	66—73	68.5—71.5	55—63	59.05—61.0	28—34	30.27—31.9
A. lancea dubius .	73—80	75.1	60—68	64.71	30—36	33.16

Considered as a whole the number of vertebrae in *Ammodytes lancea* s. lat. may thus vary from 60 to 80, D. from 51 to 68 and A from 26 to 36, a remarkably great range of variation.

It is stated for *Ammodytes lancea lancea* that it spawns in the spring (April to May) and the summer (August to September), at any rate in the Baltic and the North Sea, according to KÄNDLER[6]. I suppose that under the influence of high temperatures its young developed a lower number of vertebrae and fin rays than *A. lancea marinus*, the spawning period of which occurs in the winter (December to February) with the low temperatures (the hydrographical winter temperature falling as low as 3° C. mean or even lower). A still higher number of vertebrae and rays in dorsal and anal fins is found in *A. lancea dubius* which is a pronounced arctic form, since it occurs in Greenland; and it is the predominant form there, since 233 specimens have been available for counting the vertebrae against only 38 specimens of *A. lancea marinus*.

Simultaneously with my paper KÄNDLER published a work in which he holds the same view as in his earlier preliminary notice, viz. that *A. marinus* is a distinct species. I cannot, however, change my view[7].

By the investigation of a huge material of cod (*Gadus callarias* L.), about 20.000 specimens derived from 114 localities, JOHS. SCHMIDT[10] came to the result that the number of vertebrae, at any rate in open waters, decreases as we proceed from north to south. This applies both to the western part of the

area from Labrador and southward to U.S.A., and to the eastern part from northern Norway to the North Sea, and from Iceland to western Scotland and England. The average number of vertebrae proved to be influenced—directly or indirectly—by the temperature of the water, in such a manner, that the populations under lowest temperatures have the highest number of vertebrae and vice versa. The 2nd dorsal fin also, like the vertebrae on the whole, showed correlation between the number of rays and the temperature.

The expression "north to south" used by SCHMIDT should not be understood to mean that the geographical degree of latitude is the decisive factor, it is the temperature conditions in the locality which are of importance; this appears from SCHMIDT's own account and is also expressed in the above brief summary of his paper. A table of the average number of vertebrae with a table of the surface isotherms in the whole area discussed is given by SCHMIDT in his Pl. II, l. c.

A striking example showing that it is hydrographical conditions and not the degree of latitude which are decisive is clearly shown by SVEN RUNNSTRÖM in his paper: "Racial Analysis of the Herring in Norwegian Waters"[11], in which he writes on p. 86: "As regards the Norwegian spring-herring we found a decreasing number of vertebrae from south to north, which is apparently in contradiction to the general rule. However, we have stated that the salinity and temperature along the Norwegian coast increases northwards and thus the variation in the vertebral number is in good accordance with the presumed effect of these factors[12]. In the same manner we found that the vertebral number of the spring spawning groups in northern North Sea increases from west to east with falling salinity and temperature".

As mentioned on p. 5 I found a parallel to this in *Triglops pingelii*: In southernmost West Greenland which is strongly influenced by the cold polar current coming from East Greenland the number of vertebrae and rays in D_2 and A is greater than in central West Greenland, where the polar current is much weakened.

In several fishes a correlation has thus been proved to exist between the temperature of the sea water and the number of vertebrae, and sometimes also the rays in certain median fins.

That the number of vertebrae in races of the herring (*Clupea harengus*) may even vary with the fluctuations in environmental factors[13] has, so to speak, been proved by Nature herself in recent years by a large-scale experiment. In 1936 RUNNSTRÖM[14] called attention to the fact that the number of vertebrae in the herring of Iceland which he examined in 1932 and 1933 was lower than that found by A. C. JOHANSEN[15] on the Icelandic herring for the years 1919—1924. RUNNSTRÖM adds: "perhaps this phenomenon can be explained by changes in the hydrographical conditions on the spawning grounds". Further I may quote the following statement in RUNNSTRÖM's above-mentioned work from 1941 (pp. 92—93):

"..... the mean vertebral number of the Norwegian spring herring in present time is lower than that found by BROCH in the years 1904—1906[16]. Also the vertebral number of the Icelandic spring herring in the period 1932—34 was considerably lower than the mean found by JOHANSEN in the year 1924. Also as regards the Faroe herrings WOOD[17] found a much lower vertebral number than earlier stated by JOHANSEN, and according to LISSNER[18] the autumn spawning herring in the North Sea also seems to have a lower mean value than the commonly accepted one. Undoubtedly the northern waters have had a rather "warm" period in recent years, as pointed out by AD. S. JENSEN[19], which may have lowered the vertebral number of the herring in these waters".

It can be added that VEDEL TÅNING[20] who examined samples of herrings caught in Iceland in 1936 came to the same result as RUNNSTRÖM, viz. that the average number of vertebrae in these quite young herring, both spring and summer spawning herring, was equal to that recorded by RUNNSTRÖM in mature herring a few years ago, but lower than the value found by JOHANSEN in 1919—1924.

RUNNSTRÖM further states that ROUNSFELL and DAHLGREN[21] and TESTER[22] have found that the mean vertebral count of successive year classes of the Pacific herring (*Clupea pallasii*) along the west coast of North America varies inversely with the temperature of the water during the period of spawning and early development.

It might be asked whether the temperature is the only factor

capable of influencing the average values of the number of vertebrae and fin rays, since it might be presumed that the salinity might also be of importance. To clear up this question Johs. Schmidt examined the cod and came to the conclusion thal the salinity in the North Atlantic area does not seem to point to any closer connection between the variations of this factor and of the characters investigated, as was the case in respect of the temperature[10].

While no experiments have been made with a view to changing the number of vertebrae and fin rays by changing the temperature at a very early stage in the development of the fishes mentioned above, many investigators have studied the influence of the temperature on the rate of development of the eggs of fishes. The results all show that high temperatures increase the rate of development and that low temperatures delay it. Worth special mention is a series of accurate measurements of the average rate of development of the eggs made by Dannevig[23] on five species of salt water fishes; the results were as follows:

Temp. in °C.	−1°	3°	4°	5°	6°	8°	10°	12°	14°	
Gadus callarias	42	23	20 1/2	17 1/2	15 1/2	12 3/4	10 1/2	9 2/3	8 1/2	Time
— *merlangus*	..	..	..	15 1/3	13 1/2	10 1/4	8	6 1/2	5 3/4	of incu-
— *aeglefinus*	42	23	20 1/2	17 3/4	15 1/2	13	10 3/4	9 2/3	8 3/4	bation
Pleuronectes platessa	..	..	..	..	18 1/4	14 1/3	12	10 1/2	..	in days
— *flesus*	..	..	..	..	6 1/2	5 1/2	4 1/2	3 2/3	..	(24 hours).

Cf. also Worley whose experiments on the development of the egg of mackerel (*Scomber scombrus*) at different temperatures (10°—24° C.) showed that typical development could be realized only between 11° and 21°; the length of the developmental period increases from 49 1/2 to 207 hours when the temperature is lowered from 21° to 10° C.[24]

In this country Johansen and Krogh[25] by their studies on the influence of the temperature on the development of eggs of cod and plaice partly confirmed the result of Dannevig's experiments, partly continued them. But they explain the influence of the temperature on the rate of development of the eggs on the basis of the following quite new views: "There is no reason to

believe that a supply of energy in the form of heat could be utilized at all by the embryos. The energy, which is undoubtedly necessary for the development, is derived in the case of fish eggs, as in all other eggs, from the chemical processes involved in the metabolism of the eggs, and an outward sign of this is that a certain proportion of the nutritive material contained in the eggs, disappears during development, being oxidized to carbon dioxide and water and yielding energy. The temperature must be looked upon as a factor which will have a certain influence upon the velocity of the chemical reactions and other processes involved in the development".—As far as I know, nobody has studied this part of the problem since JOHANSEN and KROGH's paper appeared in 1914.

II.

Constant species.

In the preceding part we have mentioned fishes in which there is correlation between the temperature of the sea water and the number of vertebrae and rays in the median fins, whereby local races can be distinguished.

We shall now consider two species of fish, each having a wide geographical distribution, where the individuals live under very different conditions without forming races.

In the first place the European eel (*Anguilla anguilla* Linné[26]) should be mentioned, on which JOHS. SCHMIDT has undertaken a comprehensive racial study[27]. After examining 5—6000 specimens from its vast area of distribution—from Iceland, the Faroes and the White Sea along Europe down to North Africa and from the adjoining seas, the Baltic and the Mediterranean with the Black Sea and the Sea of Azov and from the rivers and lakes connected with all the areas mentioned here—he was able to show that all eel populations, even those from the most far-away corners of Europe displayed identical average values as regards the number of vertebrae; all European eels belong to one and the same species, within which it was impossible to demonstrate the existence of local races[28]. This, SCHMIDT says, is due to the fact that all European eels are born in the same locality. By purposeful and untiring exploration, through 25 years, of the Atlantic, from Iceland to Morocco and from Egypt to

America, SCHMIDT succeeded in demonstrating that the eels from Europe travel over the Atlantic to the Sargasso Sea where they spawn in the spring. The spawning region of the European eel lies in an area to the north-east of St. Thomas and south-east of the Bermudas, where the depth is more than 6000 m, for here, and only here, were the newly hatched larvae found, their length being 5 to 7 mm; they float 200—300 m below the surface in water layers, the temperature of which is about 20° C. The larvae (*Leptocephalus brevirostris*) gradually rise to the upper water layers, and are then carried towards Europe by the east-going movement of the North Atlantic current, and later on, when the youngest stages of development have been passed, also by their own active movements—a journey that lasts between two and three years. The first development of the eel, including the formation of the vertebrae, takes place under quite uniform hydrographic conditions[29].

The same view may be applied to the other species of *Anguilla* of the Atlantic, the American *A. rostrata* Le Sueur[30]. It occurs in south-western Greenland and Labrador; it is abundant in Canada and the United States, in the northern part of Mexico, partly also in the West-Indian archipelago; it is also found in the southern part of Mexico and in Panama and in South America in Guiana[31]. Of the American eel 863 specimens were examined and it appeared that it is distinguishable from the European eel, of which 2775 specimens were examined, by means of the average number of vertebrae: *A. rostrata* 107, 250, *A. anguilla* 114, 728[32]. In *A. rostrata* too SCHMIDT could not demonstrate races. This is due to the fact that all the American eels have common breeding grounds, situated in a small section of the warm, deep Sargasso-Sea north of the West-Indian Islands, for the most part somewhat farther west and south than the European Eel, and that they spawn a couple of months earlier in the year, as early as January and February; here hundreds of the early tiny larvae, 7—8 mm long, were caught. Gradually as the larvae (Leptocephals)[33] increase in size they spread out in the western Atlantic to the north and to a smaller extent to the south. The full development from egg[34] to elver is completed in only about one year; the elvers are then close to the American coast and seek the shores and river mouths. The European eel,

on the other hand, has many months in which to exist as larva before the metamorphosis, and during this time it is carried towards and makes for Europe[35].

III.

Correlation between temperature and number of vertebrae in genera.

In section I it was exemplified how the temperature of the water influences countable characters in certain marine fishes, viz. vertebrae and rays in the median fins, which are the characters by which races within these species are distinguishable from each other. It was also exemplified how fishes which had previously been described as distinct species, actually belong to a single species, the presumed species corresponding to races, the distinctive features of which, the number of vertebrae and fin rays, are also correlated with the different temperature conditions under which these fishes breed.

But not only this. Within certain families the genera of which are distinguishable from each other by good characters usually employed in ichthyology, some genera belong to tropical, others to temperate seas. The species within the genera distributed in warm seas have been found to possess a low number of vertebrae, while the species of genera from colder seas have a high number of vertebrae. As these investigations, which were made in the latter half of the last century, throw light on the development of higher systematic units, they will be briefly dealt with in the following.

The first to direct attention—in 1862—to this was the famous German-English zoologist Albert Günther. In the 4th volume of his monumental work in eight volumes on the fishes of the world Günther writes as follows in the introduction to the family Lip-fishes (*Labridae*): "A character which has been entirely overlooked, but which, for the further division of Labridae, is as important as that taken from the dentition or from the structure of vertical fins, is that of the number of the vertebrae, the value of which has been maintained by me on several occasions. It will be evident, from the numerous statements contained in the following pages (65—244), that in those genera

which are composed entirely or for the greater part of tropical species, the vertebral column is composed of 24 or nearly 24 vertebrae, whilst those which are chiefly confined to the temperate seas of the northern or southern hemisphere have that number increased[36]".

In the following years several of the leading ichthyologists of America, GILL, JORDAN, GOSS, GILBERT and EIGENMANN, gave notice that they too had found that there existed a correlation between temperature and vertebrae in many other families and genera of bony fishes; *Clupeidae, Labridae, Scorpaenidae, Blennidae* and *Pleuronectidae* were pointed out as examples hereof.

In 1891 JORDAN gave a statement of what was known of this subject and he drew the following conclusion from the last 30 years' investigations in this field: "In many groups of fishes the northern or cold-water representatives have a larger number of vertebrae than those members which are found in tropical regions[37]".

In these papers JORDAN has a small section on the variations in fin-rays and he writes about this: "In some families the number of rays in the dorsal and anal fins is dependent on the number of vertebrae. It is therefore subject to the same fluctuations". As examples hereof he mentions some genera within the family *Scorpaenidae.*

As a striking example showing that there is a relation between the number of vertebrae and geographical distribution JORDAN and EVERMANN in their large work mention the family *Pleuronectidae*[38] and write in the introduction (p. 2603) that in no group of fishes is it more striking that the northern representatives have the number of vertebrae increased. They give numerous examples of this and attach so much importance to the number of vertebrae that they include this character in the subdivision of the flounders into genera.

Summary.

I.

In a series of species of marine fishes, *Lycodes vahlii, Pholis gunellus, Triglops pingelii, Ammodytes lancea, Gadus callarias* and *Clupea harengus,* with a wide geographical distribution, there is

a correlation between the hydrographic conditions and the vertebrae, the latter increasing in number with decreasing temperature. Within these species local races can be distinguished which are characterized by the average numbers of vertebrae. Since the formation of the vertebrae takes place at a very early stage in the development, and as the vertebrae remain unchanged throughout life, the formation of races within each species is correlated with the temperature conditions prevailing in its different breeding areas. What has been said here of the vertebrae partly applies also to the number of rays in the dorsal and anal fins. In *Lycodes vahlii* and *Triglops pingelii* an increase of body size in relation to decreasing temperature has likewise been shown to exist.

II.

For two other species of fish which also have an enormous geographical distribution, viz. *Anguilla anguilla* and *Anguilla rostrata*, no local races can be demonstrated on the basis of the vertebral number or other characters although these species live under very different hydrographic conditions. When these fishes are going to propagate they do not breed in the places where they grew up, but all of them migrate to the same area, the Sargasso Sea, where egglaying and the very first stages of development, thus also the formation of vertebrae, take place; this explains why these fishes, no matter how far they migrate, preserve their constancy of species and do not segregate into races.

III.

In the latter half of the last century the leading ichthyologists of Europe and America called attention to the fact that in many genera of bony fishes erected on the usual good characters there is a correlation between temperature and vertebrae, so that the species within genera distributed in warm seas have a low number of vertebrae, while species of genera from cold seas have a high number of vertebrae. These conditions throw light on the development of higher systematic units.

Notes.

[1] Ad. S. Jensen: Ichthyologiske Studier, II: Om en mærkelig Variationsrække af *Lycodes vahlii* Reinh. Vidensk. Meddel. fra den naturhist. Foren. i Kbhvn. 1901, pp. 202—204.

[2] The Danish Ingolf Expedition, II. 4, 1904.

[3] Ad. S. Jensen: Contributions to the Ichthyofauna of Greenland, I—III, p. 40. Spolia Zoologica Musei Hauniensis, II, 1942.

[4] Ad. S. Jensen: Contributions to the Ichthyofauna of Greenland, IV—VI, p. 13-23. Spolia Zoologica Musei Hauniensis, IV, 1944.

[5] Ad. S. Jensen: On subspecies and races of the Lesser Sand Eel (*Ammodytes lancea* s. lat.). Kgl. Danske Vidensk. Selsk. Biologiske Meddelelser XVI, 9. 1941.—After this paper had been published I received from Mr. Paul Hansen, fishery biologist, 53 specimens of *Ammodytes*, 31.5—48.5 mm long, extracted from the stomach of the charr (*Salmo alpinus*) caught on July 17th 1932 in Amitsuarssuk, a branch of the Godthaabfjord. All these 53 specimens were examined for the number of vertebrae, and the mean figure proved to be 75.07, thus agreeing with that found for *A. dubius*; one of the specimens was however peculiar in having 80 vertebrae, while the highest number previously found in Greenland *Ammodytes* was 78.

[6] R. Kändler: Beobachtungen über die Laichzeiten der *Ammodytes*-Arten in Nord- und Ostsee. Zool. Anz., Bd. 118, 1937, p. 1.

[7] Rudolf Kändler: Untersuchungen über Fortpflanzung, Wachstum und Variabilität der Arten des Sandaals in Ost- und Nordsee, mit besonderer Berücksichtigung der Saisonrassen von *Ammodytes tobianus* L. Kieler Meeresforschungen, Bd. V, Heft 1, 1941, pp. 45—145.

As regards the adult individuals Kändler is of the opinion that the species *A. marinus* and *A. lancea* can be distinguished from each other by the colour, since the former is bluish-green on the upper side, while the latter is generally more yellow-green and lighter. It is however a well known fact that the colours in one and the same species of fish may vary very much, especially in harmony with the surroundings; as *A. marinus* is a high sea fish it is quite natural that the upper side should assume a bluish-green colour, while *A. lancea* which lives in shallow water near the shore acquires a yellow-green and lighter tinge corresponding to this area. Otherwise Kändler as well as Bruun[8] and I cannot accept Duncker and Mohr's statement that *A. marinus* differs from *A. lancea*, besides by the two ventro-lateral skin folds, by possessing a third somewhat lower skin fold in the midventral line, whereas *A. lancea* lacks such a fold; Kändler says about this that he has not been able to prove any essential difference in the numerous specimens which he had before him, a low keel-like skin fold is found in both species.

In this paper Kändler reports an interesting observation which he has made: The young stages of *A. marinus* are easy to distinguish from those of *A. lancea* by the different pigmentation; this feature is described on pp. 64—66 and illustrated by a series of text figures. Kändler attaches much importance to this and believes that the different distribution of the pigment in these larvae shows that they belong to two different species. In this connection I

call attention to the fact that JOHS. SCHMIDT in his great work: "The pelagic postlarval stages of the Atlantic species of Gadus", Part II, pp. 4—5[9] states that larvae of the common cod (*Gadus callarias*) from the southern North Sea and the inner Danish waters deviate from those of the northern North Sea, the Skagerrak, the Faroes and Iceland in that the pigment is much weaker and in that the hindmost postanal pigment band, which in the more northern sea areas in the small young of cod is an important mark of recognition, is quite lacking in several specimens. I doubt therefore that a difference in the distribution of the pigmentation in the two larval forms of *Ammodytes* should be interpreted to mean that the two forms belong to two species.

The young stages of *A. marinus* from the Baltic are considerably more slender than equally large specimens from the North Sea and than young *A. lancea* according to KÄNDLER; consequently no specific difference can be traced in that respect.

There only remain the differences in the number of vertebrae and rays in the dorsal and anal fins; as will be seen from the table on p. 6 they clearly show a division, although with transitions, of the species into three groups which I called subspecies in my earlier paper, but which I now consider it more correct to designate as races. As shown by KÄNDLER, there exist again two races of *A. lancea lancea* which have different breeding times (spring and summer) and which show considerable differences in the number of vertebrae and fin rays, although it comes within the limits for *A. lancea lancea*.

KÄNDLER studied the structure of the otoliths most thoroughly and it proved to differ according to different breeding times. Thereby it became possible to distinguish the *Ammodytes* forms from each other, and age and growth conditions could now be determined.

In 1941 (Fauna och Flora, p. 24) the Swedish zoologist YNGVE LÖWEGREN gave his view on *Ammodytes*. In catches with a seine on the south and east coast of Skåne he found 955 *A. lancea* ("*A. tobianus*") and only 29 *A. marinus*, but he emphasizes himself that this is due to the fact that *A. marinus* prefers deeper water. Only by the study of the number of vertebrae and fin rays can the two species be distinguished, in outer appearance they are practically alike, according to LÖWEGREN.—A small correction may be made in L.'s map fig. 4; it shows that *A. marinus* occurs in eastern Greenland, but in my paper I write on p. 17: "*Ammodytes* is not known from the east coast of Greenland".

[8] ANTON FR. BRUUN: The *Ammodytes lancea* group. Vidensk. Meddel. fra Dansk naturhist. Foren., Bd. 104, 1941, p. 329.

[9] Meddel. fra Kommiss. f. Havundersøgelser, Serie: Fiskeri, Bd. II, No. 2, 1906, pp. 4—5.

[10] JOHS. SCHMIDT: The Atlantic cod (*Gadus callarias* L.) and local races of the same. Comptes-rendus des travaux du Laboratoire Carlsberg, 18° volume, no. 6, p. 1—71, Pl. I—X. Copenhague 1930.

[11] Report on Norwegian Fishery and Marine Investigations, Vol. VI, No. 7, 1941.

[12] The rising temperatures and salinities of the coastal water from south to north are due to an inflow of cold Baltic water with low salinity from the south, which is gradually intermingled with warmer and more saline Atlantic water along the coast (RUNNSTRÖM l. c. p. 92).

[13] This should not be understood to mean that the vertebral number of the adult fishes can be altered; it is a well known fact that the formation of the vertebrae takes place at a very early stage of the development, and that the vertebral number hereby is fixed for the rest of the life of the fish.

[14] SVEN RUNNSTRÖM: The distribution of the Atlanto-Scandian Spring-Herring. Rapp. et Proc.-Verb. du Conseil Internat. pour l'explor. de la Mer, Vol. C, 2. Part, pp. 25—26.

[15] A. C. JOHANSEN: On the summer-spawning herring of Iceland. Meddel. fra Kommiss. f. Havundersøgelser, Bd. VI, No. 3. 1921—*Idem*: Investigations on Icelandic herrings in 1924 and 1925. Rapp. et Proc.-Verb. du Conseil Internat. pour l'explor. de la Mer, Vol. XXXIX, 1926.

[16] Hj. Broch: Norwegische Heringsuntersuchungen während der Jahre 1904—1906. Bergens Museums Årbok 1908.

[17] M. A. Wood: In "Report of North-Western Area 1934". Rapp. et Proc. Verb. du Conseil Internat. pour l'explor. de la Mer, Vol. XCIV. 1935.

[18] H. Lissner: On races of herrings. Journal du Conseil, Vol. IX, 1934.

[19] Ad. S. Jensen: Concerning a change of climate during recent decades in the Arctic and Subarctic regions, from Greenland in the west to Eurasia in the east, and contemporary biological and geophysical changes. Kgl. Danske Vidensk. Selskab, Biol. Meddel. XIV, 8, 1939.

[20] Å. Vedel Tåning: Rapp. et Proc.-Verb. du Conseil Internat. pour l'explor. de la Mer, Vol. CIX, p. 16. 1938—39.

[21] G. A. Rouncefell and E. H. Dahlgren: Fluctuations in the supply of herring (*Clupea pallasii*) in Prince William Sound, Alaska. U. S. Bur. Fish. Vol, 47, Bull. no. 9, 1932.

[22] A. L. Tester: Variation in the mean vertebral count of herring (*Clupea pallasii*) with water temperature. Journal du Conseil, Vol. XIII, 1938.

[23] Harald Dannevig: The Influence of Temperature on the Development of the Eggs of Fishes. 13. Ann. Rep. of the Fishery Board for Scotland, being for the year 1894, Part III, p. 149. 1895.

[24] Leonard G. Worley: Development of the eggs of mackerel at different temperatures. The Journal of General Physiology. Vol. 16, 1933, p. 841.

[25] A. C. Johansen and A. Krogh: The influence of temperature and certain other factors upon the rate of development of the eggs of fishes. Cons. perm. internat. pour l'explor. de la Mer, Publ. de Circonstance, No. 68, 1914.

In a succeeding paper Krogh continued the investigations on other groups of animals (eggs of frogs, an insect and a few sea urchins) and in all cases found a straightlined relation between the temperature and rate of development within the temperature interval in which development normally takes place. At the lowest temperatures at which development is possible the rate seems to be relatively a little greater. As the determination applies to very different types Krogh is of the opinion that there is reason to believe that this relation will have general validity. It is the same relation which is expressed by the so-called heat sums or day degrees. Aug. Krogh: On the influence of the temperature on the rate of embryonic development, Zeitschr. f. allg. Physiol. 16, 1914, p. 163.

[26] Synonym: *Anguilla vulgaris* Turton.

[27] Johs. Schmidt: First report on Eel investigations 1913. Rapports et Procès-Verbaux du Conseil International pour l'Exploration de la Mer, Vol. XVIII, p. 1—30 (1914).—*Idem*: Second report on Eel investigations 1915. Ibid. Vol. XXIII, p. 1—24 (1916).

[28] In a paper from 1925: On the distribution of the Fresh-Water Eels (*Anguilla*) throughout the world, II, p. 335. K. D. Vidensk. Selsk. Skr. nat.-mat. Afd. 8, X, 4) Schmidt records that from the Museum of Genova he has received a number of specimens of *Anguilla* from Massawa on the Red Sea, and that they belong to *A. anguilla*. In his paper: "A revision of the genus *Anguilla*" Vilh. Ege ("Dana-Report" No. 16, 1939, p. 149) states that he received six specimens from East Africa whose exact origin is unknown, and three specimens taken at Nairobi in Kenya; these specimens too belong to *A. anguilla*. All the East African specimens may be supposed to have immigrated from the Mediterranean through the Suez Canal. It should be mentioned in this connection that through the Suez Canal which was opened in 1869, some animals have migrated in the opposite direction, from the Red Sea into the Mediterranean. According to Steinitz ten species of fish have penetrated into the Mediterranean through the Suez Canal. Of the evertebrates the pearl-oyster (*Meleagrina margaritifera*) which occurs in great numbers in the Red Sea has migrated through the Canal into the Mediterranean and has spread there to such an extent that it is common on the North African coasts right on to Algeria. The swimming crab *Neptunus pelagicus* reached the Mediterranean at

the end of the nineties and is now so common that it is generally sold on the fish market in Alexandria. In addition immigration of the echinoderm *Ophiactis Savignyi* has been ascertained. On the other hand, no evertebrate is known with certainty to have migrated from the Mediterranean to the Red Sea through the Suez Canal. (Litt. Walter Steinitz, Publ. d. Staz. Zool. di Napoli, 8, 1927. Th. Mortensen: Dyrevandringer gennem Suez-Kanalen; Naturens. Verden, XII, 1927. Th. Mortensen: *Echinoderma*; Ministry of Commerce and Industry, Egypt, The fishing Grounds near Alexandria, XIII, 1937).

[29] Johs. Schmidt: The breeding places of the Eel. Philosoph. Transact. Roy. Soc. London, Series B, Vol. 211, pp. 179—208. 1922.—*Idem*: Die Laichplätze des Flussaals. Internat. Rev. der ges. Hydrobiol. u Hydrographie, Bd. 11, Heft 1—2, pp. 1—40, 1923.—*Idem*: The breeding places of the Eel. Smithson. Rep. for 1924, pp. 279—316 (1925). *Idem*: Danish Eel investigations during 25 years, 1905—1930. Published by the Carlsberg Foundation. Copenhagen, 1935.

[30] Syn. *Anguilla chrysypa* Rafinesque, cf. Bean in "Science" N. S. Vol. XXIX, p. 871, New York, 1909.

[31] As regards the distribution of the American Eel and its spawning region see the litterature given under Johs. Schmidt[29].—In the above mentioned paper[28] Vilh. Ege (p. 149) records that Seale "had examined eels from Panama and the West-Indies, which were said not to differ in the least from *A. anguilla*. But it became clear later that the four specimens reported as coming from Panama had really come from Cadiz in Spain, whilst an examination of the specimens from St. Thomas, West Indies, undertaken for the present work, has proved that they belong to *A. rostrata*".

[32] Later on Ege (29, p. 132) further examined one hundred *A. rostrata*, and the average number of vertebrae in the 962 specimens was so to say unchanged, viz. 107, 233. It is true that the numbers of vertebrae in the two species overlap: in *A. rostrata* 103 to 111, in *A. anguilla* (110) 111 to 119, but only very few specimens per thousand cannot be referred with certainty to the one or the other of the two species. In his comprehensive monograph on the genus *Anguilla*, which is based on a very large collection and prepared with minute care, Ege has amplified the specific distinction between *A. rostrata* and *A. anguilla* by demonstrating the following specific differences:

A. rostrata: Average maximum value of distance between verticals through anus and origin of dorsal fin, in % of total length, about 9.1. Number of prehaemal vertebrae 41—45.

A. anguilla: Average maximum value of distance between verticals through anus and origin of dorsal fin, in % of total length, about 11.2. Number of prehaemal vertebrae 44—47.

Nowhere in the paper does Ege utter the least doubt that *A. rostratra* and *A. anguilla* are good species, though in several cases he unites forms which were previously regarded as species into subspecies of a single species. Further the very essential difference should be added that the average length of fully grown larvae of *A. rostrata* is about 60—65 mm, of *A. anguilla* about 75 mm, and that the former develops from egg to elver in the course of about 12 months, whilst the latter takes 3 years to carry through the same development.

[33] The Leptocephals of the two species, like the adults, are distinguishable from each other by the number of vertebrae (or myomeres). Of the European eel more than 11000 larvae were examined, of the American eel more than 2300 larvae.

[34] When it is recorded in current literature on the basis of M. P. Fish's paper: "Contributions to the embryology of the American eel, *Anguilla rostrata*" (Zoologica, Vol. VIII, No. 5, New York, 1927) that the eggs of the American Eel have been identified, it should be pointed out that Johs. Schmidt (The Danish "Dana" Expedition 1920—22, Vol. I, No. 1, 1929, p. 16) has commented as follows on the eggs, supposed to be of *Anguilla*, collected in April—May 1923 during the work of the expedition in the Sargasso Sea: "These are altogether different from the ova which Mrs. Marie Poland Fish has referred

to the American Eel, and which, in the opinion of Dr. TÅNING and myself, have nothing whatever to do with *Anguilla*". Unfortunately Prof. SCHMIDT did not get an opportunity to revert to this matter. On my request Dr. A. F. BRUUN however informed me that most likely the eggs determined by Mrs. FISH belong to *Leptocephalus similis* Lea, which, as will be seen from BRUUN's paper (Dana Report, Vol. II, No. 9, 1937, p. 26) has the same number of myomeres as the Leptocephal of *A. rostrata*, and otherwise, to a less trained eye can easily be confounded with young Leptocephals of *Anguilla* on account of its appearance. *L. similis*, according to BRUUN (l. c.), belongs to a genus nearly allied to the *Muraena*, and it is natural to mention here that a similar confusion of a Leptocephal of this type with the *Anguilla*-Leptocephal has been made by UCHIDA, as stated by BRUUN (l. c. p. 9).

[35] The central part of the breeding area of the American Eel lies somewhat to the west and south of the central part of the breeding area of the European Eel, but the areas of the two species are apparently not separated, but seem to overlap. It is conceivable, therefore, that some larva of the American Eel took the "wrong way" to Europe, but in that case it might perhaps perish, as its pelagic larval life which only lasts 12 months would come to an end in the middle of the Atlantic ocean. Nevertheless, it has happened once that an American Eel has been found to have landed in Europe among thousands of specimens examined. A. BRUUN says in his paper: "Contributions to the life histories of the Deep Sea Eels *Synaphobranchidae*" p. 26 (Dana Report, Vol. II, No. 9, 1937), "that a specimen of *A. rostrata* was taken at San Sebastian in North Spain in December 1930; it was a glass-eel with 108 vertebrae and 68 mm long, taken along with several hundred glass-eels of *A. anguilla* sent to the late Prof. JOHS. SCHMIDT from Dr. GANDOLFI HORNYOLD". A similar case occurred in south western Greenland, where three young *Anguilla rostrata*, 109—110 mm long, were collected in 1841, according to AD. S. JENSEN (Meddel. om Grønland, Bd. 118, No. 9, p. 7, 1937); these too might have made their way to Greenland as elvers.

[36] ÅLBERT GÜNTHER: Catalogue of the Fishes in the British Museum, Volume Fourth. London 1862.

[37] DAVID STARR JORDAN: Relations of Temperature to Vertebrae among Fishes, Proc. U. S. Nat. Museum, Vol. XIV, No. 845. 1891. A modified reprint, with some additional matter, has been given by JORDAN in The Wilder Quarter-Century Book, Ithaca, N.Y. 1893.

[38] DAVID STARR JORDAN and BARTON WARREN EVERMANN: The Fishes of North and Middle America, Part III, Washington, 1898.

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HISTOTOPOGRAPHIE DES GLANDES PYLORO-DUODÉNALES

PAR

TAGE STRUNGE

KØBENHAVN
I KOMMISSION HOS EJNAR MUNKSGAARD
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Les recherches patho-physiologiques récentes sur l'anémie pernicieuse exigent une description particulière de l'anatomie normale des glandes pyloro-duodénales.

C'est CASTLE qui a démontré qu'un «facteur intrinsèque», élaboré dans l'estomac humain, peut compenser l'anémie pernicieuse, s'il est complété par le «facteur extrinsèque», ingéré par les aliments. Les expériences thérapeutiques de MEULENGRACHT et les descriptions histotopographiques de MEULENGRACHT & SØEBORG-OHLSEN localisent le facteur intrinsèque du porc à la région pyloro-duodénale et à la zone cardiaque, moins importante.

Le but de cet ouvrage est la définition anatomique des glandes pyloro-duodénales de l'homme, réalisée par des recherches cytologiques et histotopographiques. Nous avons analysé d'abord les différentes cellules épithéliales du tube digestif. Ayant constaté un même type cellulaire morphologique dans les glandes pyloriques, duodénales et cardiaques, nous avons recherché ensuite la distribution topographique de cette «cellule pyloro-duodénale». — L'examen microscopique du tube digestif fœtal complète les résultats.

Aperçu historique.

Les glandes duodénales furent découvertes par J. J. WEPFER (1677) qui, ayant examiné le tube digestif d'une femme décapitée, en publia la description suivante:

> «.... In duodeno plurimas insignes glandulas ultra palmi «longitudinem a pyloro deorsum sparsas inveni, quae de- «tracta tunica fibrosa quasi conglomeratae apparuerunt.»

JOHANN KONRAD A BRUNN (BRUNNER) découvrit en 1686 les mêmes glandes; ses résultats furent communiqués dans une thèse

(1688), et plus tard dans une monographie très importante (1715). Sa description de la zone glandulaire est devenue classique:

«.... Ventriculus abhinc cum duodeno mergatur in aquam »ferventem, ac tamdiu igni permittatur in lebete donec cor- «rugari coeperit.... ubi vero ad pylorum usque perrexeris, «statim sub limbo ejus alia rerum facies, et in conspectum «veniunt glandulae; corpuscula scilicet figurae plerumque «sphaericae, candicantia turmatim et denso agmino consita; «diversi ordinis, figurae et magnitudinis ad insertionem usque «meatus bilis: Ab initio duodeni primi ordinis et majus «culae, dimidati interdum pisi magnitudine; minores abhinc «seminum Cannabinorum, et secundi ordinis visuntur; tertii «autem, omnium minimae denso totum intestini ambitum «agmine stipant ad porum usque choledocum. Demum rari- «ores sigillatim hinc inde et sparsim disseminatae apparent, «tandemque prae exilitate visum subterfugiunt.» (Fig. 1.)

Middeldorph (1846) introduisit le terme de «glandulae Brunnianae» et s'opposa à l'opinion de Brunner qu'il s'agissait d'un «pancreas secundarium». Schwalbe (1872) documenta la structure tubulo-acineuse des glandes duodénales, dont les acini et les canaux sont revêtus d'un même épithélium cylindrique. Kuczynski (1890) examina des glandes pyloriques et duodénales, fixées au sublimé et colorées au bleu d'aniline; la ressemblance morphologique des deux types cellulaires lui fit supposer une correspondance fonctionnelle entre eux. Il observa que les glandes duodénales sont plus développées chez les herbivores (cheval, vache, porc, lapin, cobaye) que chez les carnivores (chien, chat, martre), l'homme occupant une position intermédiaire entre ces deux types. Bogomoletz (1902) se servit de la coloration: safranine-acide picrique, pour l'examen des modifications des cellules à diverses phases de la digestion. Bensley (1903) décrivit la structure des cellules glandulaires.

L'identité cytologique des glandes pyloriques et duodénales fut corroborée par le matériel d'autopsie de Paschkis & Orator (1923). Meulengracht (1934) observa la même augmentation des réticulocytes sanguins après ingestion d'une préparation séchée de paroi duodénale et de paroi pylorique à des malades, souffrant d'anémie pernicieuse.

Les glandes pyloriques ont été décrites par Bischoff (1838). Le matériel bien fixé de Toldt (1880) permit la distinction nette entre les glandes du pylore et celles du fond, séparées par une zone de transition, où s'entremêlent les deux types

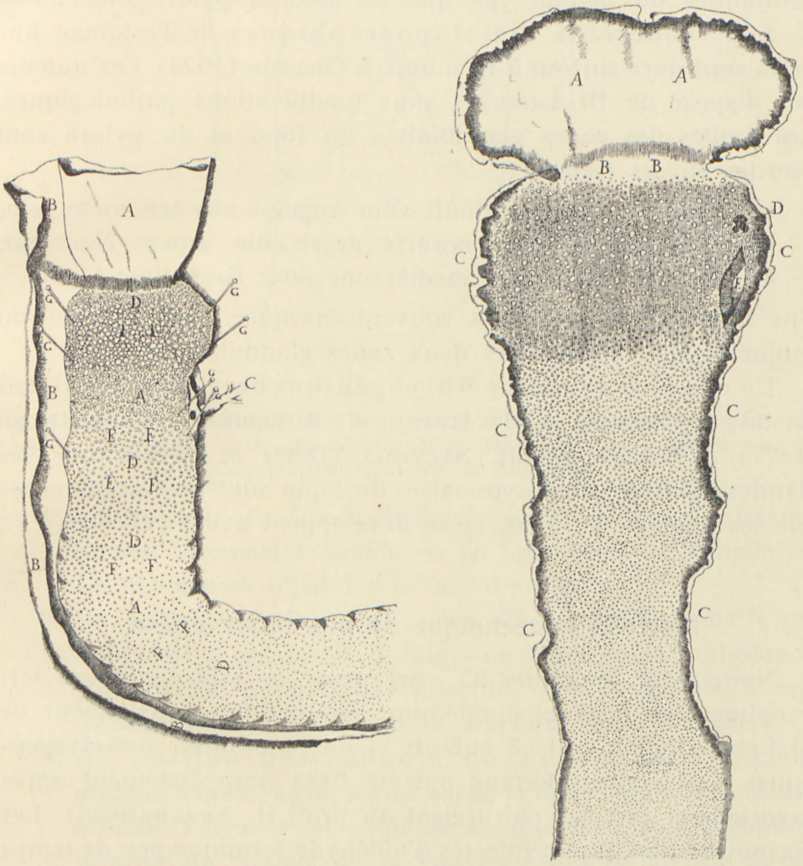

Fig. 1. La première figure des glandes duodénales. (d'après J. C. Brunner: Glandulae duodeni..., 1715).

glandulaires; les glandes pyloriques sont tubuleuses et ramifiées; elles ne contiennent pas de cellules bordantes. Stoehr (1882) trouva parfois des cellules bordantes dans les glandes du pylore; il confirma l'existence de la zone transitoire, ainsi que le firent, plus tard, Boehm & Davidoff (1895). La structure cellulaire fut décrite par Bensley (1898).

Les glandes cardiaques furent décelées par Wassmann

(1839). KOELLIKER (1863) indiqua leur distribution topographique. TOLDT (1880) définit leur structure tubuleuse, et KUPFFER rapporta une variation de cette zone glandulaire de 1 à 3 cm. PASCHKIS & ORATOR, ainsi que MEULENGRACHT, considéraient les glandes cardiaques du même type que les glandes pyloro-duodénales.

Les recherches histotopographiques de l'estomac humain sont dues surtout à PASCHKIS & ORATOR (1923). Ces auteurs ont disposé de 10 estomacs sans modifications pathologiques. Les limites des zones glandulaires du fond et du pylore sont décrites en ces termes:

«.... Die Grenzlinie verläuft vom Angulus abwärts mehr oder «minder schräg pyloruswärts gegen die grosse Kurvatur. «Sie ist gleich oft Intermediärzone oder Grenzlinie.»

Une démarcation nette est souvent marquée par une cloison conjonctivale, séparant les deux zones glandulaires.

Le développement embryogénique des glandes gastriques est mis en lumière par les travaux de KOELLIKER (1852), BRAND (1877) et TOLDT (1880). SALVIOLI (1880) a observé que les glandes pyloriques embryonnaires du lapin sont plus nombreuses que les glandes du fond, et se développent avant celles-ci.

Matériel et technique de nos observations.

Nous avons examiné 23 «organes», ce mot indiquant ici: œsophage, estomac et duodénum: ces organes proviennent de 10 fœtus humains, de 3 enfants et de 10 adultes, non-dyspepsiques. Les organes fœtaux ont été fixés immédiatement après l'avortement (service chirurgical du prof. H. ABRAHAMSEN). Les organes adultes furent injectés d'aldéhyde formique peu de temps après la mort (service médical du prof. E. MEULENGRACHT). En plus nous avons microscopié des pièces opératoires (pylorectomies), ainsi que des coupes congelées d'organes adultes en plus du matériel cité.

La fixation immédiate étant indispensable pour la méthode histotopographique, nous nous sommes servis du principe BLOCH & FABER: injection intra-abdominale du fixateur, aussitôt après la mort que possible. Pour cet examen nous avons élaboré la technique suivante (Fig. 2):

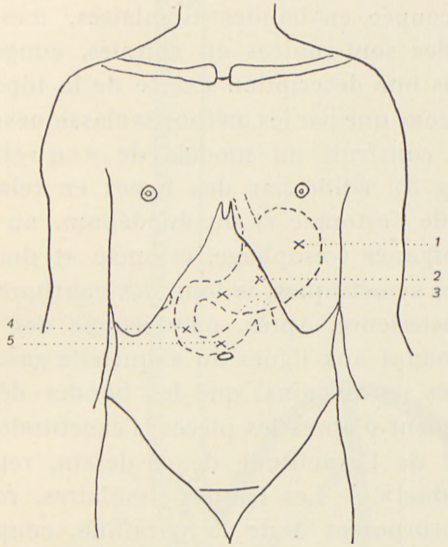

Fig. 2. Modèle schématique pour l'injection intra-abdominale afin de fixer les muqueuses gastro-duodénales. (Les contours sont modifiés d'après CUNNINGHAM: Textbook of Anatomy).

1. La région du fond: injection sagittale dans le sixième espace intercostal gauche à la ligne médio-claviculaire. Le fixateur se répand d'ici à l'œsophage.
2. Le corps de l'estomac: injection sagittale sous le rebord costal gauche, à 3 doigts de l'appendice xiphoïde.
3. La région pylorique: injection médio-sagittale, à mi-distance entre l'ombilic et la jonction xipho-sternale.
4. La portion descendante du duodénum: injection oblique, commençant au milieu de la ligne: ombilic-vésicule biliaire: l'aiguille est enfoncée en direction dorso-médiale, presque au contact de la convexité antéro-latérale de la colonne vertébrale.
5. La portion horizontale du duodénum: injection médio-sagittale à un doigt au-dessus de l'ombilic, presque au contact de la colonne vertébrale.

Nous déposons par chaque injection 100 cm^3 d'aldéhyde formique à 4 %.

Une orientation histotopographique préliminaire devient possible par la microscopie de coupes congelées: La paroi gastrique ou

duodénale est coupée en bandes tissulaires, mesurant jusqu'à 20 cm; ces bandes sont roulées en spirales, congelées, coupées et colorées. Mais une description exacte de la topographie glandulaire n'est obtenue que par les méthodes classiques de l'histologie.

D'abord on construit un modèle de «squelette gastro-duodénal» (fig. 3), édifié par des lignes en relations définies aux courbures de l'estomac et du duodénum, au pylore et au cardia. — Les organes (œsophage, estomac et duodénum) sont divisés en parties symétriques, le long des courbures: paroi antérieure, paroi postérieure. Après, on découpe des bandes tissulaires, correspondant aux lignes du «squelette gastro-duodénal». Les contours des parois, ainsi que les bandes découpées, sont dessinés directement d'après les pièces; l'exactitude de la reconstruction dépend de l'exactitude de ce dessin, représentant «le squelette individuel». — Les bandes tissulaires, roulées en spirales, seront incorporées à de la paraffine, coupées, colorées, et subiront l'examen microscopique ordinaire. Les résultats de la microscopie sont marqués au «squelette gastro-duodénal» du dessin individuel, et on peut reconstruire les diverses zones glandulaires. — L'erreur provenant de la variation en longueur de la bande pendant la préparation a été très petite; la réduction pendant la déshydratation est partiellement compensée par l'allongement causé par l'isolement de la bande de ses environs.

Nous avons coloré de préférence les coupes à l'hématoxyline -éosine et à l'azan (de Mallory-Heidenhain) modifié de la façon suivante:

Azocarmin B (solution à 1 %) à 60° C	10 min.
alcool-aniline	5—10 -
alcool acidulé par acide acétique	1 -
acide phosphoro-tungstique à 5 %	10 -
lavage à l'eau distillée	
bleu d'aniline-orange G (solution concentrée)	10 -

Cette coloration peut être précédée de

hématoxyline Hansen, très étendue	3 -

qui rend plus nets les détails de la structure nucléaire.

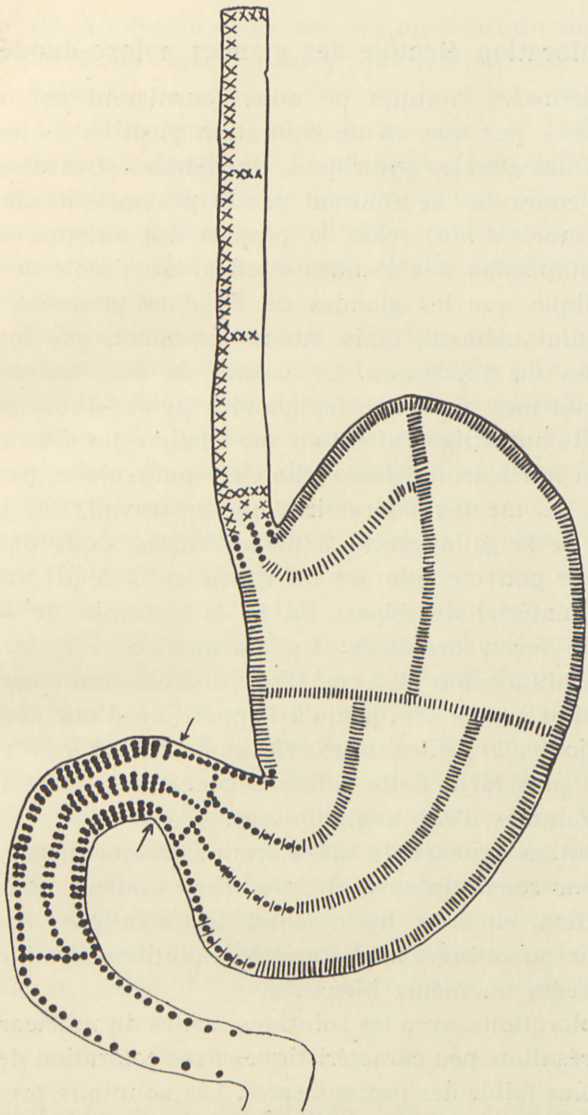

Fig. 3. Le «squelette gastro-duodénal» (paroi postérieure), édifié par des bandes tissulaires découpées et microscopiées.

||||||||||||||||||||: glandes fundiques.

●●●●●●●●●●●●●●●●●●●●: glandes cardiaques, pyloriques ou duodénales (quantité variable).

|●|●|●|●|●|●|●|: zone de transition.

xxxxxxxxxxxx: éphitelium pavimenteux (de l'œsophage).

Une coloration élective des glandes pyloro-duodénales.

Les méthodes connues ne nous fournissent aucun moyen d'identifier — par une même coloration positive — les cellules sécrétrices des glandes pyloriques, duodénales et cardiaques. Les méthodes courantes ne colorent pas le protoplasma de ces cellules. Le mucicarmin, selon la plupart des auteurs, ne colore pas le protoplasma des glandes duodénales; l'école de Bensley seule, indique que les glandes de Brunner prennent le mucicarmin simultanément, mais moins fortement, que les cellules caliciformes du duodénum. Le carmin de Best colore les cellules caliciformes ainsi que les glandes pyloro-duodénales.

Ces faits ont dirigé l'attention sur l'action des divers carmins, surtout du mucicarmin. Les recherches poursuivies par Einarsson, dans les mêmes laboratoires que ce travail, sur la coloration avec de la gallocyanine à pH différents, nous ont incité à examiner le pouvoir colorant du mucicarmin à pH variable.

Notre matériel de départ fut le mucicarmin de P. Mayer, préparé de façon ordinaire: 1 g du meilleur carmin, $^1/_2$ g de chlorure d'aluminium et 2 cm^3 d'eau distillée sont chauffés quelques minutes à petit feu, jusqu'à l'apparition d'une couleur foncée. On ajoute, à petites doses, 100 cm^3 d'alcool à 50°; filtration 24 heures plus tard. Cette solution concentrée doit être diluée avec 10 volumes d'eau avant l'usage.

La solution aqueuse du mucicarmin, presque neutre, présente une couleur rouge, intense et pure. Cette couleur varie avec la concentration en ions hydrogènes, les solutions acides étant rouge clair ou orange, tandis que les solutions alcalines deviennent violacées ou même bleuâtres.

Les colorations avec les solutions acides du mucicarmin donnent des résultats peu caractéristiques avec coloration des noyaux et teinte plus faible des protoplasmes. Les solutions presque neutres, employées d'ordinaire, colorent intensément les cellules caliciformes sans teindre ni les glandes pyloro-duodénales, ni l'épithélium superficiel gastrique (Fig. 6). Les solutions alcalines colorent d'intensité différente les cellules pyloro-duodénales ainsi que la moitié superficielle des épithéliums gastriques de revêtement (Fig. 7). Des expériences avec de nombreuses solutions alcalines ont montré que la solution suivante est préférable dans notre but:

10 cm^3 de la solution concentrée du mucicarmin sont étendus avec 100 cm^3 d'eau distillée; on y ajoute 2 cm^3 d'une solution à 5% de carbonate de soude. Le colorant est bon, si la couleur tourne au bleuâtre; mais il n'est bon à rien, si l'addition du carbonate de soude cause des floculations. (La tendance à former des floculations dépend de la composition des divers carmins; nous avons obtenu les meilleurs résultats en employant des carmins très vieux.) Le colorant alcalin possède une stabilité d'environ une semaine.

Coloration au mucicarmin alcalin: Les coupes, débarrassées de la paraffine et soigneusement lavées à l'eau distillée, séjournent de 1 à 24 heures dans une solution alcaline de mucicarmin; après, elles passent par les alcools et xylènes pour être montées dans du baume de Canada. Une coloration nucléaire, de préférence à la gallocyanine, peut précéder la coloration au mucicarmin. Les parties protoplasmiques incolores se laissent teindre par l'aurantia. (Coloration combinée à gallocyanine — mucicarmin alcalin — aurantia.) La fixation au sublimé est préférable, mais la coloration réussit aussi, le plus souvent, après fixation ordinaire à l'aldéhyde formique neutre.

Résultats de la coloration au mucicarmin alcalin: Les cellules des glandes pyloriques et duodénales paraissent rouges, et le grossissement fort décèle une structure réticulée dans leur protoplasme; les glandes cardiaques prennent la même couleur, un peu plus faible. La portion superficielle des cellules gastriques de revêtement présentent une couleur rouge plus intense et plus homogène que ne le font les glandes pyloro-duodénales. Toutes autres cellules de l'œsophage, de l'estomac et du duodénum restent incolores — les cellules caliciformes comprises (Fig. 7).

Histotopographie des glandes pyloro-duodénales.

L'examen de notre matériel post-natal, par les méthodes histotopographiques décrites, aboutit aux conclusions suivantes:

La zone des glandes pyloriques est d'ordinaire limitée à une portion modeste de l'estomac, probablement $^1/_{10}$-$^1/_{25}$ de la surface gastrique. Son étendue absolue est presque égale aux deux courbures; c'est-à-dire que l'étendue relative est plus grande

à la courbure mineure, un tiers ou même la moitié de celle-ci étant occupée par la muqueuse pylorique. — Dans un cas nous avons observé, le long de la courbure mineure, des «cellules pyloro-duodénales», situées dans les parties basales de glandes fundiques, formant ainsi une anastomose de la zone pyloro-duodénale avec la zone cardiaque. — Les variations considérables, de taille comme d'état de contraction, rendent peu exacte toute indication d'une «superficie normale» de la zone pylorique. Un estomac des dimensions moyennes citées par Hou-Jensen, doit posséder une superficie pylorique d'environ 30—40 cm^2; les glandes pyloriques s'étendent dans ce cas sur 3—5 cm le long des courbures et des parois gastriques, à partir du bord inférieur du sphincter.

Il existe parfois une limite nette entre la zone pylorique et celle du fond, mais nous n'avons jamais vu la cloison conjonctive décrite par Paschkis & Orator. L'organe pyloro-duodénal débute souvent par quelques cellules claires dans les portions basales des glandes fundiques. La proportion des éléments pyloriques augmente dans une zone transitoire de 1 à 2 cm, au-delà de laquelle les glandes fundiques sont extrêmement rares. — Une de nos préparations présente des glandes pyloriques débutant par «buissons» dans la muqueuse du fond, et devenant plus nombreuses vers le pylore.

Les limites de la zone pylorique peuvent, exceptionnellement, être très vagues; c'est quand des glandes pyloriques isolées ont été développées presque partout dans la muqueuse gastrique, mais de préférence dans la zone classique du pylore. Ces glandes isolées peuvent être entourées de fibres conjonctives. La constatation de cellules bordantes dans les glandes pyloriques n'est pas fréquente.

La zone des glandes cardiaques est minime. Elle débute, dans le chorion de l'œsophage, par quelques groupes isolés de glandes tubuleuses, contournées et ramifiées, qui atteignent leur maximum au niveau de la jonction œsophago-gastrique. Les glandes cardiaques vont jusqu'à 1 cm au-delà de la ligne sinueuse qui sépare l'épithélium pavimenteux de l'épithélium cylindrique; elles peuvent disparaître «en coin» par des cellules claires au fond des glandes fundiques le long de la petite courbure, ou le «coin cardiaque» peut anastomoser avec le «coin

pylorique». — Notre matériel n'a pas montré de glandes cardiaques situées à plus de 2 cm au-dessus de la jonction œsophago-gastrique.

Les glandes duodénales (de Brunner) apparaissent au bord inférieur du sphincter pylorique, où les glandes pyloriques percent la muscularis mucosae pour former bientôt, jusqu'au

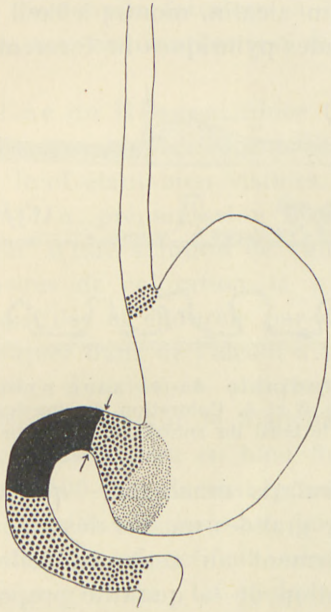

Fig. 4. Distribution topographique des glandes pyloro-duodénales (et cardiaques) d'un cas typique, dessinée d'après le «squelette gastro-duodénal» de la figure 3 (Schéma simplifié).
Zone noire: la grande masse glandulaire entre sphincter et ampoule. Points grossiers: quantités moindres de glandes pyloriques, duodénales ou cardiaques. Points fins: la zone transitoire des glandes pyloriques et fundiques.

niveau de l'ampoule de Vater, une couche compacte, épaisse de quelques millimètres, leur plus grande portion se trouvant dans la sous-muqueuse (environ $^4/_5$). Les glandes diminuent de nombre et de taille au-delà de l'ampoule, et elles se localisent de préférence dans les valvules conniventes. Vers la fin du duodénum on ne trouve que quelques tubes glandulaires isolés, dont la substance est répartie également entre chorion et sous-muqueuse. La dernière glande de Brunner se trouve souvent dans le

voisinage de l'angle duodéno-jéjunal; mais on peut, par occasion, observer quelques groupes glandulaires isolés dans le jejunum.

Quantités proportionnelles des glandes pyloriques et duodénales.

Une coupe longitudinale de la paroi pyloro-duodénale, colorée au mucicarmin alcalin, montre à l'œil nu que les portions sécrétrices des glandes pyloriques ne forment qu'une ligne mince

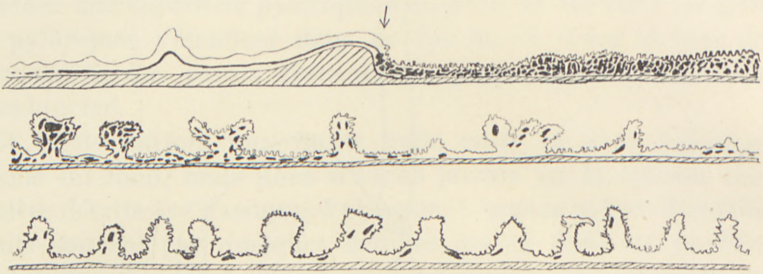

Fig. 5. Coupe longitudinale de la zone pyloro-duodénale. Même cas typique que les fig. 3 et 4. Coloration au mucicarmin alcalin. La flèche indique le bord du sphincter. Grandeur naturelle.

le long de la muscularis mucosae — quantité presque négligeable, comparée aux grandes masses des glandes duodénales, qui dépassent, probablement au centuple, celle des glandes pyloriques. Une estimation de la quantité proportionnelle des deux zones glandulaires, fondée sur leur seule projection à la surface muqueuse, serait ainsi extrêmement erronée (Fig. 5).

Nous avons constaté ainsi que la quantité principale des glandes pyloro-duodénales est localisée au duodénum, surtout entre le bord du sphincter et l'ampoule de Vater.

Observations stéréomicroscopiques des glandes gastro-duodénales.

L'observation directe des muqueuses gastriques et duodénales, colorées ou non, à l'aide du stéréomicroscope (binobjectif-binoculaire) donne l'image la plus vive de la distribution et de la quantité des glandes; une technique appropriée permet la dissection sous le contrôle du microscope.

La préparation suivante a été élaborée pour la microdissection: Les organes, fixés post mortem à l'aldéhyde formique, par l'injection intra-abdominale décrite, passent par des alcools de plus en plus concentrés (48 heures). L'alcool à 93° endurcit les formations épithéliales et musculaires, mais le tissu conjonctif reste lâche, permettant de pratiquer la microdissection en bon clivage.

L'élimination de l'aldéhyde formique à l'eau courante (24 heures) rend possible une coloration en masse par les colorants suivants:

1. L'hématoxyline de Hansen diluée (24—48 heures) est bonne pour l'orientation rapide, les contrastes entre les muqueuses du pylore et du fond étant bien visibles.

2. Le bleu gallamin, préparé selon Romeis (0,1 g de bleu gallamin pour 200 cm^3 d'une solution de chlorure d'aluminium à 5%). Après 24 heures de coloration, la pièce est soigneusement lavée à l'eau distillée et endurcie par des alcools; la microdissection est pratiquée dans de l'alcool à 93°. — Une lumière jaunâtre et concentrée, d'incidence oblique, fait ressortir les glandes pyloro-duodénales éclatant de blancheur et entourées de fibres conjonctives et musculaires en bleu foncé. L'emploi des grossissements jusqu'à 100 fois devient possible par cet éclairage, tandis que la transillumination est impraticable à cause de la colorabilité forte du conjonctif abondant.

3. Le mucicarmin alcalin: La tunique musculaire de la pièce fixée est décollée. On lave soigneusement la muqueuse à l'eau courante, ensuite à l'eau distillée. Coloration au mucicarmin alcalin pendant 24 à 48 heures (solution concentrée, étendue à 15 volumes). Les acini pyloro-duodénaux tranchés présentent des surfaces rouge foncé; est rouge aussi une ligne qui correspond à l'epithélium superficiel de l'estomac. Les autres éléments tissulaires prennent des nuances roses et rougeâtres. La microdissection des glandes est pratiquée sous une lumière blanche, d'intensité variable, et dans de l'alcool à 93°.

Nous préférons souvent disséquer la pièce, colorée et déshydratée, dans un mélange d'alcool et de xylène (le plus souvent composé de $^2/_3$ de xylène et $^1/_3$ d'alcool absolu). Ce liquide donne aux tissus une consistance très commode pour la dissection des glandes. On peut, de plus, varier son indice de réfrac-

tion: le xylène augmente la réfringence, qui peut encore être réduite par addition d'alcool absolu. — Le mélange rend transparent le tissu conjonctif, tandis que les glandes pyloriques et duodénales restent opaques. Elles peuvent, dans ce milieu, être microscopiées sous incidence oblique d'une lumière concentrée, ou bien par transillumination ordinaire.

Il faut souligner que beaucoup des détails structuraux se présentent le mieux dans des conditions physiques individualisées : indice de réfraction — couleur, intensité, angle d'incidence de la lumière.

L'image stéréomicroscopique des glandes pyloro-duodénales possède une beauté étonnante. Les formations glandulaires révèlent, en relief, leur architecture, leur répartition dans le conjonctif du chorion ou de la sous-muqueuse, leurs vaisseaux. La dissection à aiguilles pointues et aiguisées, apporte des connaissances immédiates des rapports des glandes avec les tissus environnants, conjonctifs et musculeux.

Les différences morphologiques de la zone pylorique et de celle du fond sautent aux yeux. Les dépressions superficielles, en «doigts de gant», des cryptes du fond s'opposent nettement aux cryptes pyloriques profondes, entourées de proéminences villiformes, rappelant parfois la muqueuse intestinale. Il y a toujours une transition graduée entre les deux types du relief superficiel, même si la coupe perpendiculaire correspondante présente une limite nette entre les tubes glandulaires du fond, serrés et rectilignes, et les glandes pyloriques courtes et contournées, rappelant des «buissons» dans leur interstice conjonctif abondant. — La muscularis mucosae du fond se laisse enlever par clivage, tandis que la lame correspondante du pylore est liée au chorion par de fortes cloisons interglandulaires. La muscularis mucosae isolée présente, sur la face superficielle de sa portion pylorique, de telles cloisons, qui proéminent entre les impressions des glandes. La tunique sous-muqueuse paraît lâche et forme un plan de clivage excellent — excepté une zone circulaire étroite au bord du sphincter pylorique.

Les glandes de Brunner, légèrement disséquées, présentent un haut-relief caractéristique. L'espace sous-muqueux considérable entre le bord du sphincter et l'ampoule de Vater, se montre rempli de formations glandulaires, très denses — formant presque

une masse continue. La plupart des glandes disséquées ont des formes oblongues, rappelant souvent celle d'un poisson; leur plus grand diamètre est perpendiculaire à la surface. Les glandes, en diminuant vers l'angle duodéno-jéjunal, se localisent aux valvules conniventes, et s'orientent parallèlement à la surface. Les glandes sous-muqueuses pénètrent la muscularis mucosae par un nombre de tubes glandulaires, qui se répandent parfois encore dans le chorion avant de déboucher au fond d'une crypte intestinale. La dissection permet de démontrer la subdivision des glandes duodénales en lobules, surtout dans l'espace sous-muqueux sus-Vaterien (Fig. 8).

Dénombrement des glandes pyloriques.

L'examen stéréomicroscopique a montré que presque tous les tubes glandulaires du pylore touchent la muscularis mucosae. On peut donc, après enlèvement de cette lame, dénombrer les tubes pyloriques, vus de la face profonde de la muqueuse.

La région pylorique d'un estomac fixé est marquée d'après la microscopie directe des faces tranchées, correspondant à notre «squelette gastro-duodénal». Les parties en dehors de la zone des glandes pyloriques sont découpées et enlevées. La tunique musculaire est décollée de la muqueuse pylorique, qu'on lave à l'eau et colore au mucicarmin alcalin ou à l'hématoxyline diluée. La muqueuse pylorique, isolée et colorée, est déshydratée par des alcools, et enfin mise dans le mélange xylène-alcool, qui est le milieu propre au décollage de la muscularis mucosae.

La superficie de la muqueuse pylorique immergée est mesurée, et on compte, dans ce même milieu, les extrémités profondes des tubes glandulaires, vues à travers une lame de verre, divisée en carrés de 4 mm^2 (illumination oblique). Les carrés tissulaires choisis pour l'énumération, subissent par cette méthode les mêmes modifications que la muqueuse pylorique entière. On compte ainsi les glandes pyloriques *in situ*, et leur nombre total résulte d'une multiplication simple.

Un estomac de dimensions normales et en état de contraction moyenne présenta env. 90 tubes pyloriques par mm^2, correspondant à un nombre total approximatif de 4—500.000 tubes glandulaires dans la région pylorique de l'homme.

Embryogenèse des glandes gastriques et duodénales de l'homme.

Cette portion du matériel comprend des organes de 10 fœtus humains, âgés de 2,1 - 2,5 - 2,8 - 2,9 - 4,0 - 5,0 - 5,2 - 6,4 - 7 mois lunaires. Fixation: aldéhyde formique — alcool — sublimé (1:4:5) Coloration selon Mallory-Heidenhain. Nous avons microscopié les muqueuses au niveau des courbures de l'estomac, prolongées dans l'œsophage et dans le duodénum.

Les glandes cardiaques. Le revêtement épithélial de l'œsophage fœtal est un épithélium stratifié jusqu'au 4^{e} mois. Au niveau de la transition à l'épithélium gastrique apparaissent vers le 4^{e} mois quelques cellules cylindriques grandes, claires et sans cils vibratils, avec des noyaux basals aplatis, et formant une seule couche cellulaire de la membrane basale jusqu'à la surface. Ces cellules se multiplient et s'enfoncent dans le chorion: Les glandes endo-épithéliales deviennent des glandes exo-épithéliales au commencement du 6^{e} mois. Les glandes se ramifient plus tard, mais l'enfoncement primitif peut persister comme une cavité kystique, entourée de glandes cardiaques tubuleuses (Fig. 9).

Les glandes du fond: L'épithélium cylindrique stratifié de la muqueuse gastrique se dissout, vers la fin du 3^{e} mois, en parties proéminentes, poussées par le chorion et séparées par des fentes irrégulières. Des cordes épithéliales enfoncent le chorion. Leurs cellules ressemblent aux cellules principales; les cellules bordantes apparaissent dès le 5^{e} mois. Les glandes fundiques fœtales sont courtes, parfois contournées, et peuvent, grossièrement, rappeler les glandes pyloriques de l'adulte. Elles ne surpassent les cryptes en profondeur que vers la fin de la vie fœtale (Fig. 10).

Les glandes pyloriques apparaissent au milieu du 3^{e} mois; leur développement est un peu plus avancé que celui des glandes du fond. Les cellules des glandes pyloriques sont d'abord cubiques, et peu caractéristiques; c'est le fœtus de 4 mois qui montre les signes distinctifs: protoplasma bleu clair, un peu réticulé, et noyau basal, aplati. Les glandes pyloriques du fœtus peuvent être disposées en «palissades»; elles sont plus denses et plus régulières que celles du fond. — Les cellules bordantes

ne sont pas rares dans les glandes pyloriques fœtales. Les groupes de glandes sont séparés par des cloisons, provenant d'abord du chorion, plus tard de la muscularis mucosae (Fig. 11).

Les glandes de Brunner sont observées, chez le fœtus de 4 mois, dans le duodénum entier, mais de préférence dans sa première portion. Le chorion duodénal contient quelques groupes rares de cellules cubiques entourant une lumière minime. Les glandes de Brunner se développent dès le fond des cryptes intestinales, leurs cellules claires formant parfois des «croissants» autour des épithéliums foncés des cryptes propres. Des cellules de Paneth ont été constatées chez le fœtus de 7 mois (Fig. 12).

L'histotopographie des glandes pyloro-duodénales du fœtus est donc caractérisée par la superficie considérable des zones pyloriques et cardiaques aux dépens de la zone du fond; les glandes pyloriques occupent une portion relativement grande de la courbure mineure. — La microscopie du tube intestinal entier de 2 fœtus a montré des glandes de Brunner seulement dans le duodénum, surtout dans sa première portion.

Résumé.

Une description histotopographique des glandes pyloro-duodénales est réalisée par des méthodes de l'histologie classique. La fixation est obtenue par des injections intra-abdominales d'aldéhyde formique quelques minutes après la mort et selon un schème spécial. Le mucicarmin alcalin permet une coloration élective des glandes pyloro-duodénales. — La microscopie systématique de bandes tissulaires, formant ensemble un «squelette gastro-duodénal», indique les limites des zones glandulaires de l'œsophage, de l'estomac et du duodénum.

La zone des glandes cardiaques est minime, ½—1 cm au niveau de la jonction œsophago-gastrique. — Les glandes pyloriques occupent les derniers 3—5 cm des courbures gastriques et des superficies correspondantes des parois gastriques. Une zone transitoire de 1—2 cm est fréquente; elle présente des glandes pyloriques et fundiques entremêlées. — Les glandes de

Brunner forment une couche presque compacte, dès le bord du sphincter pylorique jusqu'à l'ampoule de Vater; elles diminuent vers l'angle duodéno-jéjunal. — Des coupes longitudinales, perpendiculaires à la surface, montrent que la plus grande partie de la masse glandulaire pyloro-duodénale est localisée dans le duodénum, entre le bord du sphincter et l'ampoule de Vater.

La microdissection des muqueuses, rendues semi-transparentes dans de l'alcool-xylène, dévoile les détails de l'architecture glandulaire. Elle permet un dénombrement des tubes glandulaires du pylore.

Les glandes pyloro-duodénales ont une étendue relativement grande dans la vie fœtale, où elles se développent précocement.

La fondation P. Carl Petersen a subventionné les recherches par des fonds attribués aux investigations du professeur Meulengracht sur les origines de l'anémie pernicieuse.

Travail de
L'Institut de Pathologie de l'Hôpital de Bispebjerg, Copenhague.
Chef: M. Bj. Vimtrup, *prosecteur.*

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Après la terminaison du présent ouvrage a paru: Landboe-Christensen: The Duodenal Glands of Brunner in Man, their Distribution and Quantity. Copenhagen & London, 1944. pp. 267.

Ce livre nous donne un atlas parfait de la distribution topographique des glandes de Brunner d'après un matériel d'autopsie comprenant 53 pièces, et contient une bibliographie complète.

Indleveret til Selskabet den 9. September 1944.
Færdig fra Trykkeriet den 23. Januar 1945.

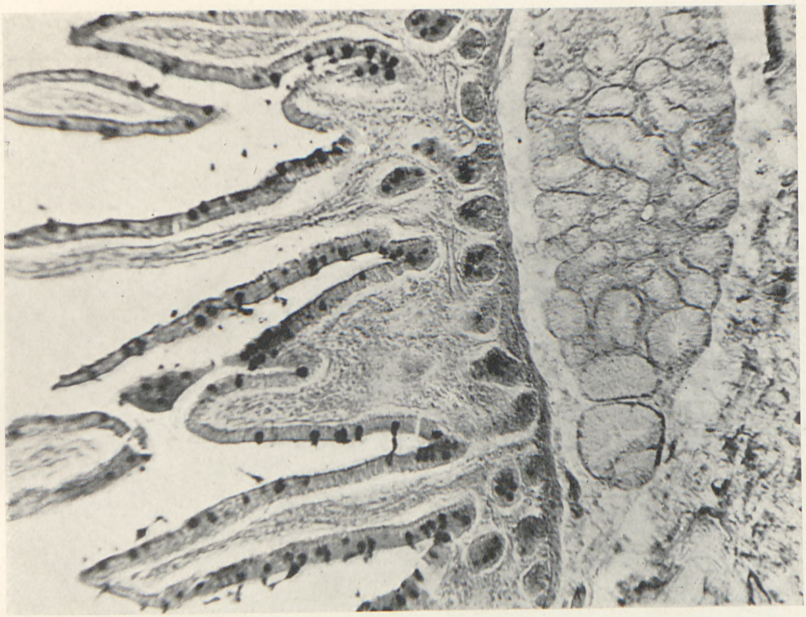

Fig. 6. Paroi du duodénum, colorée au *mucicarmin de P. Mayer* de façon ordinaire. Coloration intense des cellules caliciformes. Les glandes de Brunner ne prennent pas le mucicarmin. (Gr. 100.)

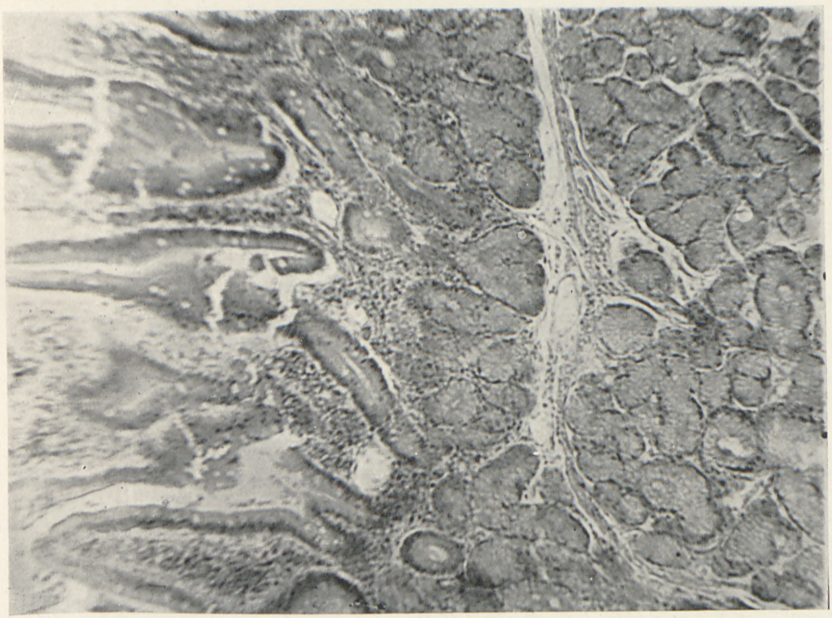

Fig. 7. Paroi du duodénum, colorée au *mucicarmin alcalin*. Les cellules caliciformes sont incolores, tandis que les glandes de Brunner prennent le mucicarmin alcalin. (Gr. 100.)

Fig. 8. *Les glandes de Brunner, aprés microdissection* du duodénum. Coloration au mucicarmin alcalin, très intense sur les acini tranchés. La flèche indique la muscularis mucosae. (Gr. 30.)

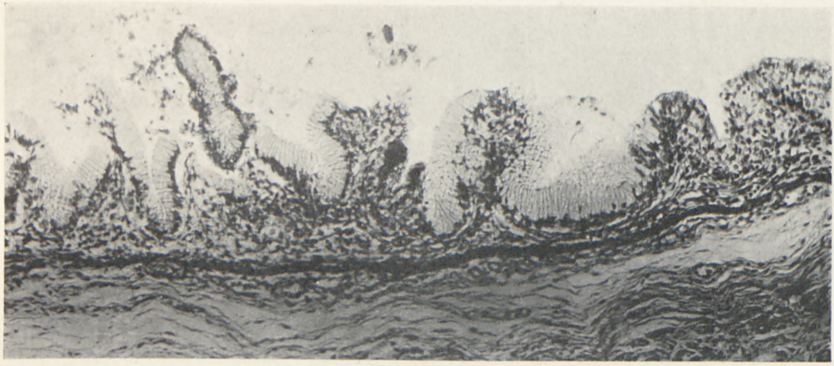

Fig. 9. *Zone cardiaque d'un fœtus humain* (7 mois). Muqueuse fundique à gauche. Epithélium stratifié vibratile de l'œsophage à droite. Un peu à droite du milieu l'épithélium cylindrique s'enfonce pour former une glande cardiaque. (Gr. 100.)

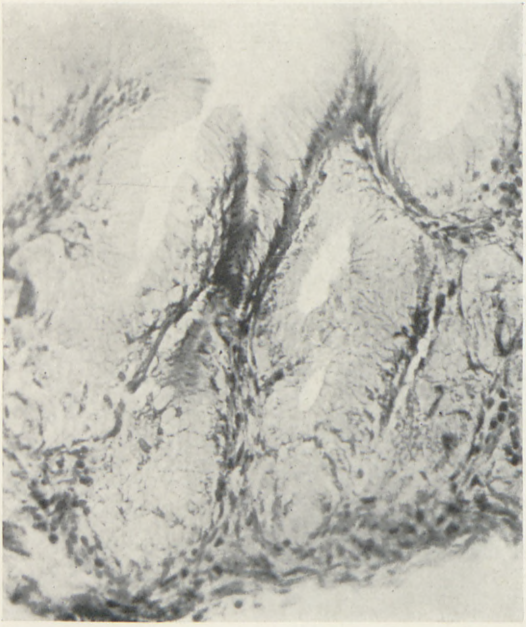

Fig. 10. *Muqueuse fundique d'un fœtus humain* (6,4 mois). (Gr. 240.)

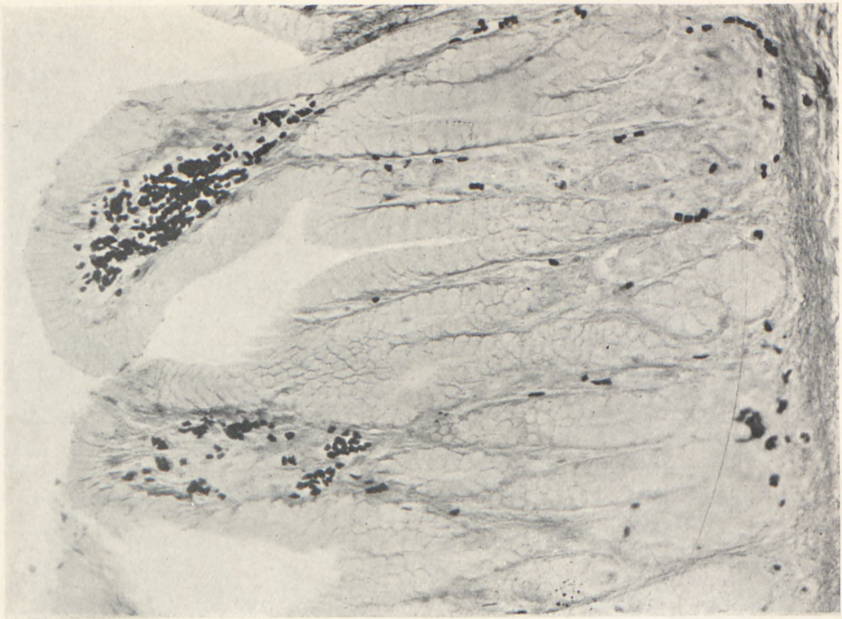

Fig. 11. *Les glandes pyloriques d'un fœtus humain* (6,4 mois). La région pylorique est plus développée que celle du fond. (Gr. 200.)

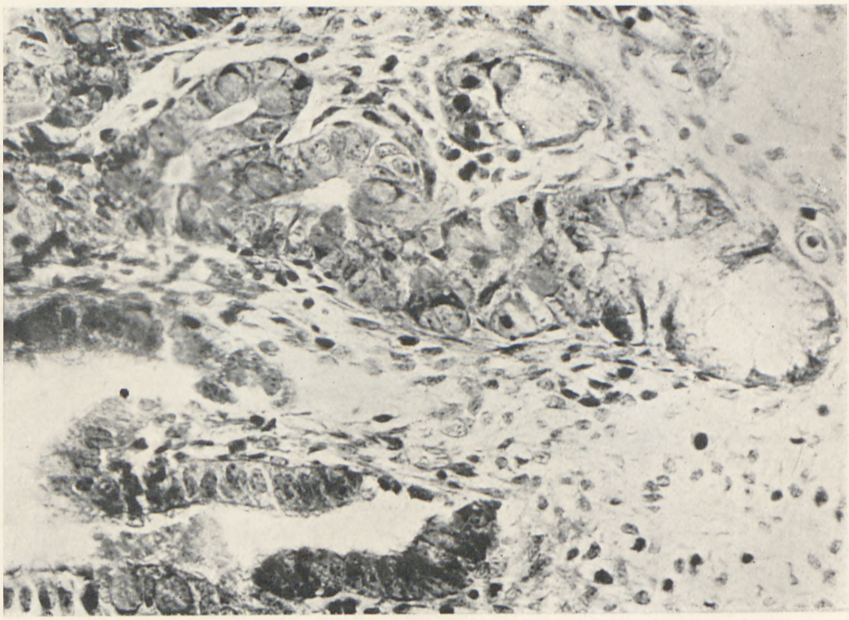

Fig. 12. *Les glandes duodénales d'un fœtus humain* (6,4 mois). (Gr. 400.)

DET KGL. DANSKE VIDENSKABERNES SELSKAB
BIOLOGISKE MEDDELELSER, BIND XIX, NR. 10

SOME MARINE ALGAE FROM MAURITIUS

III. RHODOPHYCEAE

PART 4

CERAMIALES

BY

F. BØRGESEN

KØBENHAVN
I KOMMISSION HOS EJNAR MUNKSGAARD
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In this last part of my paper: "Some Marine Algae from Mauritius" the species of the remaining order of the *Ceramiales* found in the collections are worked out.

As was the case in the former publications treating of the *Florideae*, the present part is based upon the late Dr. JARDIN's[1] collection in which the native DARUTY's gatherings are included and which belong to the Muséum National d'Histoire Naturelle, Paris, and further upon the collections of Dr. TH. MORTENSEN and Dr. R. E. VAUGHAN. And as was also the case in the former parts, some few species of algae from Réunion contained in Dr. JADIN's collection have been included in the list.

In the following list 48 species are enumerated, but later examinations will of course augment this number to a considerable degree.

As was already pointed out earlier the material is in most cases rather scarce, often a single specimen of each species; and some of it, especially that collected by DARUTY, is not always in the best condition, a circumstance which has made it impossible to determine several of the specimens.

That some later collections made by Dr. VAUGHAN have not reached me because of the war is of course a great drawback. These collections as far as I know are kept in the Kew Herbarium by Dr. COTTON.

I should also like to point out here that I have not been able to visit Lund to consult the herbarium of J. AGARDH. And likewise I wish to mention that since the outbreak of the war I have been out of touch with a great deal of my colleagues abroad and consequently without any knowledge of their publications,

[1] According to kind information Dr. FERNAND JADIN died in Montpellier on the 22. Febr. 1944.

and so also I do not know to what extent this disadvantage may affect my paper.

Though I have said above that the present part of this publication is the last, I should like nevertheless to mention that the *Chlorophyceae* and *Phaeophyceae* of Dr. JADIN's collection did not reach me until after the parts treating these groups had been published. Since at any rate the collection of *Chlorophyceae*, judging by a brief inspection, seems to contain many species of interest not mentioned in the former parts, it is not excluded that a supplementary part dealing with these groups may appear.

The algal flora of Mauritius and altogether of the Mascarene Islands seems to be very rich, a fact which indeed Dr. VAUGHAN has pointed out to me. No doubt is present that a thorough examination of the algal flora of the islands by a trained algologist will bring to light much of interest.

I am greatly indebted to Dr. HENNING E. PETERSEN who with his usual readiness has most kindly determined the few species of *Ceramium* found in the collection.

I also wish to thank Professor HARALD KYLIN of Lund University who with great kindness has given me valuable information about some few of the algae.

Copenhagen in February 1945.

VI. Ceramiales.

Fam. 1. Ceramiaceae.

Subfam. 1. Crouanieae.

Antithamnion Nägeli.

1. Antithamnion flagellatum nov. spec.

Frons pygmaea, ca. 3—4 mm. alta, caespitosa, mollissima, ex filamentis ramelliferis, in parte basali decumbentibus per haptera adfixis, sursum erectis composita.

Filamenta articulata, ex articulis in parte basali 50—80 μ lata, superne gradatim tenuioribus, diametro 3—4 plo longioribus formata.

Ramuli singuli, oppositi, verticillati, vel magis irregulariter infra apicem articulorum orti, 300—400 μ vel longiores, mollissimi et flabellati, erecti, 1—3 ies alterne ramosi, ex articulis 10—15 μ latis et 5—10 plo longioribus compositi.

Glandulae verisimiliter raro præsentes, subgloboso-depressae, in superiori latere cellulae singulae prope basem ramellorum præsentes.

Tetrasporangia oblonga, ca. 50 μ longa et 26 μ lata, cruciatim vel interdum triangule divisa, e cellulis basalibus ramulorum orta, plerumque singularia, raro bina praesentia.

Antheridia et gonimoblasti non observata.

Mauritius: Off Flat Island, dredged at a depth of 30 fathoms, 16. Oct. 1929, Th. M.

In Dr. Mortensen's collection a very little dried material occurred of a small, soft *Antithamnion*, which formed tufts about 3—4 mm. high upon a fragment of a larger alga.

The lowermost decumbent parts of the filaments are fixed to the substratum by means of hapters, the shorter ones composed of a single cell, the longer ones of several.

In the basal part of the filaments the cells are hourglass-shaped, about 50—80 μ broad and about twice as long; higher up the cells become cylindrical, about 40 μ thick and 3 to 4 times

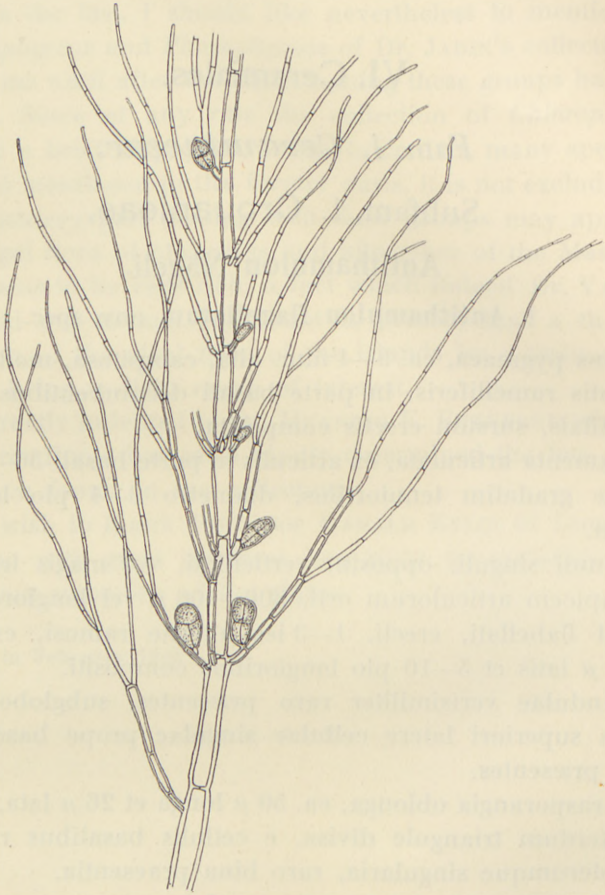

Fig. 1. *Antithamnion flagellatum* Børgs. Part of the thallus with tetrasporangia. (× about 120).

as long or longer. Near the base the cells have a thick wall, upwards this becomes gradually thinner.

From near the upper ends of the cells in the main filaments, a single or two branchlets, then but not always oppositely placed, often also three, are given off (Fig. 1); I have not seen 4 verticillate branchlets, but most probably they occur. A good many of the cells in the main filaments are without branchlets at all. Instead

of a branchlet now and then a branch is given out. The branchlets are 300—400 μ long or a little longer, slender and very flexible. Their base consists of a short cell, the next one is a little longer, whereupon the cells as a rule become about 5—10 times longer than the breadth, which is about 10—15 μ. Near the tips the filaments of the branchlets taper to about 2—3 μ only. The apical

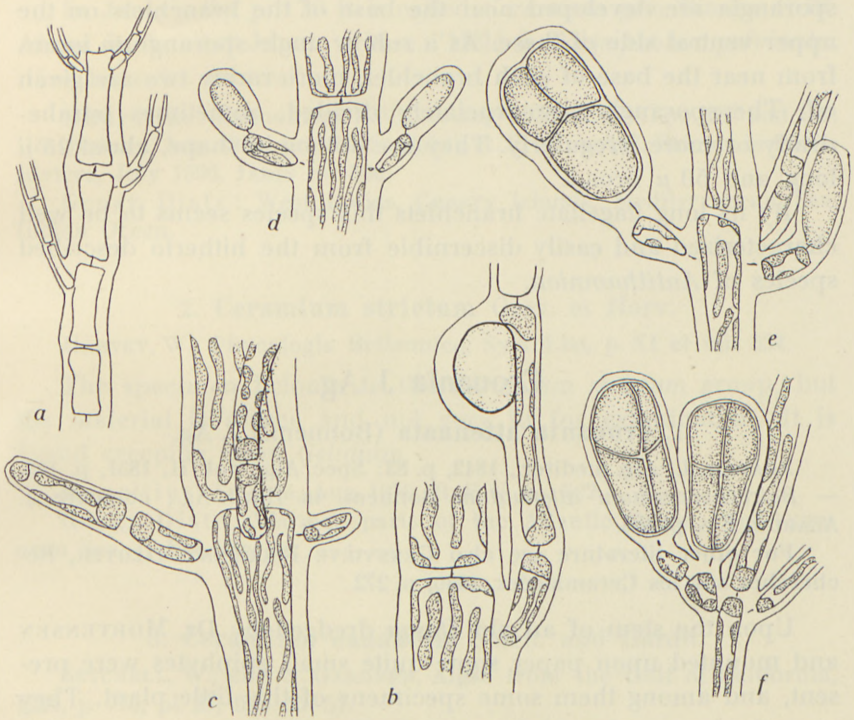

Fig. 2. *Antithamnion flagellatum* Børgs.
a, fragment of a main filament near the base; *b*, ramulus with a gland-cell; *c*, fragment of a filament with 3 ramuli; *d*, *e*, *f*, ramuli with young and ripe tetrasporangia. (*a* × 160; *b* × 350; *c*—*f* × 225).

cell is about 20 μ long with obtuse summit. The longer branchlets are subfurcated a few times near their base; the smaller ones give off a single ramulus or none at all.

Gland-cells are rather rare and in some of the specimens entirely absent. They are placed upon a cell in the lowermost parts of the branchlets on the upper side of these. They are oblong or nearly spherical; that pictured in Fig. 2*b* is 10 μ broad and 15 μ long. They have a yellowish, refractive content.

The chromatophores are irregularly ribbon-like, narrow in the older cells, broader in the young ones; in the branchlets at both ends of the cells the chromatophores in the dried condition form a dense layer, while in the middle of the cells they form broader ribbons.

Plants with tetrasporangia (Fig. 2 *d, e, f*) are found only. The sporangia are developed near the base of the branchlets on the upper ventral side of these. As a rule a single sporangium issues from near the base of each branchlet, more rarely two are given off. The sporangia are cruciately divided, sometimes tetrahedrally or more irregularly. They are oblong in shape, about 25 μ long and 50 μ broad.

By its long flagellate branchlets this species seems to be well characterized and easily discernible from the hitherto described species of *Antithamnion*.

Crouania J. Ag.

1. Crouania attenuata (Bonnem.) J. Ag.

Agardh, J., Alg. mediter., 1842, p. 83; Spec. Alg., vol. II, 1851, p. 105. — *Batrachospermum attenuatum* Bonnem. in Herb. Ag.; compare J. Agardh, l. c. p. 105.

For other literature see also Geneviève Feldmann-Mazoyer, Recherches sur les Ceramiacées, 1940, p. 272.

Upon the stem of an old *Dasya* dredged by Dr. Mortensen and mounted upon paper some quite small epiphytes were present, and among them some specimens of this little plant. They were dredged at the considerable depth of about 60 metres. The specimens were mostly tetrasporic, a single one was antheridial.

Otherwise I have not met with this species in the collections nor is it mentioned in Jadin's list.

Mauritius: Off Flat Island, Oct. 16., 1929, Th. M.

Geogr. Distr.: Most probably widespread in warm seas.

Ceramium (Roth) Lyngbye.

I am much indebted to Dr. H. E. Petersen for the determination of the species of this genus.

1. **Ceramium transversale** Collins et Hervey.

Collins, Fr., and A. B. Hervey, The Algae of Bermuda, 1917, p. 117, pl. V, figs. 29—31.

Several specimens of this species are found in the collections. Described in 1917 upon specimens from the Bermuda Island, this little species seems to be wide-spread.

In Jadin's list p. 170 it is called *Ceramium gracillimum* Griff. About its habitat Jadin writes: "Mêlé à *Polysiphonia pulvinata*, dans les eaux calmes".

Mauritius: Cannoniers Point, 5. Aug· 1933, R. E. V. no. 183 and 189. Ilôt Brocus, Aug. 1938, in "Reef pools", R. E. V. no. 198. Baie de la Petite Rivière, July 1890, Jadin no. 326.

Geogr. Distr.: West Indies, Canary Islands, Mediterranean Sea, Indian Ocean.

2. **Ceramium strictum** Grev. et Harv.

Harvey, W., Phycologia Britannica, Syst. List, p. XI et tab. 334.

The specimen belongs to the *Ceramium strictum* group, but the material is scarce and not very fit for examination. It is found creeping upon *Gelidium*.

Mauritius: Savinia, Aug. 1939, R. E. V. no. 302.

Geogr. Distr.: Warmer parts of the Atlantic Ocean, Mediterranean Sea, Indian Ocean.

3. **Ceramium caudatum** Setch. and Gardn.

Setchell, W., and N. Gardner, Algae from the Gulf of California, 1924, p. 776, pl. 27, figs. 55—57.

The specimens certainly come near to this species but any exact determination upon the rather poor material has not been possible. Some of the specimens have tetrasporangia.

The plant was twice, together with *Bornetia Binderiana* (Sond.) Zanard., found creeping between the filaments of the capitulum of *Chamaedoris Delphinii* (Har.) Feldm. et Børgs., and once imbedded more or less among the filaments of *Codium Vaughani* Boergs.

Mauritius: Gabriel Isl., March 8., 1871, Colonel Pike (Herb. Kew.). Ilôt Brocus, without dates, R. E. V. Off Flat Island, Oct. 1929, Th. M. no. 833.

Geogr. Distr.: California.

4. Ceramium elegans Ducluzeau.

Ducluzeau, J. A. P., Essai, 1805, p. 53. Agardh, J., Spec. Alg., II, p. 124; Epicr., p. 97.

A single specimen in Jadin's collection gathered by Daruty is most probably this species.

In Jadin's list p. 170 it is found as *Ceramium nodosum* Harv.

Mauritius: Without locality and date, gathered by Daruty 1892.
Geogr. Distr.: Mediterranean Sea, Cadiz.

5. Ceramium Johnstonii Setch. et Gard.

Settchel, W., and N. Gardner, Mar. Alg. Gulf of California, 1924, p. 774, pl. 76, 77.

Two specimens in Dr. Vaughan's collection are surely referable to this Californian species.

Mauritius: Black River Bay, July 9., 1939, R. E. V. no. 281. Savinia, Aug. 1939. R. E. V. no. 307.
Geogr. Distr.: California.

6. Ceramium rubrum (Huds.) Ag.

Agardh, C., Synops. alg. Scand., p. 60; Spec. alg., II, p. 146. — *Conferva rubra* Huds., Flora Angl., 1778, p. 600.

A rather large and fine specimen of this species is found in Jadin's collection.

Mauritius: Without locality and date, gathered by Daruty 1892.
Geogr. Distr.: Widespread.

Centroceras Kütz.

1. Centroceras clavulatum (Ag.) Mont.

Montagne, Exploration Scientif. de l'Algérie, Algues, p. 140, 1846. For more synonyms compare De-Toni, Syll. Alg., Vol. IV, 3, p. 1491.

Some small specimens or fragments only are found in the collections. The specimens have short spines more or less developed.

It is mentioned in Jadin's list p. 170; about its habitat he writes: "Dans les eaux calmes; à 20 centimètres au-dessous des eaux à marée basse".

Mauritius: Baie de la Grande Rivière, Oct. 1890, Jadin no. 395. Barkly Island, Aug. 1939, "in rock crevices or on rocks in exposed pla-

ces, usually incrusted with Diatoms", R. E. V. no. 337. Pointe aux Sables, Aug. 1939, "on rocks and barnacles, exposed situations", R. E. V. no. 340.

Geogr. Distr.: Warm seas.

Subfam. 3. Spyridieae.

Spyridia Harv.

1. Spyridia filamentosa (Wulf.) Harv.

Harvey, W. H., in Hooker, Brit. Flora, vol. II, 1833, p. 336. Phycologia Britannica, p. 46. J. Agardh, Spec. Alg., II, p, 340. Børgesen, Mar. alg. D. W. I., vol. II, p. 233, figs. 222–226. — *Fucus filamentosus* Wulfen, Crypt. aquat. in Roemer, Archiv f. die Botanik, III, p. 63.

The few specimens found in the collections are sterile and mostly poorly developed.

The specimens belong to the form with long thin ramuli; they have most probably been collected in sheltered localities; compare my figures 223*a* and 224 quoted above.

In Jadin's collection a single undetermined specimen gathered by Daruty is found, but in his list this species is not mentioned.

Mauritius: Grand Bay, 24. Oct. 1929, Th. M. Without locality, 1894, Daruty.

Geogr. Distr.: West Indies, warmer parts of the Atlantic Ocean, Mediterranean Sea, Red Sea, Indian Ocean, etc.

Subfam. 4. Spongoclonieae.

Haloplegma Montagne.

1. Haloplegma Duperreyi Mont.

Montagne in Ann. sci. nat., sér. 2, vol. 18, 1842, p. 258, tab. 7, fig. 1. Kützing, Spec. Alg., p. 672; Tab. Phycol., vol. XII, tab. 62. Agardh, J., Spec. Alg., II, p. 111. Weber, Alg. Siboga, p. 315. Børgesen, Some Ind. Rhodoph., I, 1931, p. 14, fig. 9.

Several specimens of this, as to anatomical structure variable, plant are present in the collections. Some of them are from shallow water and protected localities, some from more exposed ones, and in Dr. Mortensen's collection specimens dredged at a depth of 50—60 m. are also found. A comparison of these specimens has shown that the shape and development of the

assimilating branchlets and the size of the meshes in the net all vary very much. Some few figures taken from some of the specimens will show this.

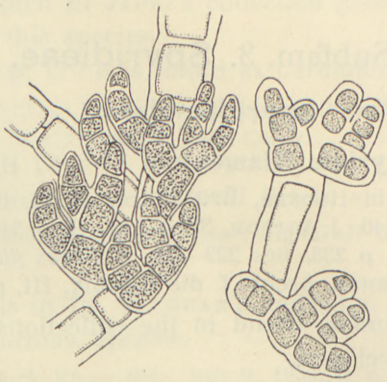

Fig. 3. *Haloplegma Duperreyi* Mont.
Fragments of the net with assimilating filaments. (× about 335).

Fig. 3 is from a specimen collected by Dr. MORTENSEN (no. 839) in shallow water; the assimilating branchlets are short and densely packed, forming small clumps about 40—50 μ long; and the meshes of the net are small for which reason the assimilating layer forms a dense tissue.

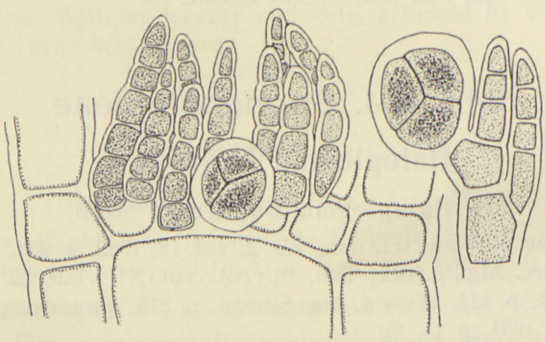

Fig. 4. *Haloplegma Duperreyi* Mont.
Fragments of the net with assimilating filaments and tetrasporangia. (× about 335).

Fig. 4 shows the assimilating filaments from another specimen found in Dr. JADIN's collection (no. 123); it is a washed up specimen gathered by DARUTY. The branchlets in this specimen are somewhat longer, up to about 100 μ long, than those of

the above-mentioned specimen but like this its branchlets are densely packed together. The meshes of the net are small. The

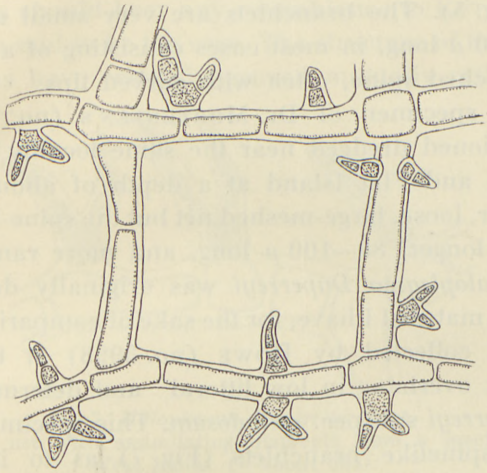

Fig. 5. *Haloplegma Duperreyi* Mont.
Fragment of the net with assimilating branchlets. (× about 335).

specimen is tetrasporic, the tetrasporangia being developed upon the branchlets. In JADIN's list p. 170 this specimen is referred to

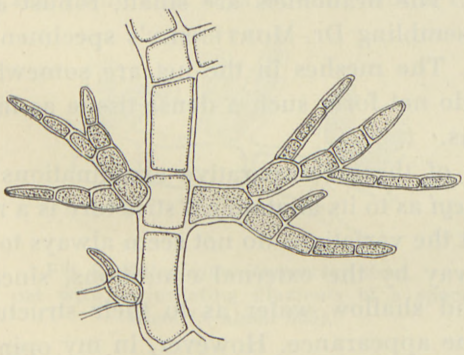

Fig. 6. *Haloplegma Duperreyi* Mont.
Fragment of the net with assimilating filaments. (× about 335).

Holoplegma Preissei Sonder; but by its long and curved assimilating branchlets, often comprising more than 20 joints and reaching a length of more than 200 μ, this Australian species is well separated from *Halop. Duperreyi.*

A plant from deep water in Dr. MORTENSEN's collection (no. 820) has a rather thin and loosely built thallus with large meshes (Fig. 5). The branchlets are very small and spine-like, about 35—40 μ long, in most cases consisting of a single, simple or two-branched spine, often with curved tips.

Another specimen of Dr. MORTENSEN's (no. 793), like the above mentioned dredged near the same locality between Gunner's Quoin and Flat Island at a depth of about 25 fathoms, had a similar, loose, large-meshed net but the spine-like branchlets were much longer, 80—100 μ long, and more ramified (Fig. 6).

Since *Haloplegma Duperreyi* was originally described upon West Indian material I have, for the sake of comparison, examined a specimen collected by HOWE (no. 3998) at the Bahamas: "under rock overhang in low littoral" and determined as *Haloplegma Duperreyi* subspec. *spinulosum*. This specimen has for the most part spinelike branchlets (Fig. 7) as to its anatomical structure reminding one of the above-mentioned plants from deep water (Figs. 5, 6).

In addition I have examined another specimen of *Haloplegma Duperreyi* dredged by HOWE at Porto Rico in 14 m. of water (no. 7637). Fig. 8 shows some few assimilating branchlets from this specimen. The branchlets are small, robust and not spine-like, much resembling Dr. MORTENSEN's specimen from shallow water (Fig. 3). The meshes in the net are somewhat larger and consequently do not form such a dense tissue as in the specimen from Mauritius.

The result of these comparative examinations is that *Haloplegma Duperreyi* as to its anatomical structure is a rather variable plant, and that the variations do not seem always to be influenced in the same way by the external conditions, since plants from deep water and shallow water as to their structure may have nearly the same appearance. However, in my opinion, the plant from Mauritius must be considered to be specifically the same as that from the West Indies; but it must of course be taken into consideration that the material upon which I have had to base this opinion was poor and consisted mostly of fragments of specimens only.

In Tabulae Phycologicae, vol. 12, pls. 62—63 KÜTZING besides *Halop. Duperreyi* and *H. Preissii* gives also some figures of the

structure of a plant he calls *Halop. africanum* from South Africa and it therefore seems natural to examine whether the specimens from Mauritius should bear any resemblance to KÜTZING's plant. This cannot be said to be the case. The structure of the specimens from Mauritius have certainly proved mutually rather variable

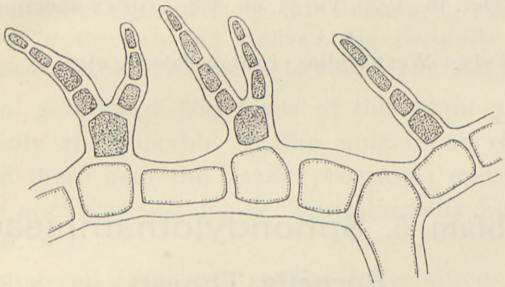

Fig. 7. *Haloplegma Duperreyi* Mont.
Fragment of the net with assimilating filaments from a specimen from the West Indies. (× about 335).

but on the other hand none of them presented any special similarity to KÜTZING's most probably rather schematic figure. Mme WEBER mentions in Algues Siboga, p. 316, that she has examined an authentic specimen of *Halop. africanum* in KÜTZING's Herbarium and arrives at the result that it is nothing but a form of *Halop.*

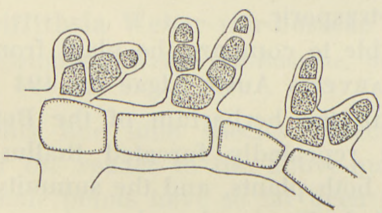

Fig. 8. *Haloplegma Duperreyi* Mont.
Fragment of the net with assimilating filaments of a specimen from Porto Rico. (× about 335).

Duperreyi; and I myself have in accordance with her statement referred a specimen from India (in Some Indian Rhodop., I, 1931, p. 14, fig. 9) showing some likeness to KÜTZING's figure to *Halop. Duperreyi.* In conformity with this I have also referred the specimens from Mauritius to *Halopl. Duperreyi*, as it seems to me that not only a renewed examination of KÜTZING's type-specimen but also an examination of good material from the

West Indies as well as from the Indian Ocean is required to settle the question.

About its habitat at Mauritius JADIN writes in his list pp. 169—170: "Mêlé à *Corallina polydactyla*; exposé à la lame forte".

Mauritius: Flat Island, Oct. 17., 1929, TH. M. no. 839. Off Flat Island, c. 30 fathoms, Oct. 16., 1929, TH. M. no. 820. JADIN's specimens are without dates.

Geogr. Distr.: West Indies, Indian Ocean, etc.

Subfam. 5. Sphondylothamnieae.

Bornetia Thuret.

Bornetia Binderiana (Sond.) Zanard.

ZANARDINI, G., Iconographia, vol. 2, 1865, p. 45, pl. 51, figs. 7, 8. J. AGARDH, Epicrisis, p. 613. — *Griffithsia Binderiana* Sonder, Nova Algarum genera etc., 1845, p. 52. J. AGARDH, Spec. Alg., II, p. 86. HARVEY, Phycologia Austral., tab. 52. KÜTZING, Tab. Phycol., vol. XII, tab. 25 a, b.

This species known so far from South-West Australia was found intermingled among the filaments of the capitulum of *Chamaedoris Delphinii* (Hariot) Feldm. et Børgs. The specimens were small but tetrasporic.

I have been able to compare the plant from Mauritius with a specimen of HARVEY's Austr. Algae no. 494 from Fremantle, W. Austr. found in the herbarium of the Botanical Museum, Copenhagen. The repeatedly furcated thallus has a breadth of about 450 μ in both plants, and the summits of the filaments are broadly rounded. The involucral rays are about 150—200 μ thick and about 300—400 μ long, being likewise broadly rounded above.

Mauritius: Off Flat Island, 17. Oct. 1929, TH. M.

Geogr. Distr.: South West Australia.

Subfam. 6. Griffithsieae.

Griffithsia C. Ag.

1. Griffithsia tenuis Ag.

Agardh, C., Spec. Alg., vol. II, p. 131. Agardh, J., Spec. Alg., vol. II, p. 81; Epicr., p. 70. Collins and Hervey, Alg. Bermuda, p. 135, pl. VI, figs. 38–39. Børgesen, Mar. Alg. D. W. I., vol. II, pag. 462, fig. 423.

In several gatherings fragments of this plant are met with; they are easily recognizable by the unicellular rhizoids given out here and there near the basal (proximal) ends of the cells; compare my above-quoted figure. The filaments had a diameter of about 150—200 μ.

All the material seen was sterile. Determined as *Griffithsia setacea* (Ellis) but referable to *Gr. tenuis* a large but sterile specimen collected by Colonel Pike is found in the collection of the Riksmuseum, Stockholm. In Dickie's list of algae from Mauritius, 1875, p. 197, it is called *Griffithsia secunda* Harv.

Mauritius: Barkley Island, Aug. 1939, R. E. V. nos. 330 and 338. Gr. River Bay, Dec. 22., 1869, Colon. Pike.

Geogr. Distr.: Widely distributed in warm seas.

2. Griffithsia Weber-van-Bosseae Børgs.

Børgesen, F., Griffithsia Weber-van-Bosseae, nov. spec. 1942, p. 15, figs. 1–3.

As regards this fine little species collected by Dr. R. E. Vaughan, I refer the reader to the above-quoted paper. Only tetrasporic and male plants have so far been found.

Mauritius: Black River Bay, July 9., 1939, R. E. V. no. 282.

Geogr. Distr.: Endemic.

In his list p. 169 Jadin mentions a *Griffithsia* spec. as occurring at Mauritius as well as at Réunion. From the last mentioned island I have seen a specimen (no. 169). An examination of it has shown that it is none of the two species mentioned above, but being sterile it is indeterminable. The plant has large oval cells about 1200 μ long and about half as broad. Of Kützing's figures in Tab. Phycol. it agrees best with that of *Gr. opuntioides* in vol. 12, tab. 27.

Subfam. 7. Wrangelieae.

Wrangelia C. Ag.

1. Wrangelia Argus Mont.

MONTAGNE, I., Sylloge gener. specierumque Cryptogamarum, Paris, 1856, p. 444. BØRGESEN, Mar. Alg. D. W. I., vol. II, 1916, p. 116, figs. 125, 126. — *Griffithsia Argus* Mont. in WEBB et BERTHELOT, Hist. nat. iles Canaries, vol. III, Sect. III, 1836–50, p. 176, tab. 8, fig. 4. *Wrangelia plebeja* J. Ag., Spec. Alg., vol. II, 3, 1863, p. 707; Epicr., 1876, p. 623.

Some small specimens of *Wrangelia* found in JADIN's collection and in his list, p. 163, determined as *Wrangelia plebeja* are partly this species, partly the following one. The material was not suitable for examination and a more detailed comparison with West Indian material has not therefore been made. Mme WEBER mentions this species in her "Liste", p. 220, as found in the Malayan Archipelago and says about her specimens that they are in good accordance with West Indian ones.

Tetrasporic plants were met with only; the sporangia have a diameter of about 60 μ, thus the same as in the West Indian plant.

Mauritius: Mahébourg, Sept. 1890, JADIN nos. 444, 449.

Geogr. Distr.: West Indies, Canary Island, Malayan Archipelago, India.

2. Wrangelia penicillata C. Ag.

AGARDH, C., Spec. Alg., II, p. 138. AGARDH, J., Spec. Alg., II, 3, p. 708; Epicr., p. 623. BØRGESEN, Alg. Mar. D. W. I., vol. II, p. 120, figs. 131–132, where the literature is quoted. KYLIN, H., Über *Wrangelia penicillata* und ihre systematische Stellung, 1928, p. 1, figs. 1–3. — *Griffithsia penicillata* Ag., Systema Alg., p. 143.

A single small specimen is present in JADIN's collection; in his list, p. 163, it is referred to *Wr. plebeja*. It is not very well suited for microscopical examination, but it seems to agree with West Indian material.

The specimen is tetrasporic.

About its habitat JADIN writes: "Croissant sur les récifs exposés aux grosses lames".

Mauritius: Mahébourg, Sept. 1890, JADIN no. 470.

Geogr. Distr.: Warmer parts of the Atlantic Ocean, Mediterranean Sea, Malayan Archipelago, Japan, Australia.

Subfam. 8. Callithamnieae.

Aglaothamnion Feldmann-Mazoyer.

1. Aglaothamnion monopodon nov. spec.

Frons exiguissima, usque ad 1 mm. alta, ecorticata, erecta, per cellulam basalem in cuticulam hospitis deorsum plus minus penetrantem adfixa.

E cellula basali filum singulum erectum gignit. Filum erectum inferne nudum, ex media parte sursum ramosum, ramis alternatim ortis aut magis irregulariter, superne subdichotomis.

Filum erectum, in parte basali ca. 20 μ latum, in media parte ca. 30 μ; dein ad apicem versus filamenta gradatim attenuata, superne ca. 7 μ lata.

Pili non observati.

Cellulae uninucleatae, chromatophora irregulariter vittaformia continentes.

Tetrasporangia sessilia, ovoidea, ca. 30—35 μ longa et 27—30 μ lata, solitaria aut 2—3 seriata, superne in latere superiore cellularum orta.

Corpuscula antheridiorum praecipue in superiore parte thalli discos oblongos formantia.

Gonimoblasti gemini, subglobosi, plus minus ovati aut magis irregulares, ca. 70 μ longi et 55 μ lati, in media parte thalli evoluti.

Mauritius: Flic-en-Flac, December 31., 1938, R. E. V. no. 249.

A small *Aglaothamnion* 600—700 μ high rarely up to 1 mm. occurred upon the thallus of a specimen of *Gracilaria lichenoides* preserved in formol.

The plant (Fig. 9) is attached to the host by means of a short basal cell of which the lower half is immersed in the thick peripheric layer of the host in much the same way as is the case for instance in *Acrochaetium unipes* Børgs. (Mar. Alg. D. W. I., vol. II, p. 35). From the upper end of the basal cell issues a single erect, in its lower part unbranched, main stem; lowermost in this stem the cells are short with thick walls but they soon become longer, often up to 20 times their own breadth, while at the same time the walls become gradually thinner. Near the base the main stem is about 20 μ and the lumen of the cells

reaches a breadth of 4—5 μ only; upwards the stem grows slowly thicker to about 30 μ below the first ramification. The surface of the wall in the lower part of the thallus is often unevenly

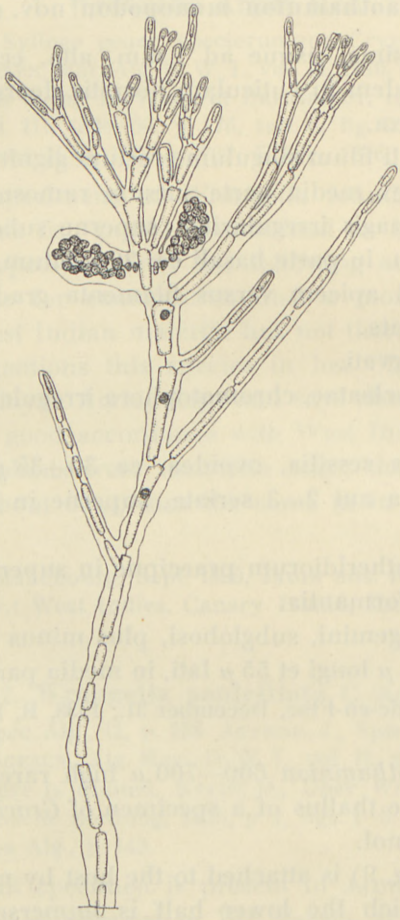

Fig. 9. *Aglaothamnion monopodon* Børgs. A young female plant. (× about 165).

wavy; at the joints a slight narrowing is more or less observable. No cortical layer formed by rhizoids is present.

The plant is alternately ramified, now and then also more irregularly; in the upper parts of the thallus the ramification is subdichotomous and the branches issue at acute angles.

In the female plant the unbranched basal part of the main

stem often becomes proportionally shorter than that of the male and tetrasporic plant, because several straight, obliquely upwards directed, and mostly unramified branches are given off from some of the segments below the first fertile segment (Fig. 9).

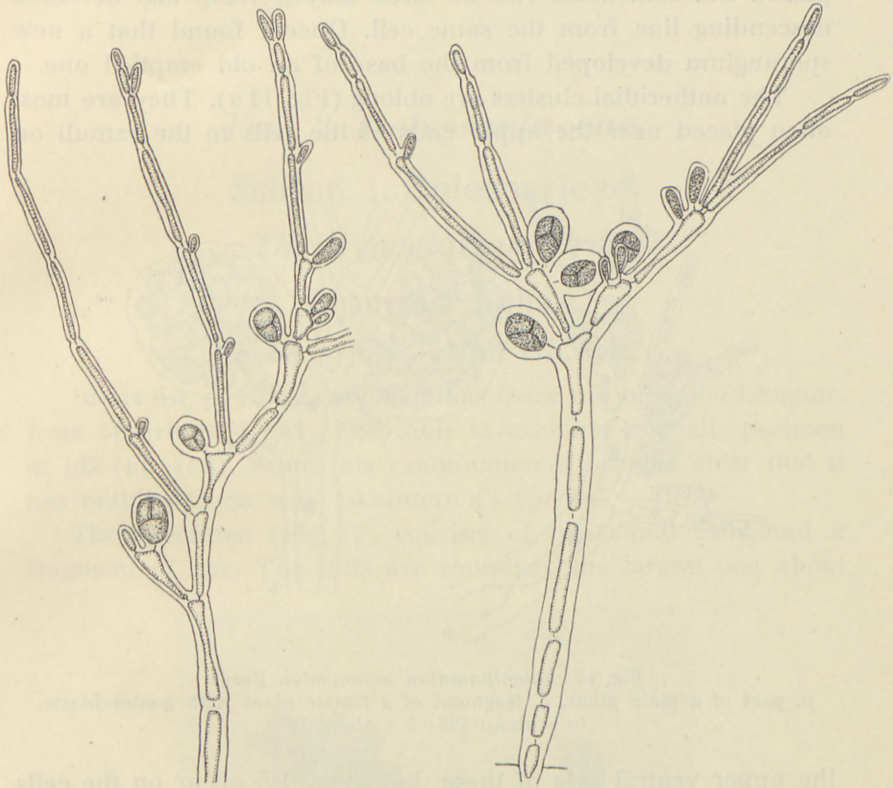

Fig. 10. *Aglaothamnion monopodon* Børgs. Specimens with tetrasporangia. (× about 165).

When the ramification begins the breadth of the filaments decreases slowly upwards to about 7 μ in the uppermost branchlets.

In the older cells the chromatophores are irregularly ribbon-like, in the younger ones short staff-like or more roundish and crowded together in a reticular manner.

The cells contain a single nucleus each; it is placed a little above the middle of the cell.

Tetrasporic as well as male and female specimens were found.

The tetrasporangia (Fig. 10) are formed near the summits of the cells on their upper ventral side; they are sessile, when young lanceolate, when ripe broadly oblong to ovate, about 30—35 μ long and 27—30 μ broad. They are mostly solitarily placed but sometimes two or three may develop together in a descending line from the same cell. Once I found that a new sporangium developed from the base of an old emptied one.

The antheridial clusters are oblong (Fig. 11 *a*). They are most often placed near the upper ends of the cells in the ramuli on

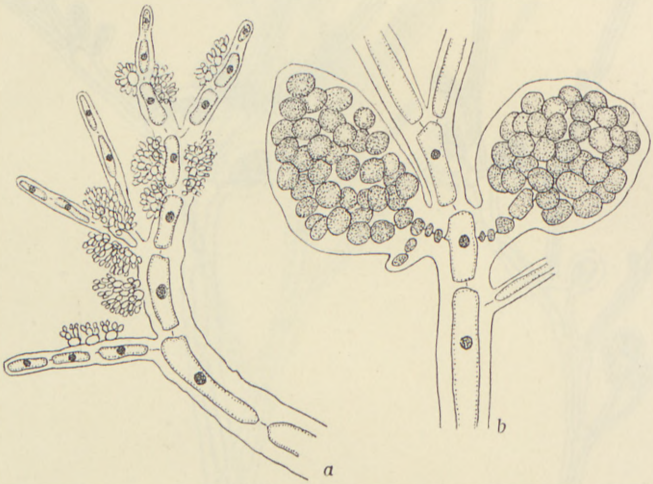

Fig. 11. *Aglaothamnion monopodon* Børgs.
a, part of a male plant. *b*, fragment of a female plant with gonimoblasts. (*a* × about 335; *b* × about 165).

the upper ventral side of these, but they also occur on the cells of the main filaments, and altogether rather irregularly, for instance also on the dorsal lower side of the ramuli.

In the female plants (Figs. 9 and 11 *b*) the gonimoblasts are developed in accordance with Oltmann's description of *Callithamnion corymbosum* (1898, p. 115) and Kylin's of *Callithamnion Furcellariae* (1923, p. 56). They are formed near the middle of the plant but two or three pairs may be developed above each other. Their shape is roundish-polygonal or more oblong; perfectly ripe fruits have not been observed.

In two cases a small basal gonimolobe was developed from the auxiliary cell; Kylin, l. c., p. 57, mentions a similar case in

Callithamnion Furcellariae; compare Fig. 11 *b*, the gonimoblast to the left.

Because of its small size and especially because of its peculiar base, this tiny species is easily distinguishable from the species hitherto known of *Aglaothamnion*.

Fam. 2. ***Delesseriaceae.***

Subfam. 1. Delesserieae.

a. *The Hypoglossum-Group.*

Chauvinia Kylin.

1. **Chauvinia Jadinii** nov. spec.

In his list, p. 167, JADIN mentions *Delesseria ruscifolia* Lamour. from Mauritius. I have been able to examine a small specimen of his (no. 465). From this examination it became clear that it has nothing to do with LAMOUROUX's species.

The specimen (Fig. 12) consists of two small tufts and a fragment of one. The tufts are roundish, the largest one about

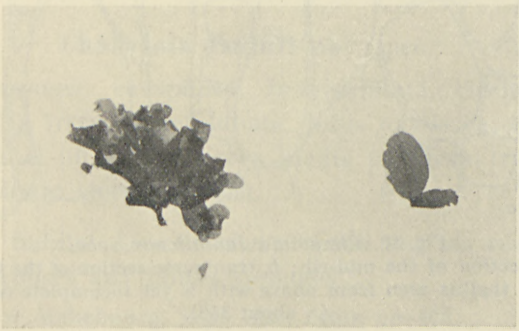

Fig. 12. *Chauvinia Jadinii* nov. spec. (×1).

$1^1/_2$ cm. high issuing from a quite short stipe from the upper ends of which numerous short leaflike lobes are given out. One of the largest lobes is oval, nearly 1 cm. long and half as broad, with broad apex, the margin is entire and does not seem to have been waved. A midrib is easily observable with the naked eye.

The colours of the plant is rosy-red. With some minor exceptions this description of the appearance of the plant may be said to correspond fairly well to that of a small specimen of *Delesseria ruscifolia*. But the anatomical structure shows immediately that we have quite another plant before us.

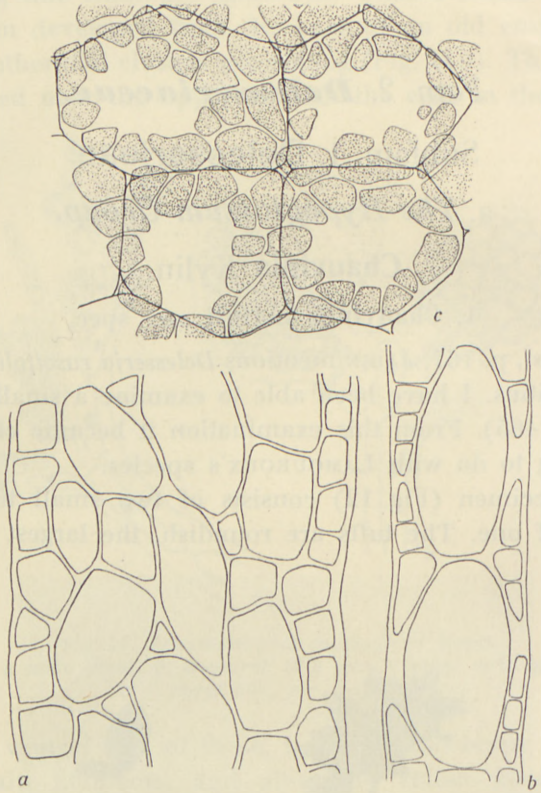

Fig. 13. *Chauvinia Jadinii* nov. spec.
a, transverse section of the mid-rib; *b*, transverse section of the thallus; *c*, fragment of the thallus seen from above with a yet incomplete cortical layer. (× about 350).

The leaflike thallus consists of a single layer of large cells covered more or less completely by a cortical layer of small cells on both sides (Fig. 13 *b*, *c*). A transverse section of the midrib shows that it is composed of several cells, upto 5—6, above each other (Fig. 13 *a*); rhizoids are present between the cells. Lateral microscopical veins are not found. The prolifications

are developed from the midrib. All the top-cells of the cell-rows of the 1—3 order reach the margin of the thallus and all the cells of the second order carry a side-branch; compare KYLIN's figure 52a of *Hypoglossum Woodwardii* (1923, p. 81) and of *Chauvinia coriifolia* (1924, p. 12, fig. 6a). From this description of the anatomical structure of the plant it becomes evident that it points very clearly in the direction of the group *Hypoglossum*.

Upon my inquiry whether any of the *Hypoglossum*-species could have a cortical layer Professor KYLIN who some years ago worked out the above quoted very valuable monograph on the *Delesseriaceae* (1924) has most kindly communicated to me that the species of the genus *Hypoglossum* have always a monostromatic thallus. The genus *Chauvinia* Kylin, also belonging to the *Hypoglossum*-group, with the only known species *coriifolia* (Harv.) Kylin (=*Delesseria coriifolia* Harv. Phyc. Austr., pl. 150) has, on the other hand more than three layers of cells in its thallus, and since the plant from Mauritius is otherwise built in complete conformity with that genus, it might be referred to it as a new species.

This new species I propose to name *Chauvinia* JADINII Børgs. in memory of the late Dr. FERNAND JADIN who by his collections of algae from the Mascarene Islands has contributed so very much to our knowledge to the algal flora of these islands.

Chauvinia Jadinii nov. spec.

Frons pygmaea, caespitosa, brevistipitata, circiter $1^1/_2$ cm. alta et ultra(?), irregulariter lobata, lobis ovatis ca. 1 cm. longis et $^1/_2$ cm. latis et ultra(?), e nerva media prolifera, tristromatica. Specimen unicum sterilem adest.

About its habitat JADIN writes: "Croit sur les récifs ou sur les rochers exposés aux lames violentes".

Mauritius: Mahébourg, Sept. 1890, JADIN no. 465.

b. *The Claudea-Group.*

Compare PAPENFUSS, 1937, p. 60.

Caloglossa (Harv.) J. Ag.

1. Caloglossa Leprieurii (Mont.) J. Ag.

var. *Hookeri* (Harv.) Post.

POST, E., Systemat. u. pflanzengeogr. Notizen zur *Bostrychia-Caloglossa*-Assoziation, 1935, p. 53. BØRGESEN, *Catenella Nipae* used as Food in Burma, 1938, p. 267, fig. 2. — *Caloglossa Hookeri* Hook. fil. & Harv., 1845, p. 270.

Well developed, tetrasporic material of this interesting variety is found in Dr. VAUGHAN's collection. Together with *Caloglossa* and attached to it is found a small alga; compare *Polysiphonia* spec. mentioned later p. 36.

In JADIN's collection I have further seen a small sterile specimen of this variety. In his list p. 167 it is called *Caloglossa amboinensis.*

It is found in a "Trou d'eau douce à Flacq". As to its interesting habitat JADIN points out that it grows in perfectly fresh water. I refer the reader to JADIN's detailed description and to Miss POST's remarks about it (1943, p. 203, the note).

In the collection of the Riksmuseum, Stockholm, a tetrasporic specimen is found. It was collected by Col. PIKE in "Mt. stream of Ponce", Jan. 26. 1870. In DICKIE's list, p. 193, it is mentioned as *Delesseria Leprieurii* Mont.

Mauritius: Ilôt Brocus, Aug. 1938, R. E. V. no. 192. Flacq, June 1890, JADIN no. 512.

Geogr. Distr.: Widely distributed in warm seas.

Vanvoorstia Harv.

1. Vanvoorstia spectabilis Harv.

HARVEY, W. H., Short characters etc., 1854, p. 144; Ceylon Alg. Exs. no. 3. KÜTZING, Tab. Phyc., vol. 19, tab. 56. WEBER v. BOSSE, Algues Siboga, p. 390, fig. 141. BØRGESEN, Contributions II, 1937, p. 344, fig. 15. PAPENFUSS, The Structure and Reproduction of *Claudea multifida, Vanvoorstia spectabilis* etc., 1937, p. 31.

Based upon well preserved material collected by SVEDELIUS during his stay in Ceylon in 1903 PAPENFUSS has in the above-

cited paper given a thorough description, accompanied by instructive figures, of the structure and development of this elegant alga and of its reproductive organs.

In JADIN's collection a single but well prepared female specimen is found. It is included in JADIN's list p. 168, being mentioned here for the first time as occurring at the Mascarene Islands.

About its habitat JADIN writes: "Abondant sur les parties verticales du récif qui reçoivent les courants des grosses lames venant se briser sur les coraux; toujours recouvert par le flot."

Mauritius: Mahébourg, Sept. 1890, JADIN no. 441.

Geogr. Distr.: Ceylon, Malayan Archipelago, Japan, South Africa, Mauritius.

Subfam. 2. Nitophylleae.

Martensia Hering.

1. **Martensia elegans** Hering.

HERING, Diagnoses Alg., 1841, p. 92. Flora, 1844, II, no. 47, p. 803, pl. VII. HARVEY, Nereis Australis, 1847, p. 73. pl. 43. SVEDELIUS, Martensia, 1908. For further literature comp. DE-TONI, Syll. Alg., vol. IV, p. 616.

Of this species, first described by HERING upon specimens from Port Natal, two quite small specimens are present in the collections. One of these was dredged at a depth of about 25 fathoms by Dr. MORTENSEN, the other one is found in Dr. JADIN's collection and mentioned in his list p. 167. About its habitat JADIN writes: "Un seul exemplaire recueillit dans un bassin aux eaux tranquilles dans les rochers."

Mauritius: Flacq, July 1890, JADIN no. 265. Between Gunners Quoin and Flat Island, 25 fathoms, Oct. 15., 1929, TH. M.

Geogr. Distr.: South Africa, Australia, Malayan Archipelago.

Fam. 3. ***Dasyaceae.***

Dasya C. Ag.

1. **Dasya scoparia** Harv.

HARVEY in J. AGARDH, Symbolae, 1841, p. 34. HARVEY, Nereis Austr., 1847, p. 62, tab. 21. KÜTZING, Tab. Phyc., vol. 14, tab. 65, fig. d, e. AGARDH, J., Spec., II, 3, p. 1221.

In JADIN's list p. 169 *Dasya arbuscula* Ag. is mentioned. Two specimens referred to this plant, one no. 122 from Réunion, the other no. 493 from Mauritius, are present in his collection; an examination of these has shown that the plant in question is *Dasya scoparia* Harv.

While that from Mauritius is sterile, the other one from Réunion is tetrasporic. The stichidia are sessile with only one sterile basal cell in the stichidium. I mention this because HARVEY in his description in Nereis says about the stichidia that they are "breve pedicellatis", and his figures 3 and 4 also show two rather long cells in the stalk of the stichidia.

About the habitat of this plant JADIN writes: "Cueilli sur des rochers exposés aux lames violentes."

Mauritius: Flacq, Sept. 1890, JADIN no. 493. Saint-Gilles, April 1890, JADIN no. 122.

Geogr. Distr.: South Africa, Japan.

2. **Dasya pedicellata** Ag.

AGARDH, C., Systema Alg., 1824, p. 211. COLLINS and HERVEY, Algae of Bermuda, 1913, p. 130. — *Dasya elegans* (Mart.) Ag., Spec. Alg., vol. II, 1828, p. 117. *Dasya villosa* Harvey, Algae of Tasmania, 1844, p. 433. For other synonyms compare DE-TONI, Syll. Alg., vol. IV. p. 1201.

JADIN in his list, p. 169, has both *Dasya pedicellata* and *D. villosa* as found at the island, and of both species I have seen specimens of his, that of *D. pedicellata* being cystocarpic while that of *D. villosa* has stichidia. And if these two forms are regarded as different species, JADIN's determinations are quite correct. However, my view of these species is that they can hardly be kept distinct.

What may point in the direction of the presence of two separate species, leaving out the geographical distribution, is that the thallus in *Dasya villosa* seems to be more robust and has a darker purple-violet colour, while the thallus of *Dasya pedicellata* is somewhat slender, more soft and has a more rose-red colour; but variations from this in the one or the other direction may be found in specimens from the Atlantic as well as in those from the Indian-Pacific Oceans.

Some few years ago the Chinese algologist C. K. TSENG, 1938,

p. 601, in connection with Yendo's statement, 1916, p. 262, after the examination of a large material, arrived at the result that some differences between the two species are to be found in the placing and shape of the stichidia; while these in *Dasya villosa* should be sessile and always have coordinate ramulets, those in *D. pedicellata* should always be stalked, not terminated with mucrons or filaments. And in the cystocarps similar differences should be found. But a comparison of the two specimens from Mauritius with material of *Dasya pedicellata* from the West Indies has not confirmed this. Thus the stichidia in the small specimen from Mauritius are very like those in the West Indian material, being sessile upon the pseudo-branchlets and adventitious branchlets in both plants (compare ROSENBERG, p. 50, fig. 15). And as to the cystocarps, when those of the specimen from Mauritius are compared with West Indian ones I have found them very much alike, the cystocarps in both specimens being placed on a pedicel having the length of about a third part of the length of the cystocarps.

To me the facts seem to correspond what is the case with *Asperagopsis Sanfordiana* Harv. from the Pacific-Indian Oceans and *Asparagopsis taxiformis* (Del.) Collins and Herv. from the Atlantic which until lately, mainly because of differences in the size and colour of the thallus in connection with the geographical distribution, have been considered as separate species, but about which Mme and Dr. JEAN FELDMANN (1942, p. 82) in their highly interesting paper on the alternation of generations of the *Bonnemaisoniaceae* with reference to earlier pronouncements and especially to that of LUCAS (1935, p. 222), have now established the fact that *Asparagopsis Sanfordiana* plainly is to be considered as a synonym only of *Aspar. taxiformis*.

JADIN in his list, p. 169, about the habitat of *Dasya villosa* says: "Cueilli sur des rochers."

Mauritius: Mahébourg, Sep. 1890, JADIN no. 485. The other specimen is without locality, gathered by DARUTY 1892.

Geogr. Distr.: Warmer parts of the Atlantic and Indian-Pacific Oceans.

Dictyurus Bory.

1. Dictyurus purpurascens Bory

in Belanger, Voyage Ind. orient., p. 170, tab. 1 (after De-Toni, Syll. Alg., IV, p. 1173). Agardh, J., Spec. Alg., II, 3, p. 1245. Falkenberg, Rhodomelaceen, p. 675, tab. 17, figs. 10–24.

A few specimens are found in Dr. Jadin's and Dr. Vaughan's collections.

About its occurrence at the islands Jadin writes p. 169: "Cueilli dans les bassins rocheux, à l'abri des grosses lames, mais recevant les eaux bouillonnantes et très aérées des vagues venant battre sur les rochers."

Mauritius: Ilôt Brocus, R. E. V. no. 196 (cast ashore). Flacq, Oct., 1890, Jadin no. 482.

Geogr. Distr.: Indian Ocean.

Fam. 4. ***Rhodomelaceae.***

Subfam. 1. Polysiponieae.

Polysiphonia Greville.

1. Polysiphonia mollis Hook. fil. et Harvey.

Harvey, W. H., Nereis Austr., 1847, p. 43. Agardh, J., Spec. Alg., vol. II, 3, 1863, p. 968.

On pieces of an indeterminable seagrass a small *Polysiphonia* is found in Dr. Mortensen's collection, growing sociably and forming roundish tufts about 4 cm. high. This plant I think is referable to *Polys. mollis* Hook. fil. et Harv.

This species which seems to be widespread in the Indian Ocean is described by Hooker and Harvey and next by J. Agardh who gives a somewhat more detailed description of it. Later it has been examined by various investigators, for instance Askenasy (1894, p. 13), Yendo (1916, p. 261), and Weber (1923, p. 356), but all have placed a ? after their determinations. In "Alg. Bombay", 1935, pp. 60—62 I have mentioned these earlier examinations at the same time adding some comparative remarks on Harvey's species and on *Polys. platycarpa* established by myself. Because of the new material from Mauritius I have

again taken up the question of the relationship of the two species and shall first give a description of the specimens from Mauritius which I presume to be referable to *P. mollis*.

These specimens have no cortical layer. They are attached to the host plant by means of a small disc (Fig. 14) which may be strengthened by means of hapters issuing from the lowermost cells in the erect main filaments (Fig. 14*a*). Near the base the filaments are about 200—300 μ thick and the segments from

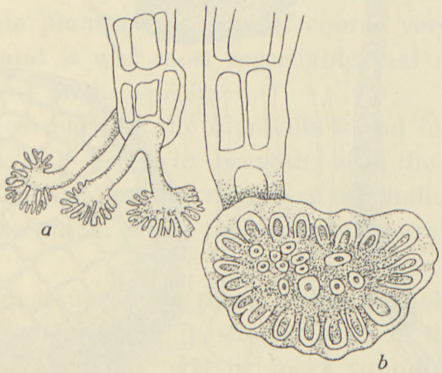

Fig. 14. *Polysiphonia mollis* Hook. fil. et Harvey.
Bases of two specimens. (× *a* about 50, *b* about 150).

somewhat shorter than the breadth up to a little longer than this. The peripheral wall in the cells near the base is thick, and the segments may be a little narrowed in the middle.

At some distance from the base the main axis becomes divided and gradually indistinct, and because the angles of the branches are nearly right angles the branches are spreading; higher up in the thallus the branches issue at acute angles and the filaments are therefore placed more closely together.

In the middle of the thallus the breadth of the filaments decreases to about 100 μ and the segments at the same time become about double as long as the breadth. Towards the tips the filaments gradually taper still more, the segments at the same time becoming shorter.

The trichoblasts are formed in a screw to the left with a $^1/_4$ divergence; now and then a branch is developed instead of a trichoblast.

Most of the specimens are tetrasporic, some are cystocarpic; of androphores I have seen only some loose lying ones adhering to other specimens. The tetrasporangia are developed in the upper branches and branchlets (Fig. 15*a*); in the lowermost fertile parts of the branches, the sporangia are often solitary or occur a few together interrupted by a few sterile segments; higher

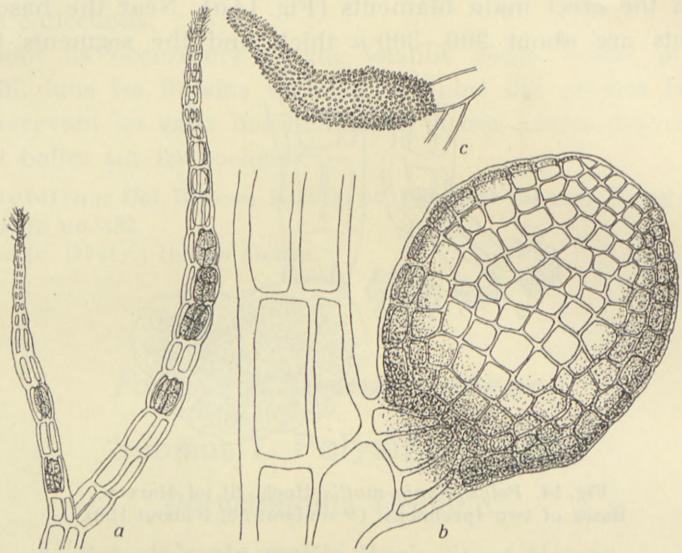

Fig. 15. *Polysiphonia mollis* Hook. fil. et Harvey.
a, fragment of the thallus with tetrasporangia. *b*, an antheridial body. *c*, a cystocarp. (*a* × about 50, *b* and *c* × 120).

up they are present in longer coherent rows placed more or less distinctly in a screw. The sporangia are oval c. 90—110 μ long and 70—90 μ broad.

The cystocarps (Fig. 15*b*) are urceolate with a small not especially marked ostiole above; they are about isodiametric, length and breadth about 330 μ, sometimes a little broader than long or the reverse.

The androphores (Fig. 15*c*) are elongated-subcylindrical, tapering gradually upwards; they are about 200—250 μ long, and about 50 μ broad near the base. The androphores are formed of the trichoblasts with the exception of two basal sterile cells, from the uppermost of which a sterile, several times forked, ramulus is given off on its right side. No sterile cell is present at the top of the androphores.

Because of its scutate base and the short basal segments in the primary erect filaments, I think this plant is referable to *Polys. mollis*; it is in good accordance with the descriptions of HARVEY and J. AGARDH. In connection with his description AGARDH refers to HARVEY's Australian Algae Exsicc. no. 168 from Fremantle, West Australia as being the type of the species. A single specimen of this is found in the Botanical Museum, Copenhagen, and from an examination of it I have found that the specimen contained not only pieces of tetrasporic but also of male and female plants. This was of course very valuable but on the other hand it was most regrettable that no base of the plant was present in the specimen.

The lowermost parts of the filaments found in this specimen measured from 200—275 μ in breadth, and the segments had about the same length. Near the middle of the thallus the filaments were about 100 μ thick, while the segments were about 3 times longer.

The branches issue at the place of the trichoblasts.

The tetrasporangia occur in shorter rows in the upper parts of the filaments; they are oval or more roundish, about 50—72 μ broad and about 85 μ long. The androphores are built entirely in conformity with those of the plant from Mauritius.

Of cystocarps I have seen only rather few and young ones; they are urceolate of shape about 240 μ broad and a little longer, thus a good deal smaller than those found in the specimens from Mauritius; but the cystocarps of a specimen of the same number of HARVEY I once examined in the Herbarium of the Kew Gardens was much larger, having a breadth of 460 μ and a length of 420 μ.

When the descriptions of the plant from Mauritius and of that of HARVEY are compared it must be admitted that they are in good conformity and that it is justifiable to refer the plant from Mauritius to *Polys. mollis*.

Finally if we compare it with *Polysiphonia platycarpa* Børgs., described upon specimens from Bombay, it cannot be denied that this species, when its base is left out of consideration, bears a great resemblance to *Polysiphonia mollis* with regard to the build of the thallus and its fruiting organs. On the other hand it must be said to be easily discernible from *Polys. mollis* by its

creeping basal filaments composed of segments about double as long as broad.

In JADIN's collection there are two small specimens no. 233 and 513 which I think are referable to this species. The specimens have a basal disc, being attached to a seagrass. They are tetrasporic. JADIN in his list, p. 169, refers them to *Polysiphonia pulvinata* Harv.

Mauritius: Cannoniers Point, Oct. 26., 1929, TH. M. Baie de la Petite Rivière, July 1890, JADIN no. 233. Mahébourg, Oct. 1890, JADIN no. 513.
Geogr. Distr.: Tasmania.

2. **Polysiphonia platycarpa** Børgs.

BØRGESEN, F., Some Indian Rhodophyceae, 1934, p. 23, figs. 15–17.

To this species described upon material from Bombay I have referred some few specimens found in Dr. JADIN's and Dr. VAUGHAN's collections.

The species very much resembles the above mentioned *Polys. mollis* but by its decumbent filaments it is decidedly distinct from it. In the sparse material specimens with tetrasporangia, cystocarps and androphores are found.

A small specimen in Dr. JADIN's collection in his list, p. 169, referred to *Polys. pulvinata* is, I think, this species.

Mauritius: Black River Bay, July 9., 1939, R. E. V. no. 282. Barkly Island, Aug. 1939, R. E. V. no. 333. Port-Louis, 1890, JADIN no. 371.
Geogr. Distr.: Indian Ocean.

3. **Polysiphonia ferulacea** Suhr, J. Ag.

SUHR in AGARDH, J., Spec. Alg., vol. II, 3, p. 980. BØRGESEN, Mar. Alg. D. W. I., vol. II, p. 277, figs. 277–279.

On a piece of *Turbinaria* preserved in alcohol there was a small specimen of a *Polysiphonia*, of which Fig. 15 shows a fragment.

The habit of the plant shows such a great similarity to a *Polysiphonia* from the West Indies which I have called *P. ferulacea* that I do not hesitate to refer it to this species. To be sure, the plant is somewhat smaller than the West Indian plant, thus the cystocarps are about 225 μ broad and 300 μ long and the

breadth of the thallus about 200 μ only, but it must be taken into consideration that the whole specimen was only some few millimetres long.

Mauritius: Flic-en-Flac, Jan. 2., 1939, R. E. W. no. 259.
Geogr. Distr.: West Indies, Mexico, Australia, Sandwich Islands etc.

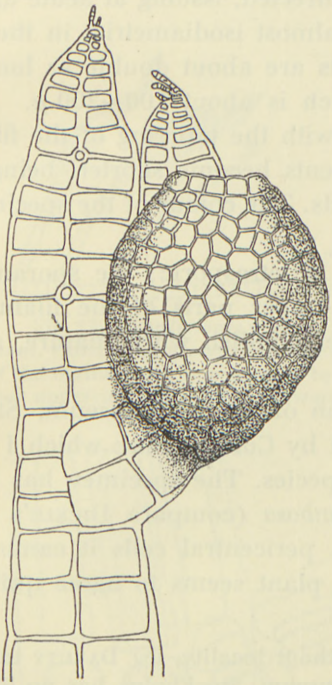

Fig. 16. *Polysiphonia ferulacea* Suhr, J. Ag.
Fragment of the thallus with a cystocarp. (× about 125).

4. **Polysiphonia variegata** (C. Ag.) Zan.

Zanardini, Synopsis Algarum, 1842, p. 162. Agardh, J., Spec. Alg., vol. II, p. 1030. Falkenberg, Rhodomelaceen, p. 119, tab. 21, fig. 30. Børgesen, Some Indian Rhodophyc., IV, 1934, p. 26, fig. 18.

A specimen in Jadin's collection in rather a bad condition gathered by Daruty seems to agree quite well with Falkenberg's description of this species and with Indian specimens referred by me to this species.

The specimen has 7 pericentral cells and no cortical layer; the trichoblasts are not much developed. The mutual arrangement

of the trichoblasts and branches is in accordance with the description of FALKENBERG. Near the base the filaments are about 400—500 μ thick, rather stiff, and the branches are given off at nearly right angles; higher up the filaments taper much, become very flabby and much ramified, while at the same time the branches become upwards directed, issuing at acute angles. Near the base the segments are almost isodiametric, in the middle of the filaments the segments are about double as long as the breadth of the filaments, which is about 100—200 μ.

In connection with the tapering of the filaments towards the summits the segments become shorter, being broader than long near the apical ends. The colour of the specimen is dark reddish-brown.

The specimen is tetrasporic; the sporangia occur in rather long rows in the upper parts of the filaments; they are oval when young, more roundish when mature, about 80 μ long and 70 μ broad.

In the collection of the Riksmuseum, Stockholm, there is a specimen collected by Colonel PIKE which I presume is likewise referable to this species. The specimen has been determined by DICKIE as *P. corymbosa* (compare DICKIE's list, p. 192) but as this species has 4 pericentral cells it cannot be referred to it. On the whole the plant seems to agree quite well with that in JADIN's collection.

Mauritius: Without locality, leg. DARUTY in Herb. JADIN. The specimen in the Riksmuseum, Stockholm, has no locality either.

Geogr. Distr.: Mediterranean Sea, European and North American Atlantic coasts, West Indies, Indian Ocean.

Polysiphonia spec.

The collection of Dr. VAUGHAN contains a small *Rhodomelaceae* reminiscent of *Lophosiphonia* in its way of growing while its ramification is like that of *Polysiphonia*, but because of its complete sterility I have preferred to let it remain undetermined. Owing to its peculiar occurrence, creeping upon the thallus of *Caloglossa Leprieurii* collected on mangrove-roots, and also because it might be the representative of a new genus I propose to give a short description of it accompanied by some figures.

The plant (Fig. 17) is radiate, has 4 pericentral cells and no cortical layer; no strongly pronounced dorsiventrality is present

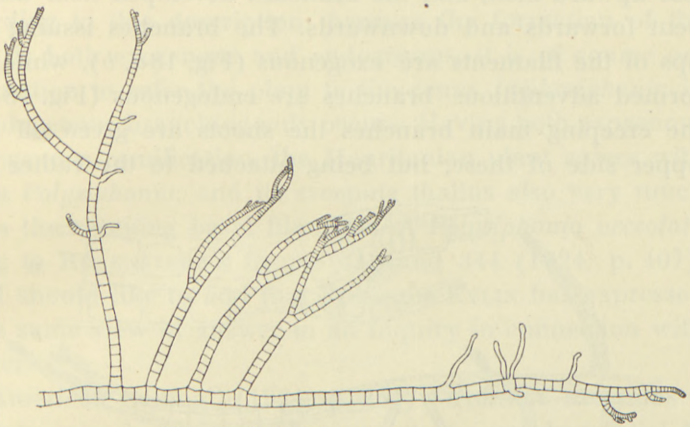

Fig. 17. *Polysiphonia* spec.
Habit of a piece of the plant. During the growth because of the movable substratum the foremost part of the thallus must be presumed to have been turned round so the former upperside has come downwards. (× 20).

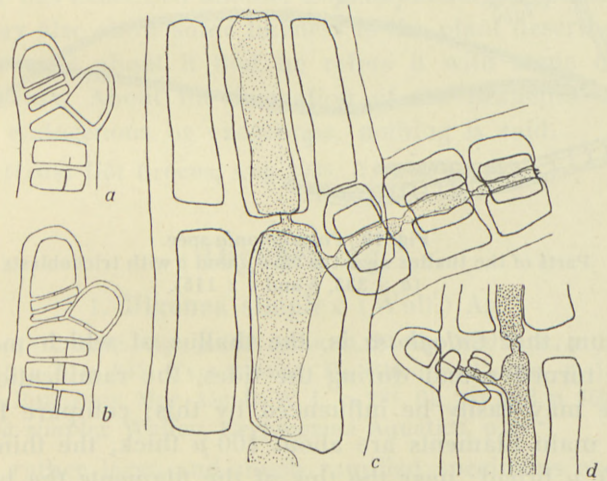

Fig. 18. *Polysiphonia* spec.
The figures show the different ways of branching found in the plant.
a, *b*, exogenous branching; *c*, *d*, endogenous branching; the central cells are dotted. (× 500).

nor any marked differentiation between long and short shoots, all the branches, when the proper conditions are present, being able to attach themselves to the substratum. The creeping filaments

are fastened by means of unicellular rhizoids given out from the pericentral cells. The tips of the creeping filaments are more or less upward-bent, and the branches developed near the tips are bent forwards and downwards. The branches issuing near the tips of the filaments are exogenous (Fig. 18*a*, *b*), while the later formed adventitious branches are endogenous (Fig. 18*c*, *d*). On the creeping main branches the shoots are given off from the upper side of these, but being attached to the rather movable

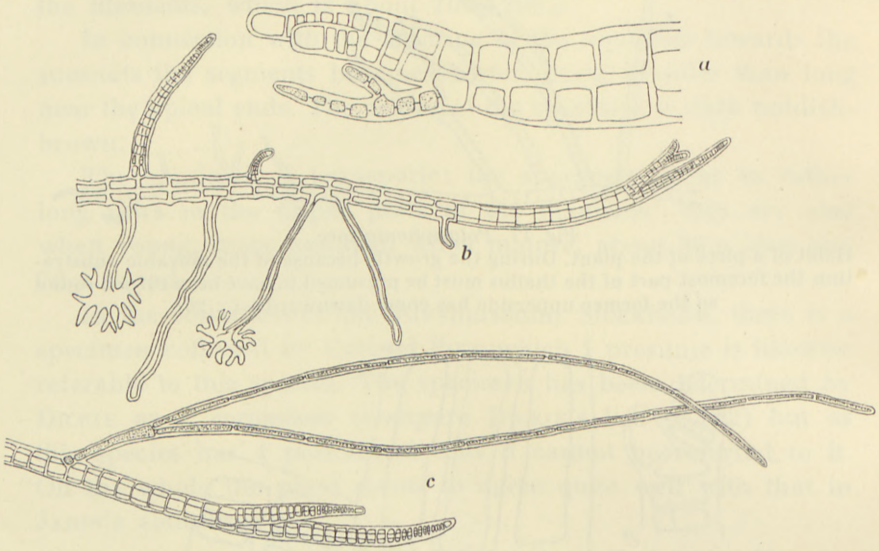

Fig. 19. *Polysiphonia* spec.
Parts of the thallus near the tips. *a* and *c* with trichoblasts.
(*a* × 335, *b* and *c* × 115).

substratum that *Caloglossa* is, the thallus of which may easily become turned round during the tides, the ramification of the epiphyte may easily be influenced by this; compare Fig. 17.

The main filaments are about 100 μ thick, the thinner ones about 70 μ broad; near the tips of the filaments the breadth is only 10 μ. The segments are mostly almost isodiametric; sometimes their length is up to about twice their breadth. In the main filaments the cells are more or less narrowed in their middle.

Trichoblasts (Fig. 19*a*, *c*) are rarely developed; in most cases they consist of a single long filament but one or two side-branches may be found. They grow very long, often more than 1 mm.,

and are often directed straight forward along the mother branch. The trichoblasts are composed of long cells, near their base they are about 15—16 μ broad, tapering upwards to about 4 μ.

According to this description, because the formation of the branches is both exogenous and endogenous, it is of course out of the question to refer the plant to the genus *Lophosiphonia* in which endogenous branching only occurs. Having both exogenous and endogenous ramification, the Mauritanian plant agrees with the genus *Polysiphonia*, and its creeping thallus also very much resembles the creeping basal filaments of *Polysiphonia urceolata* according to ROSENVINGE's figures 342 and 344 (1924, p. 407).

And I should like to add that Professor KYLIN has expressed much the same view in answer to an inquiry in connection with this matter.

In "American Samoa" (1924, p. 254) SETCHELL mentions a small *Lophosiphonia* spec. which seems to be nearly related to the plant from Mauritius.

And in "Tahitian Algae", 1926, p. 103, pl. 21, figs. 3 and 4 SETCHELL has described another *Lophosiphonia* (*L. sparsa* Setch.) which may also show some likeness to the plant described here. SETCHELL says about it that he refers it with some doubt to *Lophosiphonia*. About the formation of the branches, whether they are endogenous or exogenous, nothing is said.

Mauritius: Ilôt Brocus, Aug. 1938, R. E. V. no. 192.

Digenea Ag.

1. **Digenea simplex** (Wulf.) Ag.

AGARDH, C., Spec. Alg., p. 389. AGARDH, J., Alg. Mediterr., p. 147; Spec. Alg., vol. II, p. 3, p. 845. FALKENBERG, P., Rhodomelaceen, p. 159, pl. 9, figs. 25—29. BØRGESEN, Mar. Alg. D. W. I., p. 281, fig. 281 and p. 469, fig. 427. — *Conferva simplex* Wulfen, Cryptogama Aquatica, p. 17.

Some rather large and much ramified specimens are found in the collections. Besides sterile and some few tetrasporic specimens a single antheridial and another cystocarpic one are present.

The antheridial bodies, compare my figure l. c., p. 469, fig. 427, as well as the cystocarps are formed near the tips of the branchlets.

Digenea simplex is mentioned by JADIN in his list, p. 169. As to its habitat he writes: "Croissant dans le sable, dans les eaux tranquilles".

Mauritius: Pointe aux Roches, Febr. 7., 1939, R. E. V. no. 265. Flic-en-Flac, without date, R. E. V. no. 251. Some specimens collected by DARUTY are without locality and date.

Geogr. Distr.: Widely distributed in warm seas.

Subfam. 2. Herposiphonieae.

Herposiphonia Nägl.

1. Herposiphonia tenella (Ag.) Ambr.

AMBRON, H., Bilateralität bei den Florideen, 1880, p. 197. FALKENBERG, P., Rhodomelaceen, p. 304. BØRGESEN, F., Mar. Alg. D. W. I., vol. II, p. 286, figs. 287—289, and p. 472, figs. 430—431. — *Hutchinsia tenella* Ag., Spec. Alg., vol. II, p. 105. *Polysiphonia tenella* J. Ag., Alg. Mediterr., p. 123; Spec. Alg., vol. II, 3, p. 919.

A single undetermined specimen in JADIN's collection has a ramification like the typical *Herposiphonia tenella*. The plant has mostly 7—8 pericentral cells, but 9 and 10 are also found, and in more poorly developed branchlets 6 only are present. The decumbent, creeping main filaments are from about 90 μ up to 170 μ thick and the segments about 140—220 μ long. The branchlets have a diameter of about 60 μ and the segments are about 100 μ long.

Unfortunately the specimen was sterile.

Mauritius: Without locality and date, collected by DARUTY, 1893, in Herb. JADIN.

Geogr. Distr.: Mediterranean Sea, Morocco, West Indies, Malayan Archipelago, Ceylon etc.

2. Herposiphonia secunda (Ag.) Ambr.

AMBRON, H., Ueber ein. Fällen von Bilateralität bei den Florideen, 1880, p. 197. FALKENBERG, Rhodomelaceen, 1901, p. 307, pl. 3, figs. 10—12. BØRGESEN, Mar. Alg. D. W. I., vol. II, p. 469, figs. 428—429. *Hutchinsia secunda* Ag., Systema Alg., 1824, p. 149. *Polysiphonia secunda* Zanard., Synops. Alg. Adriat., 1841, p. 64. J. AGARDH, Alg. Mediterr., p. 122; Spec. Alg., vol. II, 3, p. 921. For further literature comp. DE-TONI, Sylloge Alg., vol. IV, p. 1052.

Upon fragments of an old *Turbinaria* preserved in alcohol there occurred an *Herposiphonia* which instead of the normal ramification of *Herposiphonia secunda* had quite the same arrangement of long and short shoots as was found in a specimen from the West Indies described in my above-quoted paper and of which a diagrammatic figure is given on p. 472, fig. 429 above. The only difference from the West Indian plant was that the specimen from Mauritius as a rule had 4 naked segments together, and not 3 as was the case in the West Indian plant.

The specimen was sterile.

Mauritius: Flic-en-Flac, Jan. 1939, R. E. V. no. 259.
Geogr. Distr.: Widely distributed in warm seas.

Subfam. 3. Lophotalieae.

Murrayella Schmitz.

1. **Murrayella periclados** (Ag.) Schmitz.

Schmitz, Fr., Die Gattung *Lophothalia* J. Ag., p. 227. Falkenberg, P., Rhodomelaceen, p. 563, pl. 12, figs. 24–25. Børgesen, Mar. Alg. D. W. I., vol. II, p. 314, figs. 318–320. Post, Erica, *Bostrychia-Caloglossa*-Assoziation, 1936, p. 29. — *Hutchinsia periclados* Ag., Spec. Alg., vol. II, p. 101. For more synonyms compare Post, l. c.

Fine fruiting specimens are found in Dr. Vaughan's collection. Now and then the stichidia have side-branches as in my Fig. 320 l. c. In some stichidia an often long monosiphonous filament is found at their upper ends.

The plant was growing on Mangrove roots.

Mauritius: Ilôt Brocus, Aug. 1938, R. E. V. no. 191.
Geogr. Distr.: Widespread in warm seas.

Bostrychia Montagne.

1. **Bostrychia Moritziana** (Sond.) J. Ag.

Agardh, J., Spec. Alg., II, 3, p. 862. Analecta algolog., cont. IV, 1897, p. 77. Post, E., *Bostrychia-Caloglossa*-Assoziation, 1936, p. 10; Weitere Daten z. Verbreit. d. Bostrychietum, III, 1939. — *Polysiphonia Moritziana*

Sonder in KÜTZING, Spec. Alg., p. 838. For further literature comp. DE-TONI, Syll. Alg., vol. IV, p. 1158.

This species is mentioned by Miss POST (1939, p. 15) as found in a collection of mangrove algæ from Mauritius sent to her by Dr. VAUGHAN.

Mauritius: "Small island near Mauritius," R. E. V.
Geogr. Distr.: Widely distributed in warm seas.

2. **Bostrychia tenella** (Vahl) J. Ag.

AGARDH, J., Spec. Alg., vol. II, p. III, p. 869. FALKENBERG, P., Rhodomelaceen, p. 515. BØRGESEN, F., Mar. Alg. D. W. I., p. 300, figs. 299–303. POST, E., *Bostrychia-Caloglossa*-Assoziation, 1936, p. 25; Weitere Daten zur Verbreitung des Bostrychietum III, 1939, p. 22. — *Fucus tenellus* Vahl, Endeel kryptog. Planter fra St. Croix, 1802, p. 45.

In Dr. VAUGHAN's collection is found a small gathering containing this species. The monosiphonous branchlets in this specimen are very long, often composed of more than 50 cells. The specimen was sterile.

It was growing on mangrove roots.

Mauritius: Ilôt Brocus, Aug. 1938, R. E. V. no. 193. Miss POST (1939, p. 23) mentions the locality: "Small island near Mauritius."
Geogr. Distr.: Widespread in warm seas.

Subfam. 4. Polyzonieae.

Leveillea Decsne.

1. **Leveillea jungermannioides** (Mart. et Her.) Harv.

HARVEY, W. H., Mar. Bot. West Austr., 1855, p. 539. FALKENBERG, Rhodomelaceen, p. 392, pl. 6, figs. 1–13; pl. 14, figs. 18–27. — *Amansia jungermannioides* Martens et Hering, in Flora 1836, p. 481, figs. 1–4. *Polyzonia jungermannioides* (M. et Her.) J. Ag., Symbolae, 1841, p. 25.

Several specimens of this small, elegant alga are found in the collections. The specimens met with crept on various algae, for instance *Turbinaria*, *Sargassum* etc. JADIN in his list, p. 169, calls it *Polyzonia jungermannioides*; he found it on *Dasya arbuscula* and *Udotea flabellata*.

A specimen gathered in January had tetrasporangia.

As to its habitat JADIN writes: "Dans des bassins recevant l'eau fortement aerée".

Mauritius: Flic-en-Flac, Jan. 1939, R. E. V. no. 259. Mahébourg, Sept. 1890, JADIN no. 263.

Geogr Distr.: Red Sea, Indian Ocean, Australia.

Subfam. 5. Amansieae.

Amansia Lamour.

1. Amansia glomerata Ag.

AGARDH, C., Systema Alg., 1824, p. 247. AGARDH, J.. Spec. Alg., II, 3, p. 1111. FALKENBERG, P., Rhodomelaceen, p. 416, tab. 1, figs. 20—21, tab. 6, 14—29. — *Delesseria rhodantha* Harv., Alg. Mauritius, 1834, p. 151, tab. 126. *Amansia fasciculata* Kütz., Tab. Phyc., vol. XV, tab. 4, fig. a—d.

Several specimens of this species, known from the island from earlier investigations, are found in the collections. A specimen collected by Dr. VAUGHAN in August has tetrasporangia.

Besides *Amansia glomerata* JADIN in his list, p. 168, also mentions *Amansia multifida*, known from the tropical Atlantic Ocean, as occurring at Mauritius. I have been able to examine a specimen of his (determined as *Amansia multifida*) which was collected by DARUTY in 1892. It must be admitted that this specimen has a somewhat deviating appearance from the typical form of *A. glomerata* but nevertheless it seems to me to be nothing but a form of this species. In this specimen the flat main branches are somewhat narrower but especially longer than those in the normal form, and the characteristic arrangement of the branches in rosettes is less marked. Furthermore the marginal endogenous branchlets, the length of which, according to FALKENBERG, 1901, p. 416, when they are most vigorously developed, does not surpass the breadth of the flat main shoots, are still more developed, the longest ones attaining a length of about 4—6 mm., while the flat main shoots are only about 3 mm. broad, rarely more. These long marginal branchlets are given out in groups only here and there, most of them remaining short. This somewhat differing shape of the specimen is most probably due to the less favourable conditions of life to which it has been exposed, for instance a more quiet locality or at a greater depth. The specimen has also a lighter colour which may suggest this. In the Botanical Museum, Copenhagen, there is a rather similar specimen from

the Hawaiian Islands. It has been determined as *Amansia glomerata* by REINBOLD who remarks about it: "junge langgestreckte Exemplar an *A. Dietrichiana* erinnernd".

About the habitat of this species JADIN writes: "Croit en touffes roses sur les récifs exposés aux lames fortes."

Mauritius: Flacq, June 1890, JADIN no. 259. Cannoniers Point, Oct. 1929, TH. M. Pointe aux Sables, Aug. 1939, R. E. V. no. 346.

Geogr. Distr.: Pacific and Indian Oceans.

Vidalia Lamouroux.

1. **Vidalia fimbriata** (R. Br. mscr.) J. Ag., Falkenb. emend.

FALKENBERG, P., Rhodomelaceen, p. 433. J. AGARDH, Spec. Alg., II, 3, 1863, p. 1124. — *Fucus fimbriatus* R. Br. in TURNER, Fuci, vol. III, 1811, p. 87, tab. 170. *Amansia Melvilli* J. Ag., Till Algernes System, 4. afd., VII, Florideae, 1884, p. 110. *Vidalia Melvilli* (J. Ag.) Schmitz, Mar. Florideen von Deutsch-Ostafrika, 1895, p. 159–160.

The material contains some few and poorly developed specimens. In his list, p. 168, JADIN calls this species *Vidalia obtusifolia*, using the name BORNET (1885, p. 19) gave some specimens of this species from Madagascar. But, as is pointed out later by SCHMITZ (l. c., p. 159) who has examined BORNET's plant from Madagascar, this is identical with specimens from Dar es Salaam and like these referable to *Vidalia Melvilli*. However, FALKENBERG according to later examinations embodied in his monograph on the *Rhodomelaceae*, after a comparison of the East African plant with the Australian *Vidalia fimbriata* arrived at the conclusion that in reality they all belong together, being referable to *Vidalia fimbriata*.

In the sparse and fragmentary material I have had for examination the specimens showed some variations. Thus a specimen in JADIN's collection (no. 267) on the flat ca. 4 cm. broad shoots has two rows of well-separated small adventitious branchlets in conformity with FALKENBERG's figure 19, pl. 7 of *Vidalia fimbriata* var. *neocaledonica*. And another specimen with a narrower thallus (scarcely 3 mm. broad) had also adventitious branches from the surface of the flat shoots, but these were more irregularly placed and more vigorously developed; this specimen was tetrasporic.

On the other hand, some small specimens in Dr. VAUGHAN's

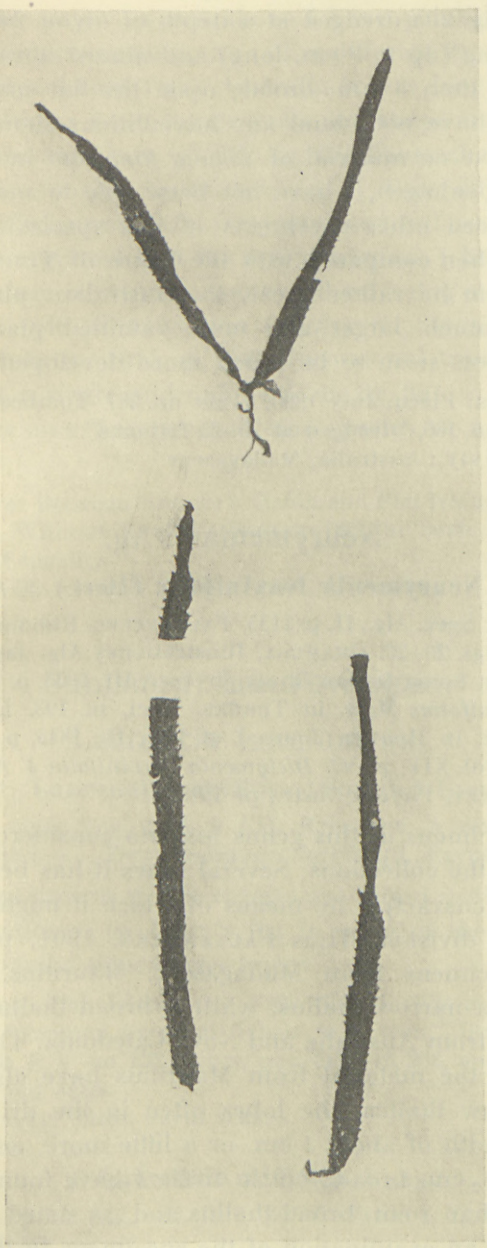

Fig. 20. *Vidalia fimbriata* (A. Br.) J. Ag.
Specimens with long, narrow and almost unramified thallus.
(× 1).

collection (Fig. 20) dredged at a depth of about 20—25 fathoms have very long (up to 8 cm. long) and almost unramified shoots a little more than 3 mm. broad; upon the flat surface of these specimens I have not found any adventitious shoots at all.

As we have no material of *Vidalia fimbriata* in the Botanical Museum, Copenhagen, I have not been able to make any comparison between other specimens of this species and those of Mauritius. When compared with the figure of TURNER the difference seems to be rather great, the Australian plant being for instance a much larger and more ramified plant, while the marginal shoots seem to be much more developed.

Mauritius: Flacq, July 1890, JADIN no. 267. Tombeau Bay, Dec. 8., 1932, R. E. V. no. 166, "dredged at 20—25 fathoms".

Geogr. Distr.: Australia, Madagascar.

Neurymenia J. Ag.

1. Neurymenia fraxinifolia (Mert.) J. Ag.

AGARDH, J., Spec. Alg., II, p. 1135. FALKENBERG, Rhodomelaceen, 1901, p. 444, tab. 7, figs. 20—29. OKAMURA, Illustrationes Alg. Jap., I, tab. XIII, 1901. BØRGESEN, Some Indian Rhodophyceae, III, 1933, p. 137, figs. 17—20. — *Fucus fraxinifolius* Mert. in TURNER, Fuci, pl. 193. *Epineuron fraxinifolium* Harv. in HOOKER Journal of Bot., IV, 1845, p. 532. KÜTZING, Tab. Phycol., vol. XIV, pl. 99. *Dictymenia fraxinifolia* J. Ag. in Linnæa, XV, p. 27. HARVEY, Phycol. Austr., pl. 124.

Some specimens of this genus hitherto considered monotypic are found in the collections. Several times it has been attempted to find some characters by means of which it might be possible to manage a division. Thus FALKENBERG, 1901, p. 444, points out that specimens from Madagascar, Mauritius, India, and Ceylon have a narrow thallus, while a broad thallus is peculiar to specimens from Australia and Nova Caledonia. The rather few specimens in the material from Mauritius have also a proportionally narrow thallus, the lobes often in the dried condition having a breadth of about 1 cm. or a little more, only some few lobes were $1^1/_2$ cm. broad; but in India I have found specimens with a more than 2 cm. broad thallus and, as stated in my paper (1933, p. 41), an examination of the specimens in the Kew Herbarium does not seem to speak in favour of a division based upon the dimensions of the thallus.

In the same paper quoted above I have pointed out that the shape of the stichidia seems to be rather different from the different localities; in specimens from Australia it was long and slender, while in specimens from South India the shape was broad and short; compare my figure p. 138, fig. 18. These characters might perhaps offer a possibility of division, if these differences hold good when sufficient material from various localities is examined.

It is to be regretted that the few specimens I have seen from Mauritius are not tetrasporic, most of them being sterile; a single one is cystocarpic. COTTON was the first to describe the cystocarps (Kew Bulletin, 1913, p. 254). In material from South India I have found a single androphore (l. c. fig. 20).

Dr. MORTENSEN has dredged this species at a depth of 25 fathoms.

Mauritius: Between Gunner's Quoin and Flat Island, 15. Oct., 1929, TH. M. no. 802. Without locality, DARUTY 1892 in herb. Jadin. R. E. W. no. 234 without locality.

Geogr. Distr.: Indian Ocean, Japan, Australia.

Subfam. 1. Laurencieae.

Laurencia Lamouroux.

1. Laurencia papillosa (Forssk.) Grev.

GREVILLE, Algae Brit., 1830, p. LII. BØRGESEN, A revision of Forsskåls Algae, 1932, p. 6. — *Fucus papillosus* Forssk., Fl. Ægypt. — Arab., p. 190.

Several specimens of this species are found in the collections. It is not mentioned in JADIN's list in spite of the fact that his collection contains typical specimens.

Mauritius: Flat Island, Oct. 1929, TH. M. Flic-en-Flac, Oct. 31., 1938, R. E. V., "common on coral debris in lagoon." Black River Bay, July 7., 1939, R. E. V. no. 282. JADIN, nos. 213, 216, 268, and 474, all without localities and dates.

Geogr. Distr.: Most warm seas.

2. Laurencia nidifica J. Ag.

AGARDH, J., Spec. Alg., vol. II, 3, p. 743. YAMADA, Y., Notes on *Laurencia*, 1931, p. 202.

JADIN's collection has two small specimens (nos. 79 and 112) from Réunion of a small elegant *Laurencia*; in his list, p. 168,

they are referred to *L. obtusa*. Very probably they are like HARVEY's *Laurencia obtusa*? var. *nana* (1834, p. 152) which DE-TONI in Sylloge Alg., vol. IV, p. 785, refers though with a note of

Fig. 21. *Laurencia nidifica* J. Ag. Specimens in natural size.

interrogation to *Laur. nidifica* J. Ag.; HARVEY's description also seems to agree fairly well with the specimens.

Unfortunately I have not been able to compare the specimens

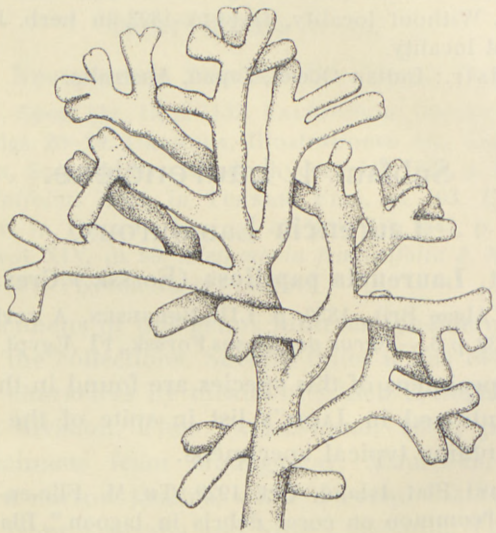

Fig. 22. *Laurencia nidifica* J. Ag. Fragment of the thallus. (× 8).

with original material but they seem to agree quite well with AGARDH's description.

As to the habit of the plant (Figs. 21 and 22) the thallus is terete, c. 600 μ thick, and irregularly ramified as the branchlets issue in all directions with a longer or shorter distance between them, sometimes several are given off at nearly the same height,

though without being opposite or verticillate. The branchlets are again provided with irregularly placed ramuli especially towards their tips.

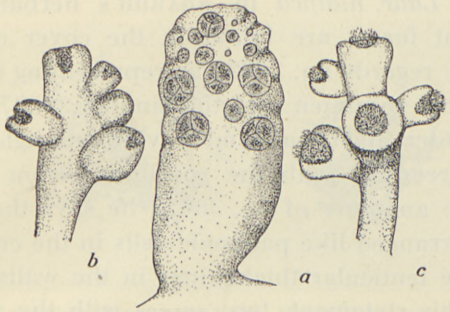

Fig. 23. *Laurencia nidifica* J. Ag.
Branchlets with tetrasporangia (*a*), cystocarps (*b*), and antheridial bodies (*c*). (× about 110).

In the tetrasporic plant the ramuli (Fig. 23*a*) are nearly cylindrical or taper a little towards their base, upwards with unevenly waved surface; in their upper half the tetrasporangia

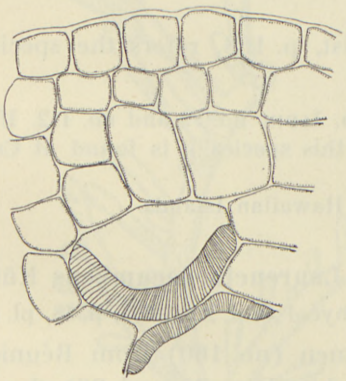

Fig. 24. *Laurencia nidifica* J. Ag.
Part of a transverse section of the thallus. (× about 225).

are developed. The latter are nearly globular with a diameter of about 100 μ or a little more.

The cystocarps (Fig. 23*b*) are developed in the upper half of short branchlets; they are urceolate or subcylindrical, tapering towards the base and summit.

The ramuli carrying the antheridial bodies (Fig. 23*c*) are

topshaped, like the cystocarps they issue several together from the upper half of short branchlets.

According to YAMADA (l. c., p. 202), who has examined the specimens of *Laur. nidifica* in AGARDH's herbarium in Lund, rather different forms are found in the cover comprising this species, but he regards no. 36628 as representing the type. About the habit of this specimen and the similar 36627 he writes that they are "slender and weak and having entangled bases"; this is in good agreement with the specimens from Réunion. And concerning the anatomy of no. 36628 he says that "the surface cells are not arranged like palissade cells in the cross-section and there are some lenticular thickenings in the walls of the medullary cells". This statement, too, agrees with the anatomy of the specimens from Réunion (Fig. 24). The peripheric cells in these specimens are almost isodiametric, c. 30 μ in diameter; the medulla consists of roundish not very large cells, some few in the middle of the thallus are provided with thickenings of the wall.

Apparently allied to this species is *Laurencia elegans* Lucas, 1935, p. 222, but this plant has no thickenings in the medullary layer.

JADIN in his list, p. 168, refers the specimens to *Laurencia obtusa*.

Réunion: Herb. JADIN no. 79 and no. 112. If HARVEY's *Laurencia obtusa* var. *nana* is this species it is found at Cap Malheureux, Mauritius.

Geogr. Distr.: Hawaiian Islands.

3. **Laurencia decumbens** Kütz.?

KÜTZING, Tab. Phycol., vol. XV, 1865, p. 18, pl. 51.

A small specimen (no. 160) from Réunion in JADIN's collection as to its habit (Figs. 25 and 26) shows much similarity to KÜTZING's above-quoted figure drawn from a plant collected in New Caledonia. YAMADA (1931, p. 195) has examined the unique specimen in KÜTZING's herbarium and points out that it has quite another anatomical structure than that of *Laurencia perforata* (Bory.) Mont. to which species J. AGARDH in Epicrisis, p. 649, believed it was related because of its very similar habit. While *Laur. perforata* has palissade-like epidermal cells KÜTZING's specimen has not such cells. But concerning the specific

value of KÜTZING's plant YAMADA adds: "But because KÜTZING's species is represented by only one sterile specimen, it is very difficult to obtain an exact idea of it."

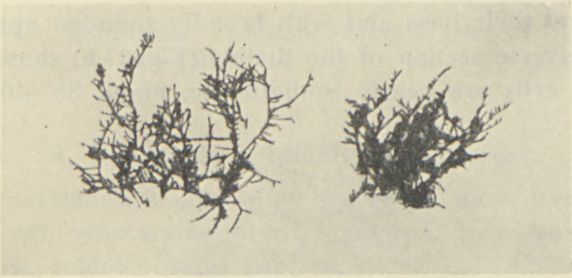

Fig. 25. *Laurencia decumbens* Kütz. Habit of the specimens. (× 1).

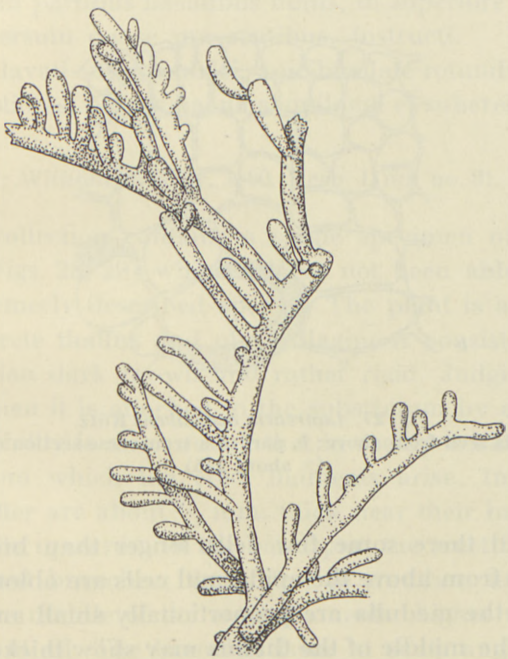

Fig. 26. *Laurencia decumbens* Kütz. Fragment of the thallus. (× about 6).

The specimen from Réunion forms small tufts about 2 cm. high, the thallus attaining a breadth of about $^1/_2$ mm. only. A characteristic feature of the plant is that the main branches are often much curved, and that the branchlets and ramuli issuing

from them are unilaterally placed upon their upper convex side. The branchlets are of variable size, shorter or longer; the shorter ones are most probably later developed, adventitious ones. The branchlets are upto about 2—3 mm. long and 400 μ broad, narrowed at their base and with broadly rounded apices.

A transverse section of the thallus (Fig. 27 *b*) shows that the epidermal cells are nearly isodiametric, about 35—40 μ broad,

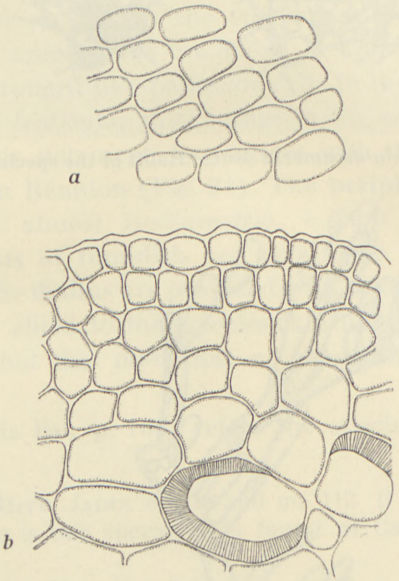

Fig. 27. *Laurencia decumbens* Kütz.
a, surface cells seen from above; *b*, part of a transverse section of the thallus. (× about 225).

but here and there some few cells longer than broad may be found. Seen from above the epidermal cells are oblong (Fig.27 *a*). The cells of the medulla are proportionally small and some few of these in the middle of the thallus may show thickenings of the wall (Fig. 27 *b*). YAMADA does not mention if such are present in KÜTZING's specimen.

That the specimen from Réunion, which like that of KÜTZING is sterile, shows a great similarity to that of KÜTZING, cannot be denied; but to arrive at an exact conclusion a comparison with KÜTZING's specimen is necessary. Since this is out of the question

at present and because of the distant locality of KÜTZING's plant I have placed a ? after the specific name.

JADIN in his list, p. 168, refers this plant to *Laur. perforata* (Bory) Mont.

Réunion: Saint-Gilles, Apr, 1890, JADIN no. 160.
Geogr. Distr.: New-Caledonia.

4. **Laurencia columellaris** nov. spec.

Frons caespitosa, usque ad 10 cm. alta et ultra, teres, in parte basali $^3/_4$ cm. crassa, irregulariter ramosa, in sicco subrigida, cartilaginea, colore obscure-rubro.

Rami subpauci, erecti, angulis acutis emissi, irregulariter egredientes, in partibus basalibus nudis, in superiore parte ramulis, quoqueversum dense praesentibus, instructi.

Ramuli clavati-subcylindrici, apicibus late rotundis, ad basem leniter angustiores, omnes paene aequilongi et suberecti, ca. $1^1/_2$—2 mm. longi.

Réunion: Without locality, 1890, Herb. JADIN no. 91.

JADIN's collection contains a single specimen of an elegant *Laurencia* (Figs. 28, 29) which I have not been able to identify with any formerly described species. The plant is about 10 cm. high with terete thallus and of cartilaginous consistency, in the dried condition dark brown and rather rigid. Judging from the single specimen it is attached to the substratum by a small disc, but the possibility is also present that in reality it has decumbent filaments from which the erect filaments arise. In dried condition the latter are about $^3/_4$ mm. thick near their base, tapering only very little upwards. In the basal part the erect filaments are naked, without branches; these, few in number, are given out from near the middle of the erect filaments; higher up there are few or none at all. The branches issue at acute angles being all straight and directed upwards. They are naked in their basal part, higher up ramuli issue in all directions. The ramuli are $1^1/_2$ to 2 mm. long, obliquely upward-directed, subclavate of shape, increasing slowly from the narrow base to the obtuse rounded apex.

The specimen is sterile.

In a transverse section of the thallus (Fig. 30) it is seen that the surface cells are mostly about quadratic, having a breadth of about 15—20 μ; some of them are, however, broader, others

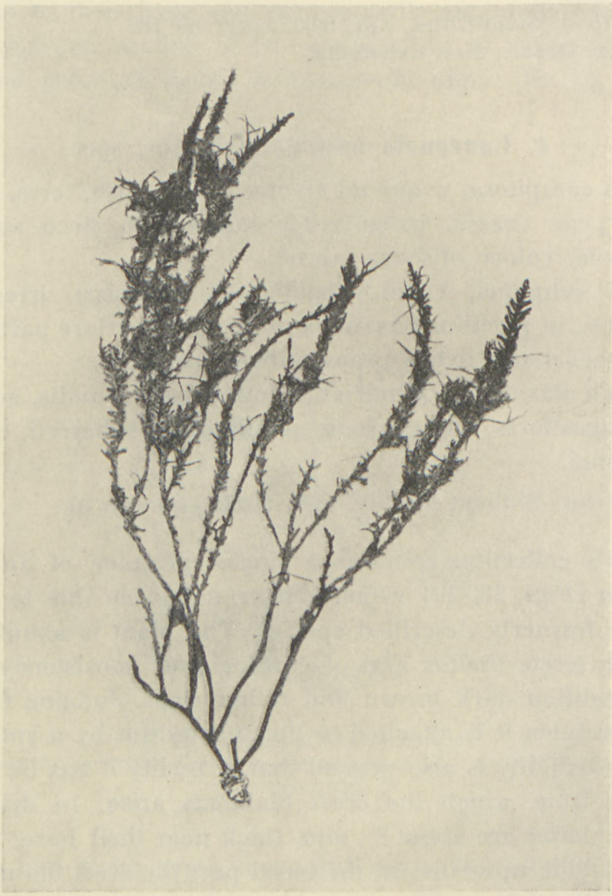

Fig. 28. *Laurencia columellaris* Børgs. Habit of the original specimen. (× 1).

narrower than these. The cells of the medulla are small, the largest have a breadth of about 40—50 μ. Their walls are proportionally thick, but any local thickenings of the wall are not met with. When the surface cells are viewed from above they are roundish and of variable size.

As to the arrangement of the ramuli this species may show some similarity to *Laurencia tropica* Yamada (1931, p. 233, fig. Q)

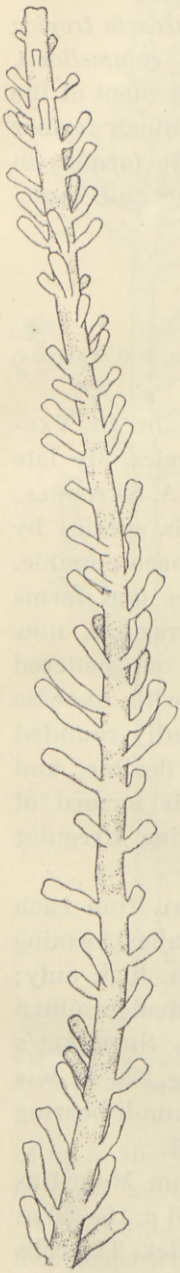

Fig. 29. *Laurencia columellaris* Børgs.
The upper end of an erect main filament with branchlets.
(× about 6).

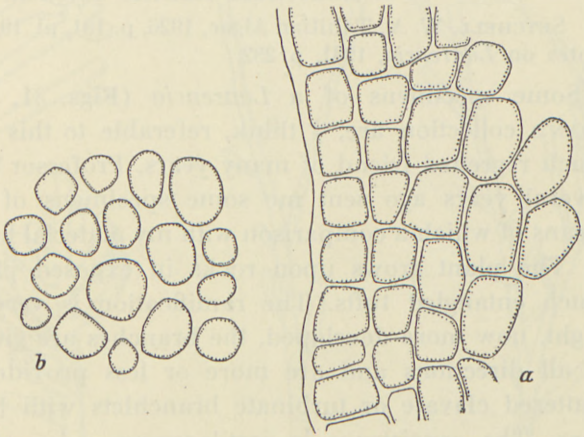

Fig. 30. *Laurencia columellaris* Børgs.
a, part of a transverse section of the thallus. *b*, surface cells seen from above. (× about 320).

but a more detailed comparison will soon show that essential differences are present. Thus the ramification of *Laurencia tropica* is very irregular and quite different from that of *L. columellaris*, and even if there is some resemblance in the arrangement of the ramuli, these are more densely placed and occur much farther down the filaments of the latter species than in the former, in which also the ramuli are of more variable shape and sometimes divided.

5. **Laurencia flexilis** Setchell.

Setchell, W. A., Tahitian Algae, 1926, p. 101, pl. 19, figs. 1–6. Yamada, Notes on *Laurencia*, 1931, p. 232.

Some specimens of a *Laurencia* (Figs. 31, 32) in Dr. Vaughan's collection are, I think, referable to this species. My late much regretted friend of many years, Professor W. A. Setchell, several years ago sent me some specimens of this species by means of which a comparison with my material has been possible.

The plant grows upon rocks in exposed places and forms much entangled tufts. The ramification is very irregular, now slight, now more developed, the branches are given out scattered in all directions and are more or less provided with likewise scattered clavate or turbinate branchlets with broadly rounded apex. The consistence is cartilagenous, wiry and flexible, and the colour is dark-red. The base of the thallus is formed of decumbent branches which gradually fuse, forming irregular discs or rather clumps.

The specimens from Mauritius differ somewhat from each other. Two of them (nos. 255 and 261, fig. 31) are smaller, forming tufts intermingled with *Corallinaceae* about 4—5 cm. high only; the erect filaments in these collections are a good deal ramified near their summits, showing much similarity to Setchell's figures 1 and 4. The other gathering (no. 348, fig. 32) is less ramified with often quite few and remotely placed ramuli bearing a greater resemblance to Setchell's figures 2, 3, 5.

As to the anatomical structure of the plant from Mauritius (Fig. 33), the surface cells are about as long as broad c. 15—20 μ; the peripheric walls and the walls on the whole are thick. The cells of the medulla are proportionally small, rarely attaining a diameter

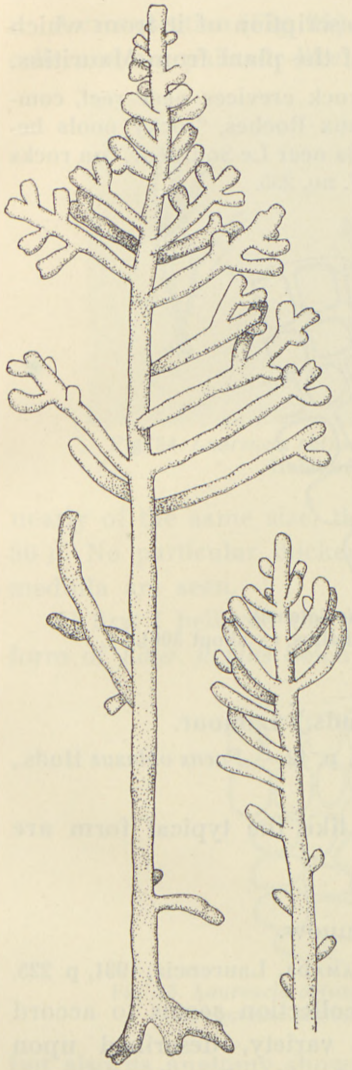

Fig. 31. *Laurencia flexilis* Setchell. Parts of the thallus (no. 261). (×8).

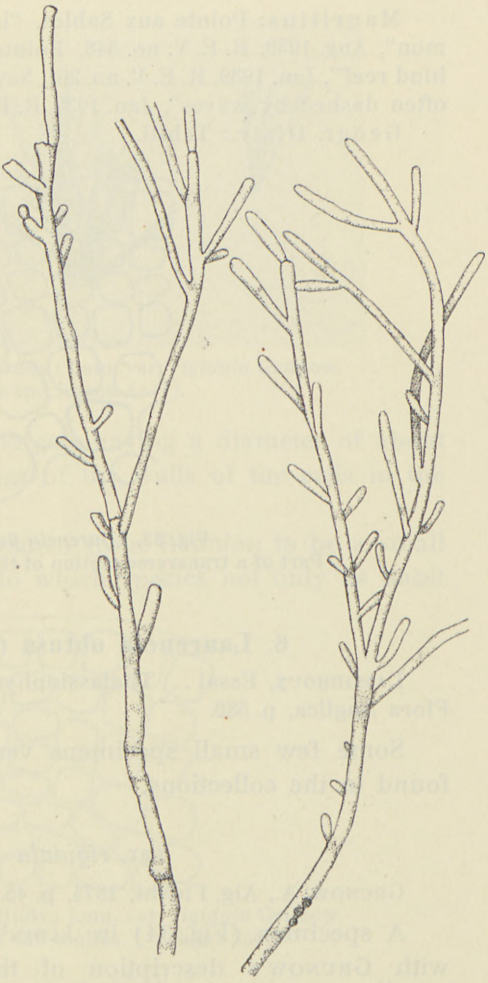

Fig. 32. *Laurencia flexilis* Setchell. Parts of erect filaments (no. 348). (× about 3).

of up to 50—60 μ; they have thick walls but no special thickenings of these have been found.

Setchell does not himself give any description of the anatomical structure of the plant, but Yamada (1931, p. 232) having examined the type specimen in the herbarium of the University

of California has published a short description of it from which it is evident that it is quite like that of the plant from Mauritius.

Mauritius: Pointe aux Sables, "in rock crevices near reef, common", Aug. 1939, R. E. V. no. 348. Pointe aux Roches, "rocky pools behind reef", Jan. 1939, R. E. V. no. 261. Savinia near Le Souffleur, "on rocks often dashed by waves", Jan. 1939, R. E. V. no. 255.

Geogr. Distr.: Tahiti.

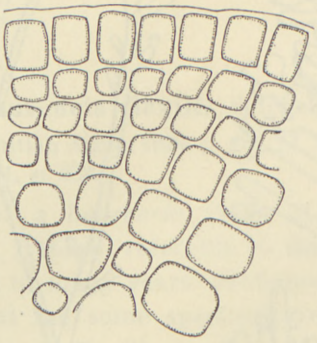

Fig. 33. *Laurencia flexilis* Setchell.
Part of a transverse section of the thallus. (× about 300).

6. **Laurencia obtusa** (Huds.) Lamour.

Lamouroux, Essai ... Thalassiophytes, p. 42. — *Fucus obtusus* Huds., Flora Anglica, p. 586.

Some few small specimens very like the typical form are found in the collections.

var. *rigidula* Grunow.

Grunow, A., Alg. Fidshi, 1874, p. 45. Yamada, Laurencia, 1931, p. 225.

A specimen (Fig. 34) in Jadin's collection seems to accord with Grunow's description of this variety, described upon specimens from the Samoa Islands. As is pointed out by Grunow, this variety looks rather like Kützing's figure of *Laurencia corymbifera* Kütz. in Tab. Phycol., vol. 15, pl. 56, from the West Indies, but like the plant from the Pacific Ocean the specimen from Réunion is more robust and the branchlets more broadly clavate. The thallus is rigid and dark-red and forms 2—3 cm. high dense tufts of erect filaments emerging from the basal disc-like filaments.

A cross section (Fig. 35) of the thallus shows that the epidermal cells are not palisade-like; those of the medulla are small, all

Fig. 34. *Laurencia obtusa* (Huds.) Lam. var. *rigidula* Grunow. Habit of the specimens. (× 1).

nearly of the same size, the largest having a diameter of about 50 μ. No particular thickenings of the walls of the cells in the medulla are seen.

At first I believed the specimen from Réunion to be a small form of *Laur. flexilis* Setch., to which species not only its habit

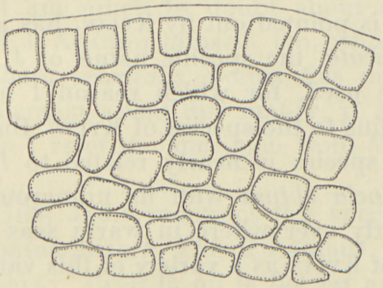

Fig. 35. *Laurencia obtusa* (Huds.) Lam. var. *rigidula* Grunow. Transverse section of the thallus. (× about 250).

but also its anatomy shows some similarity, but the ramuli are broader upwards and in this respect agree with *Laur. obtusa*. JADIN in his list, p. 168, calls it *Laurencia corymbifera* Kütz. and writes about its habitat: "Abondant sur les récifs et sur les rochers où la lame frappe violemment".

var. *natalensis* (Kylin) Børgs.

Laurencia natalensis Kylin, Rhodophyceen von Südafrika, 1938, p. 24, pl. 8, fig. 21.

Dr. MORTENSEN has collected some specimens of a small *Laurencia* which seem to agree quite well with KYLIN's description and figures of this plant originating from Durban and Port Elisabeth in South Africa.

The specimens from Mauritius are about 3—5 cm. high, of a rather soft consistency adhering strongly to the paper. They have an irregular raceme-like ramification; a main axis is as a rule rather clearly discernible.

The specimens being gathered in October, have tetrasporangia as well as cystocarps and androphores.

A transverse section shows that the surface cells are not palisade-like and no thickenings of the wall of the cells in the medulla are present.

At first I had named these specimens *Laurencia obtusa*, var. *divaricata* (J. Ag.) Yamada, Notes on Laurencia, 1931, p. 223, the *Laurencia divaricata* J. Ag. being described upon specimens from the Red Sea. According to KYLIN, who has been able to examine the specimens of *Laurencia divaricata* in the herbarium of J. AGARDH in Lund, his *Laurencia natalensis* differs only by its smaller size from AGARDH's specimens; but since SUHR in the year 1840, as is pointed out by KYLIN, has already described a *Laurencia divaricata*, the specific name of J. AGARDH for this species cannot be used, for which reason I have referred the plant from Mauritius to the species of KYLIN. But KYLIN does not conceal that this species is nearly related to *Laur. obtusa*, and seeing that *Laurencia obtusa* with its numerous varieties so to speak occurs nearly everywhere in warm seas I prefer to consider the species of KYLIN as a variety of this variable and widely distributed species.

Mauritius: Forms near the typical form: Flacq, July 1890, JADIN no. 256. Ilôt Brocus "in Reef pools", Aug. 1938, R. E. V. no. 198. Var. *natalensis* (Kylin) Børgs.: Flat Island, Oct. 16., 1929, TH. M.

Réunion: Var. *rigidula* Grunow: Saint-Gilles, 1890, JADIN no. 133.

Geogr. Distr.: Widely distributed in warm seas.

Subfam. 2. Chondrieae.

Acanthophora Lamour.

1. Acanthophora spicifera (Vahl) Børgs.

Børgesen, West Indian Florideae, II, 1610, p. 201, figs. 18–19. Mar. Alg. D. W. I., vol. II, 1915–20, p. 259, figs. 253–58. — *Fucus spiciferus* Vahl, Endeel kryptog. Planter fra St. Croix, 1802, p. 44. *Acanthophora Thierii* Lamour., Essai sur les genres . . . Thalassioph. non artic., 1813, p. 44. For further literature compare my above-quoted papers.

Several specimens are found in the collections but all are sterile except a tetrasporic specimen collected in December by Dr. Vaughan.

The stichidia are like my figures quoted above of specimens from the West Indies, especially those shown in Fig. 257 *C*, in which the sporangia are found in the upper cupola-like, bare apex of the branchlets, the spines first appearing below this fertile part; according to J. Agardh, Spec. Alg., II, p. 816, this feature should be characteristic of *Acanthophora orientalis* J. Ag.; compare also his definition of this species. But since I have found the stichidial branchlets to be of rather variable shape in West Indian material I am much in doubt about the distinction of these species, and J. Agardh himself, too, when describing (l. c. p. 821) *Acanthophora orientalis*, points this out; compare also Falkenberg's statement about this matter, p. 231.

For this reason I refer the specimen from Mauritius to *Acanthophora spicifera*.

In his list, p. 168, Jadin mentions this species using the formerly employed name *Acanth. Thierii* Lamour. About the habitat at the island he says: "Croit là où le flot est assez violent, mais pas sur les récifs, ni aux endroits où la vague est très forte".

Mauritius: Flacq, June 1890, Jadin no. 201. Port-Louis, Aug. 1890, Jadin no. 370. Grand Bay, Oct. 25., 1929, Th. M. Flic-en-Flacq, Dec. 31., 1938, R. E. V. no. 247.

Geogr. Distr.: Widely distributed in tropical seas.

Chondria Harv.

Subgenus 1. ***Euchondria*** *Falkenb.*

1. **Chondria tenuissima** (G. et W.) Ag.

Agardh, C., Spec. Alg., p. 352; Systema Alg., p. 205. Thuret et Bornet, Études phycolog., p. 88, tab. 43—48. Falkenberg, P., Rhodomelaceen, p. 195. — *Fucus tenuissimus* Good. et Woodw. in Transact. Linnean Soc., vol. III, 1797, p. 215, tab. 19. For further literature compare De-Toni, Sylloge, vol. IV, p. 834.

A well prepared female specimen of this species is found in Jadin's collection.

Mauritius: Without locality, collected by Daruty 1892.

Geogr. Distr.: Warmer parts of the Atlantic coasts of Europe and America, Mediterranean Sea, Malayan Archipelago, Japan, Australia.

Subgenus 2. ***Coelochondria*** *Falkenb.*

2. **Chondria dasyphylla** (Woodw.) Ag.

Agardh, C., Spec. Alg., p. 350; Systema Alg., p. 205. Falkenberg, P., Rhodomelaceen, p. 197, pl. 22, figs. 4—18. — *Fucus dasyphyllus* Woodw. in Transact. Linnean Soc., vol. II, 1794, p. 239, pl. 23, figs. 1—3. *Chondriopsis dasyphylla* J. Ag., Spec. Alg., II, p. 809. For further literature compare De-Toni, Sylloge, vol. IV, p. 834.

Upon pieces of a sea-grass several small fertile specimens were found intermingled with *Polysiphonia mollis* Hook. fil. et Harv.

Tetrasporic as well as female and male specimens were met with.

Mauritius: Cannoniers Point, Oct. 26., 1929, Th. M. Barkly Island, Aug. 1939, R. E. V. no. 330.

Geogr. Distr.: Warmer parts of the Atlantic coasts of Europe and America, Mediterranean Sea, Indian Ocean.

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Index of Species

with some few synonyms, the latter printed in italics.

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DET KGL. DANSKE VIDENSKABERNES SELSKAB
BIOLOGISKE MEDDELELSER, BIND XIX, NR. 11

FACULTATIVE PARTHENOGENESIS AND HAPLOIDY IN *EPIPACTIS LATIFOLIA*

BY

O. HAGERUP

KØBENHAVN
I KOMMISSION HOS EJNAR MUNKSGAARD
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1. Introduction.

In *Orchis maculatus* it is a normal occurrence that some few ovula are not fertilised but develop haploid embryos. In addition it may happen that a few female nuclei in the same ovary are fertilised by more than one male nucleus, and in this way polyploid embryos may arise beside the normal diploid ones (HAGERUP, 1944).

It would be of interest to ascertain whether these changes in the degree of polyploidy are peculiar to this one observed species, or whether we are here concerned with universally valid laws. This can only be decided by investigations on many other species. The present notice must be regarded as part of such a series of studies which it is to be hoped can in due course be continued by investigations on other plants.

The orchids are particularly well suited for studies on fertilisation, both because they are very easy to fix and stain, and also because their ovaries contain a greater number of ovula than most other plants. To this must be added that the formation of species is probably livelier within the orchids than in any other family, for which reason we may here expect to find species well suited for researches on the significance of polyploidy for the formation of species in nature.

Next to *Orchis maculatus* among our native orchids *Epipactis latifolius* is the orchid which is most polymorphous, for there are great variations both in the form, colour, and pubescence of the floral and the vegetative organs. But notably there are striking differences in the size of the individuals, both giant and dwarf forms occurring under partly ecologically different conditions. The individuals employed for this investigation all belonged to a medium type; unfortunately I had no opportunity of observing

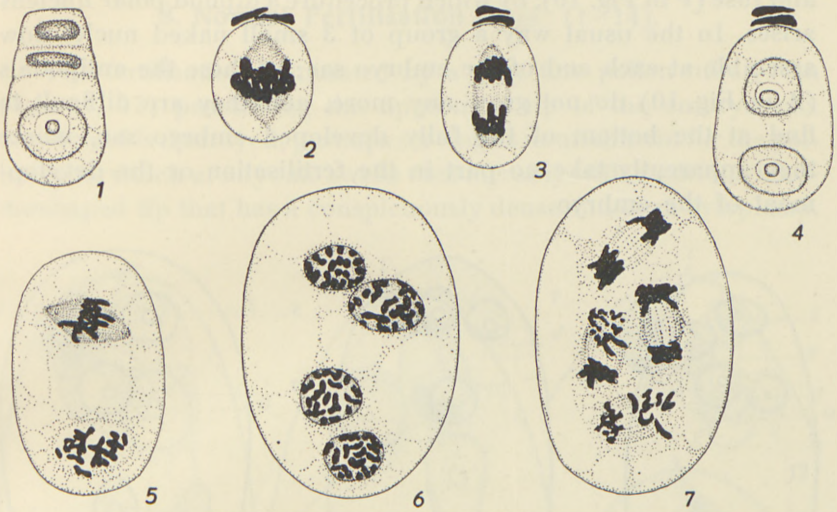
extremes. But such individuals would be particularly valuable and ought to be studied.

All my material has been collected in nature, partly on Funen and partly in the environs of Copenhagen (Kongelunden). Meiosis is found in the anthers in the middle of July and the fertilisation takes place only a couple of days after the pollination in the first half of August, when the ovary is 3—4 mm. thick. The ovaries must be pruned as thoroughly as possible and treated for a short time beforehand with CARNOY'S liquid, so that the ovula die quickly. Then the actual fixing is done according to LEVITZKY, by which method remarkably good results are obtained when the specimens are left for several days in the fluid, which should be renewed a couple of times.

Haematoxylin is not quite good for this object. But the sections should be treated for half an hour in normal hydrochloric acid heated to 60°, and should then be stained according to FEULGEN. After this they are stained $1^1/_2$—2 minutes in "Lichtgrün". In this way a result approaching the ideal can be obtained, which makes both *Orchis maculatus*, *Epipactis*, and especially *Listera* perhaps the best suited pedagogic specimens for embryological studies (EFTIMIU-HEIM). The fertilisation itself, so difficult to observe in most other plants can also very easily be followed with the above-mentioned technique; the male nuclei are large and stain such a vivid deep red that they are at once seen already under low enlargement, because they often lie in the green cytoplasm of the pollen tube.

The embryo sacs are very large and the sections should therefore be thick (25—35 μ). So as to be able also to investigate interesting exceptional cases I examined a very large quantity of material and studied about 35.000 embryo sacs containing male nuclei, so presumably my investigations are based on a fairly reliable numerical material.

As an object of study the plant, however, suffers from an essential defect, the chromosomes being difficult to count even if they are well fixed. This is partly because they are remarkably long and provided with constrictions, partly because often the large chromosomes do not lie in the same plane but curve irregularly. In spite of this it is, however, as a rule fairly easy to see whether a less good mitosis is diploid or haploid.



Figs. 1—7. First stages in the development of the embryo sac. Figs. 1—4 above show the dark remnants of 2 degenerated macrospores. × 700.

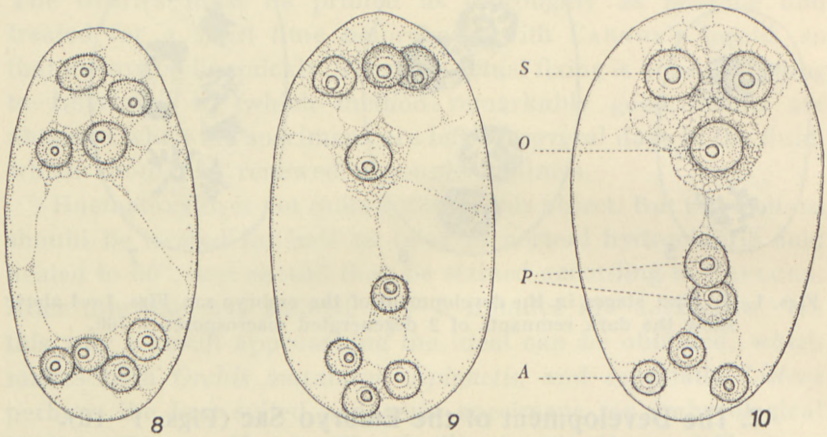
2. The Development of the Embryo Sac (Figs. 1—10).

VERMOESEN (1911) as well as BROWN and SHARP (1911) have already described the development of the embryo sac and found almost the same as I have. It will therefore suffice here to give a brief account supported by the subjoined figures.

Nearly always three macrospores only are formed (Fig. 1), of which the inner one develops into an embryo sac. This mother cell gradually increases considerably in size and soon supplants the other two macrospores, which can long be seen above the embryo sac as two compressed dark remnants (Figs. 2—4).

When the mother cell of the embryo sac has attained a certain size its nucleus will divide in two in the usual way (Figs. 2—4), though no cell wall is formed between them. The two daughter nuclei soon continue the division (Fig. 5), young embryo sacs being thus produced first with 4 (Fig. 6), later with 8 nuclei (Figs. 7—8). Originally there is a group with 4 nuclei at each end of the young embryo sac (Fig. 8), but from each of these two groups a nucleus soon wanders towards the centre of the embryo sac (Fig. 9). These two nuclei draw near each other

and fuse (P in Fig. 10), by which procedure a diploid polar nucleus arises. In the usual way a group of 3 small naked nuclei now assemble at each end of the embryo sac; of these the antipodals (*A* in Fig. 10) do not grow any more, and they are difficult to find at the bottom of the fully developed embryo sac, where they apparently take no part in the fertilisation or the development of the embryo.

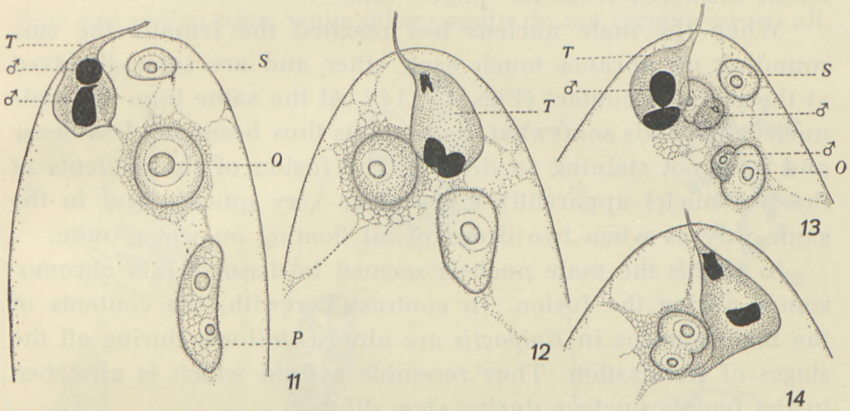


Figs. 8—10. Young embryo sacs with 8 nuclei, which are taking their final positions. *S*, synergid. *O*, egg. *P*, polar nucleus. *A*, antipodal. × 700.

When the embryo sac is fully developed so that it can be fertilised it is often fairly easy to identify the different nuclei by means of their size, colour, and position. The synergids (*S* in Figs. 10, 11, 13) are both the smallest nuclei at the upper end of the embryo sac, they are almost globular and slightly stained. A little below these lies the female nucleus itself (*O* in Figs. 10, 11, 13) which is comparatively large and also stains relatively faintly. It is surrounded by plenty of cytoplasm but has no wall. The polar nucleus (*P* in Figs. 10, 11), on the other hand, is comparatively dark, often somewhat flat and situated below the egg cell and not exactly in the middle of the embryo sac. In contrast with the other nuclei it contains 2—3 nucleoli. It can be decided whether the female nucleus is fertilised by ascertaining how many nucleoli it contains, since every nucleus brings with it its nucleolus; thus the fertilised female nucleus will contain 2 nucleoli.

3. Normal Fertilisation (Figs. 11—14).

The fertilisation is initiated by a very fine pollen tube (*T* in Figs. 11, 13) penetrating the uppermost tip of the embryo sac. A synergid is destroyed, perhaps serving as nourishment for the pollen tube, which at any rate swells considerably with a curious bladdershaped tip that has a conspicuously dense and dark cytoplasm.



Figs. 11—14. Early stages of normal fertilisation. *S*, synergid. *O*, egg. *P*, polar nucleus. *T*, pollen tube. ♂, male nucleus. × 700. See also text.

Often the vegetative nucleus of the pollen tube does not enter the embryo sac but remains outside the ovula in the narrow part of the pollen tube. Not rarely, however, the said nucleus is found in the embryo sac with the male nuclei (♂ in Figs. 11, 13) which may give rise to confusion, since the vegetative nucleus also stains very darkly. But it is often recognisable by the difference in size (either larger or smaller); and in addition it is angular, showing that it is decaying and out of function.

The tip of the pollen tube immediately grows towards the female nucleus and soon gets into contact with the cytoplasm of the latter. The male nuclei were long and narrow when they were in the pollen tube, where there was very little room. But as soon as they enter the embryo sac, where there is plenty of room, they at once change their form, become short, discoid, and usually furnished with 1—3 angles. As soon as the pollen tube has approached the female nucleus, the male nuclei pass out through small openings in the side or tip of the pollen tube

in a place lying as near as possible to the female nucleus, in the cytoplasm of which they are soon to be found.

The conveyance of the male nuclei probably takes place by means of currents in the cytoplasm which is extended in long strands between the various parts of the embryo sac. It is remarkable that the two male nuclei nearly always reach the two nuclei to be fertilised at the same time, though these are found at different distances from the pollen tube.

When the male nucleus has reached the female, the surrounding membranes touch each other and are soon dissolved at the place of contact (Figs. 13, 14). At the same time the male nucleus expands somewhat, its contents thus becoming less dense and now not staining so deeply. The fusion of the contents of the two nuclei apparently takes place very quickly and in the same way as when two drops of oil floating on water unite.

In *Orchis* the male nucleus seemed to dissolve into chromosomes during the fusion. In contrast herewith, the contents of the male nucleus in *Epipactis* are almost uniform during all the stages of fertilisation. They resemble a fluid which is absorbed in the female nucleus during slow dilution.

In the more advanced stages of the fusion the male nucleus is placed on the outside of the female as a slight hump, the contour of which is gradually smoothed out, until it is quite flush with the membrane of the egg cell. The respective nucleoli of the nuclei do not fuse at this stage, but later.

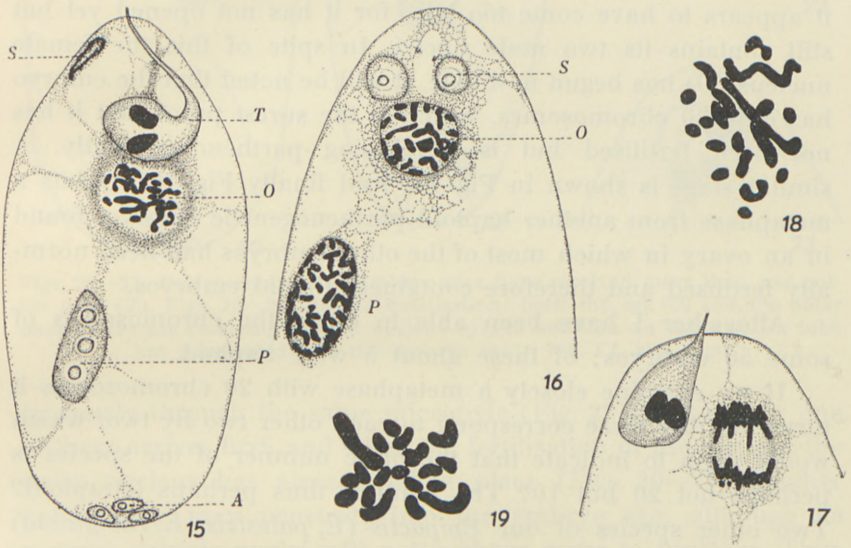
When the female nucleus has been fertilised, there is still a live male nucleus in the embryo sac, and the later fate of this may vary a great deal. In some cases it will not function, as was also the case in *Orchis*.

But in other cases (Fig. 13) this second male nucleus will quickly reach the polar nucleus with which it fuses similarly as in the fertilisation of the female nucleus. In that case the polar nucleus will soon begin to divide, and it is common to find an endosperm consisting of either 2, 4 or 6 but very rarely of more free cells. In most other orchids the endosperm has not so many cells, and most frequently it is entirely absent, thus for instance in *Orchis* and *Listera*.

4. Facultative Parthenogenesis and haploid Embryos

(Figs. 15—19).

At pollination an ovary as a rule receives an enormous amount of pollen. What causes the equal distribution of the pollen tubes among the ovula is not known, but at any rate this task is not always discharged without error. For many ovula receive more than one pollen tube, while others again do not receive any at all.



Figs. 15—19. Facultative parthenogenesis. Eggs (*O*) are developed without fertilisation and are haploid. Figs. 15 and 17, the embryo sac has received a pollen tube (*T*) after the embryo (*O*) has begun to develop. Fig. 16, the embryo sac has not received any pollen tube, and yet egg and polar nucleus are developing. × 700. Figs. 18—19. Metaphases with $2n = 20$ in the first division of the egg. × 1500. Cf. text.

As is well known, the pollen tubes have a double function; not only do they accomplish the fertilisation but they also excrete a substance which makes the ovary grow. This impulse to growth affects not only the wall of the ovary and the fertilised ovula, but all parts of the ovary begin to grow and this also applies to the ovula which have not received any pollen tube and thus have not been fertilised. Such an embryo sac is shown in Fig. 16. Above it is seen that both the synergids (*S*) are intact, which is a sure sign that no pollen tube has penetrated into the embryo

sac. Directly below the synergids lies the female nucleus (*O*) which nevertheless has begun to divide. It is in prophase and contains 20 chromosomes, which is the haploid number of the species.

Below in Fig. 16 is seen the polar nucleus (*P*) which also develops without fertilisation, and therefore only contains 40 chromosomes and is diploid.

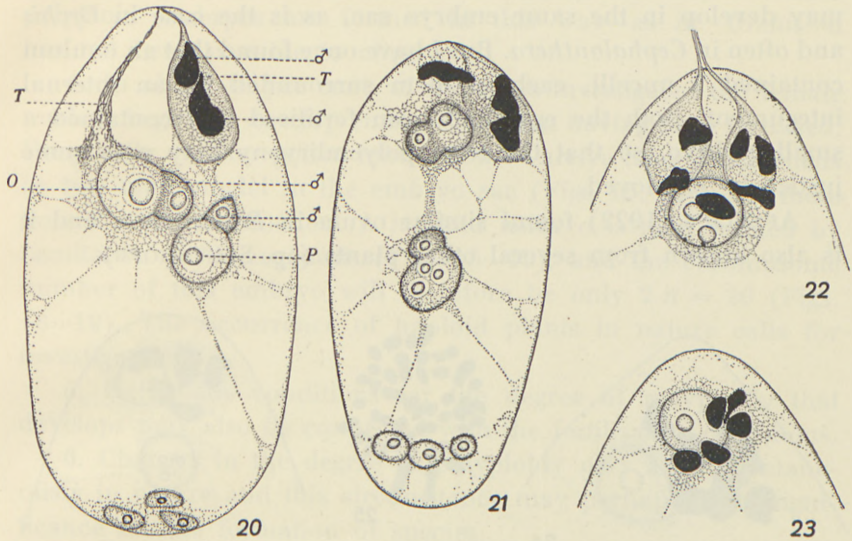
Fig. 15 shows another case of facultative parthenogenesis. It is true that the embryo sac has received a pollen tube (*T*); but it appears to have come too late, for it has not opened yet but still contains its two male nuclei. In spite of this the female nucleus (*O*) has begun to divide, it will be noted that the embryo has only 20 chromosomes, which is the surest proof that it has not been fertilised but is developing parthenogenetically. A similar stage is shown in Fig. 17. And finally Fig. 19 exhibits a metaphase from another haploid parthenogenetic embryo, found in an ovary in which most of the other embryos had been normally fertilised and therefore contained diploid embryos.

Altogether I have been able to count the chromosomes of some 50 embryos; of these about 5 were haploid.

If we examine closely a metaphase with 20 chromosomes it turns out that these correspond to each other two by two, which would seem to indicate that the basic number of the species is perhaps not 20 but 10? The plant is thus perhaps tetraploid? Two other species of our *Epipactis* (*E. palustris*, *E. rubiginosa*) have also $n = 20$. It would be of value to know the chromosome number of the slender *E. microphylla* as well as the more robust *E. violacea* from more southerly latitudes; it is possible that these might have other degrees of polyploidy?

5. Polyspermaty (Figs. 20—26).

The distribution of the pollen tubes among the ovules is also irregular seeing that more than one pollen tube can penetrate into an ovulum. This is so frequent that even about half of the ovula of an ovary can be "superfertilised" in this way. Quite frequently 3 pollen tubes penetrate into an ovulum, and once I have even found four. 2 pollen tubes may very well pass simul-



Figs. 20—23. Polyspermaty. The embryo sacs have received more than one pollen tube (*T*). Figs. 20—21. Double fertilisation: both the egg (*O*) and the polar nucleus (*P*) are receiving a male nucleus (♂); in addition an extra pollen tube has penetrated into the embryo sac. × 700. Cf. also text.

taneously through the same micropyle (Fig. 22); but mostly one of them arrives first, and when the fertilisation of the egg and the polar nucleus has already taken place (Figs. 20—21) another pollen tube may penetrate into the embryo sac, although its content of male nuclei will not perhaps come to function. Thus Figs. 22, 23, 24, 26, show examples of how the female nucleus may be surrounded during the fertilisation by 5—8 active male nuclei. How many of these fuse with the female nucleus I have not been able to observe. The only sure proof that a superfertilisation may facultatively take place is in the chromosome number of the embryos, but this is very difficult to establish in *Epipactis*, especially as regards high numbers. The species therefore is not well suited for elucidating the problem as to whether polyploid embryos can arise from superfertilisation. That this may in fact happen was, however, shown by *Orchis* in which I was able to observe that 2 male nuclei may penetrate simultaneously into the same egg; and polyploid embryos also occurred.

I have never in *Epipactis latifolia* seen that two embryos may develop in the same embryo sac, as is the case in *Orchis* and often in *Cephalanthera*. But I have once found that an ovulum contained 2 nucelli, each of them surrounded by an internal integument. Both the eggs had been fertilised and contained a small embryo, so that the term polyembryony may with some justice be employed.

Afzelius (1922) found similar ovula in *Platanthera*, and it is also known from several other plants (cp. Schürhoff).

Figs. 24—26. The egg is surrounded by several active male nuclei derived from several pollen tubes. × 700. Fig. 25. Metaphase from the first division with 2 $n = 40$ × 1500. See also text.

These investigations on the rise and disappearance of polyploidy in nature will be continued with other plants (*Listera*, *Papaver*).

I owe cordial thanks to Professor C. A. Jørgensen for examining several of my preparations and for his critical remarks.

My respectful acknowledgements are due to the Carlsberg Foundation which has supported my studies for several years.

Summary.

1. Every fruit contains several thousand ovula which are fertilised in very different ways.

2. Most of the eggs are normally fertilised (Figs. 11—14) by a single male nucleus and the embryo will then have the normal diploid number of chromosomes, 2 $n = 40$ (Fig. 25).

3. Often more than one pollen tube enters an ovulum (frequently 2—3). Therefore there will be several male nuclei at

disposal to carry out the fertilisation (Figs. 20—26). Whether polyploid embryos are formed in this way—as in *Orchis*—it has not been possible to observe.

4. In about 10 per cent. of the cases investigated the female nucleus begins to develop embryos without having been fertilised. Frequently another pollen tube will then arrive later which sheds its two male nuclei in the embryo sac (Figs. 15, 17); but these do not come to function. A haploid embryo is then formed by facultative parthenogenesis (Figs. 15—19), and the chromosome number of this embryo will therefore be only $2n = 20$ (Figs. 18—19). The occurrence of haploid plants in nature calls for investigation.

5. Hence the conditions for the degree of polyploidy that develops may also be connected with the fertilisation conditions.

6. Changes in the degree of polyploidy may arise spontaneously in nature and this circumstance may perhaps be of significance for the formation of species.

While this paper was in press I collected living material of *E. microphylla* in Møn. This species too has $n = 20$.

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THE HEMOLYMPH NODES OF THE RAT

BY

ERIK ANDREASEN and OLE GOTTLIEB

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In man, and probably in all mammals we find organs which resemble ordinary lymph nodes, but are red and contain blood in the sinus; such organs have been termed hemolymph nodes. Opinions are strongly divergent as to whether these nodes are organs *sui generis* or ordinary lymph nodes in the sinuses of which the red blood cells are present as a result of congestion, stasis, diapedesis, or resorption of extravasates. The structural resemblance of some hemolymph nodes to the spleen and the fact that this type of nodes is lacking afferent lymphatics are the principal reasons why many authors take them to be specific organs, closely related to the spleen. Other investigators regard the hemolymph nodes as modified lymph nodes because gradual transitional structures can be demonstrated between ordinary lymph nodes and hemolymph nodes. The great divergence in the views concerning the nature of the hemolymph nodes is undoubtedly due to the fact that most authors have been inclined to generalize their observations in a single or a few species. The term "hemolymph nodes" can hardly be said to denote an anatomical unity but more likely a common designation for two different forms of organs: (1) hemolymph nodes that are lacking afferent and efferent lymphatics and thus are situated in the blood stream exclusively; (2) hemolymph nodes which have afferent and efferent lymphatics, and are situated in the lymph stream as ordinary lymph nodes. Functionally the hemolymph nodes appear to constitute a unity, as their main function seems to be a phagocytic decomposition of red blood corpuscles (a review with a comprehensive list of literature on the hemolymph nodes has been given by WELLER, 1938).

Among the animals whose hemolymph nodes most frequently have been the subjects of investigation, the white rat holds a prominent place. The regular occurrence of the nodes is evident from works by VINCENT and HARRISON (1897), DRUMMOND (1900),

Lewis (1902), Weidenreich (1902), Keller (1922), Macmillan (1928) and many others. Lewis failed to find communications between the sinuses of the nodes and neighbouring lymphatic channels, but later investigators have established the presence of lymphatics (Helly, 1902; Weidenreich, 1905; Meyer, 1913; Keller, 1922). On the other hand there is much difference of opinion in respect to the interpretation of the blood-vascular connections of the sinuses. The description given by various authors of the more detailed structures of the nodes is largely the same. Selye and Foglia (1939), however, state that the hemolymph nodes of the rat do not contain blood, but only pigment-storing phagocytes in their sinuses and reticulum, and that these "iron pigment lymph nodes"—as the two authors propose calling them—are rarely if ever observed in immature animals.

In a fairly comprehensive work on the lymphatic system we have had good opportunities of observing the hemolymph nodes in rats at different ages. As thus we were able to ascertain some interesting features of the hemolymph nodes that have not been reported in the literature, we decided to carry out a systematic microscopic examination of these nodes. The result of this work supplemented with experimental studies on the mechanism by which blood seeps into the sinuses will be reported in what follows.

Gross Anatomy.

The hemolymph nodes of the rat are situated retroperitoneally in the brown adipose tissue on the posterior abdominal wall between the inferior vena cava and the cranial pole of the kidney. On the left side the nodes are easily accessible, whereas on the right side it is necessary first to detach the liver from its attachment to the kidney and the adrenal. One or two nodes are found on each side. As a rule they are oval, sometimes spherical. The longest diameter varies between 1 and 4 mm. They are slightly flattened with a smooth surface, and do not differ from the ordinary prevertebral lymph nodes (the lumbar lymph nodes and the cisternal group) except by being mottled or red.

This description makes it evident that it is the nodes here mentioned, from one or both sides, that have been the subject

of the studies on the hemolymph nodes of the rat cited in the literature. A few authors think that also certain other groups of lymph nodes ought to be classified with the hemolymph nodes. Thus VINCENT and HARRISON (1897) and LEWIS (1902) reported that a group of small hemolymph nodes can be demonstrated in the fold of the peritoneum between the spleen and the stomach; these nodes are very diminutive and often it is impossible macroscopically to distinguish them from ordinary lymph nodes. In some cases we have looked for this group but we have never observed a red colour of the nodes. KELLER (1922) states that in a few cases he found some hemolymph nodes along the thoracic vertebrae, and MACMILLAN (1928) and SELYE and FOGLIA (1939) classify the lymph nodes of the thymus as hemolymph nodes. In our material we have submitted these groups of lymph nodes to a very thorough inspection, but never found them to be mottled or red. Nor have we found on microscopic examination that these nodes differ from the ordinary lymph nodes.

Our investigations include observations made on about 300 albino rats; for details as to the strain of rats, their nutrition and growth, see ANDREASEN, 1943. As a rule the nodes were dissected out in animals killed by bleeding under ether anesthesia. In many cases we further observed and dissected out the nodes in living animals (under light ether anesthesia) without being able to demonstrate any characteristic differences in the colour of the nodes in living and dead animals.

Our investigations showed that the hemolymph nodes do not appear till the latter half of the first month of life. Prior to this age, it is true, lymph nodes were found corresponding to the location, but microscopically these nodes did not differ from ordinary lymph nodes. Among 17 animals that were from 29 to 31 days old we found that these nodes were definitely red in 16 animals; they were perfectly white only in 1 animal (one of the smallest of the examined animals in the 1 month group).

In all the older animals examined the nodes were constantly mottled or red. In the animals of the 1 month group, one of the poles as a rule was mottled. With advancing age the red areas increase in size (cf. Fig. 1.) so that in the animals of the 1-year and 2-years groups the red colour often was predominant.

The weight of the nodes was recorded in 125 normal animals. The age and sex distribution is given in Table 1.

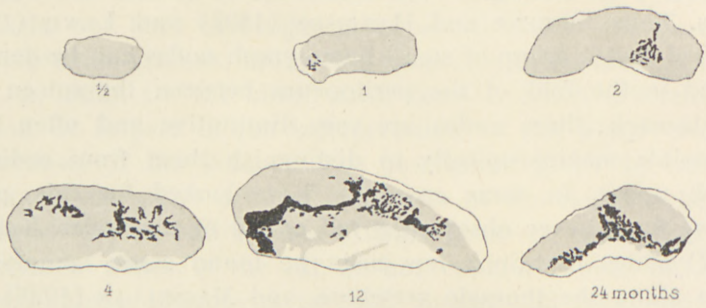

Fig. 1. Camera lucida drawings of sections of hemolymph nodes from rats of different ages, showing the characteristic development of the blood-filled sinuses with increasing age.

A graphical presentation of the individual weights of the hemolymph nodes is given in Fig. 2; and weight curves are plotted on the basis of the average weights for the age groups examined. From these diagrams it is evident that in the females the growth of the organs is conluded at the age of 2 months, in the male at the age of 4 months, after which the weight of the organs largely appears to remain stationary throughout life. In the males the weight appears to fall slightly towards the age of 2 years, but no reduction in the weight can be demonstrated statistically.

Table 1.

Age and Sex Distribution of Normal Animal Material.

Age	Designations of groups	Females	Males
1 month	I	7	9
2 months	II	11	11
3 —	III	12	10
4 —	IV	13	11
5 —	VI	9	10
1 year	XXI	2	4
2 years	XXIV	9	7
Total	..	63	62

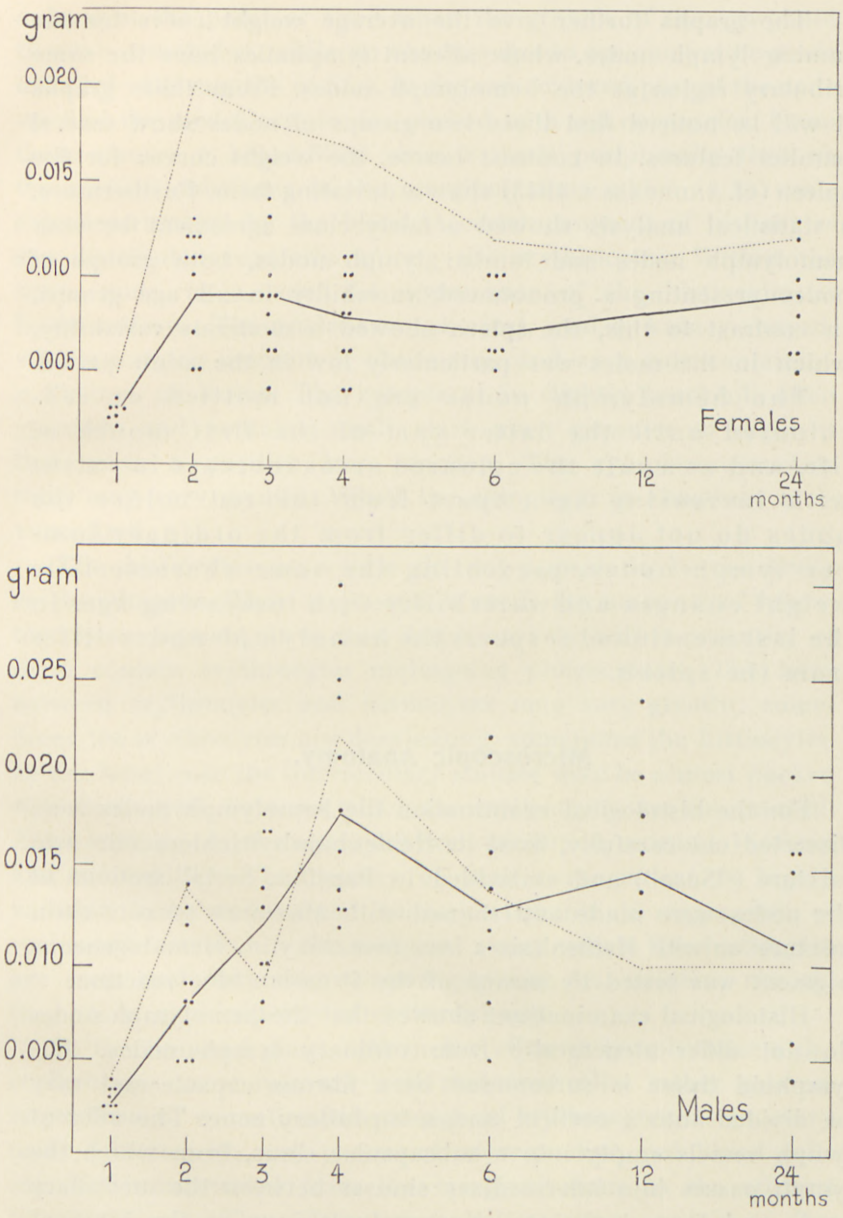

Fig. 2. Graphical presentation of the individual and the average weights of the hemolymph nodes in females and males. The graphs further give the average weight curves for the ordinary lumbar lymph nodes, represented by the dotted lines. The weights of the organs are plotted as ordinates while the age groups are plotted along the axis of abscissa.

The graphs further give the average weight curve for the lumbar lymph nodes, whose afferent lymphatics have the same tributary region as the hemolymph nodes. From these graphs it will be noticed that these two groups of nodes show several parallel features. In contrast hereto, the weight curves for the spleen (cf. ANDREASEN, 1943) show a deviating form. Furthermore, a statistical analysis showed a fairly close agreement between hemolymph nodes and lumbar lymph nodes, both groups of nodes presenting a pronounced variability in all age groups. In contrast to this, the spleen showed a moderate variability, which in the males was particularly low in the adult period.

The hemolymph nodes are not mottled or red-coloured until the latter part of the first month of life, and as a rule the coloured areas increase in extent with increasing age. Apart from the red colour the nodes do not appear to differ from the ordinary lumbar lymph nodes, presenting the same characteristic weight changes and variability with increasing age. In the last mentioned respects the hemolymph nodes differ from the spleen.

Microscopic Anatomy.

For the histological examination the hemolymph nodes were dissected out carefully, fixed in Heidenhain's trichloracetic acid mixture ("Susa") and embedded in Paraffin. Serial sections of the nodes were made and stained with Maximow's azure-eosin mixture or with Heidenhain's iron hematoxylin. Hematogeneous pigment was tested by means of the Prussian-blue reaction.

Histological examinations showed that the hemolymph nodes do not differ structurally from ordinary lymph nodes. The lymphoid tissue is surrounded by a fibrous capsule and may be divided into a cortical and a medullary zone. The afferent lymph vessels empty into a subcapsular sinus, from which the lymph passes into intermediary sinuses between the medullary cords and from here into the terminal sinus at the hilus of the node and then into the efferent lymph vessels.

For the description of the features characteristic of the hemolymph nodes we shall pick out a typical node removed from an

animal at the point of time when the growth of the lymphoid tissue is concluded, while no changes due to age can yet be made out (3rd—4th months of life). In the red-coloured part of the node we find the medullary sinuses packed with red corpuscles. From these sinuses a blood-filled sinus extends peripherally through the cortical substance to the marginal sinus which is to some extent permeated by the blood. A certain amount of histiocytes are distributed regularly among the erythrocytes. Within the same node, as a rule, the appearance of the histiocytes is fairly uniform. The nucleus is spherical, oval or kidney-shaped, with a varying amount of chromatin, and as a rule containing a distinct nucleolus. The cytoplasm is slightly basophil, often greenish, and contains small, dark-green granules of pigment that gives a positive iron reaction. The cytoplasma-nuclear ratio may vary greatly. When the cytoplasm is abundant it is vacuolized.

The histiocytes as a rule do not appear to be markedly erythrophagous. They may contain moderate amounts of hematogeneous pigment, it is true, but as a rule only a minority of them contain erythrocytes undergoing phagocytosis. The ratio between erythrocytes and histiocytes may vary greatly; sometimes the erythrocytes are dominating, sometimes the histiocytes. In the latter case the intermediary sinuses may be almost packed with histiocytes, between which the erythrocytes are compressed. As a rule the erythrocytes are well-preserved, normal in form and stainability. The density of their arrangement is highly variable from well-defined solitary elements to aggregates of eosinophil masses. In the marginal sinus only a few histiocytes are seen, and they may contain pigment, but seldom; nor do they show any evidence of erythrophagy.

In the terminal sinus and in the efferent lymph vessels the erythrocytes as a rule are well preserved. One rather gets an impression of a continuous passage of erythrocytes, as in every node sinuses with well preserved, close-packed, red blood cells can be demonstrated from the marginal sinus to the efferent lymph vessels. The efferent lymphatics often contain a few histiocytes that appear to be carried along by the lymph stream. In the bends of the blood-filled intermediary sinus one now and then has the impression of a marked retardation or complete

cessation of the flow; here, as a rule, the erythrocytes are relatively scanty, often sticking to the histiocytes in a rosette pattern, often in close relation to mast cells that have migrated into the sinus from the adjacent lymphoid tissue. Further the pigmentation of the histiocytes is farther advanced here than in the other parts of the sinus.

The lymphoid tissue that is in direct contact with the blood-filled sinus shows a characteristic differentiation, as it is made up of plasma cells—in contrast to the rest of the lymphoid cortex in which plasma cells are not represented.

The microscopic picture of the hemolymph nodes presents such characteristic changes with increasing age that it is practicable from the histological examination of the nodes with a fair degree of accuracy to determine to which age group the animal belongs.

In infantile animals—in the first two weeks of life—red or mottled nodes, as mentioned, were never observed. Microscopy of the renal lymph nodes will now and then reveal a few erythrocytes in the marginal or intermediary sinuses, and they may stick to the histiocytes in a rosette-like pattern. Otherwise, no evidence of erythrophagy is seen; nor do the histiocytes contain any hematogeneous pigments. The presence of a few erythrocytes in the sinuses is not characteristic of the renal lymph node and is seen just as often in the other lymph nodes of the rat. In older infantile rats (about 1 month old) blood-filled sinuses in a small area of the node are practically always found. The blood admixture is not massive, and as a rule the histiocytes make up the dominant component in the blood-filled sinus. Erythrocytes and histiocytes are rather close-packed. The histiocytes are large, with light basophil protoplasm, in which a few granules of pigment are found, although exceptionally. Most of the specimens show no evidence of erythrophagy, but in a few of them some histiocytes are seen to contain red cells. Free erythrocytes are found in the terminal sinus and efferent lymphatics, so there can be no doubt that a flow of blood takes place.

In somewhat older animals (2 months old) the blood admixture to the sinuses becomes more abundant. The erythrophagy has become more lively, and the number of pigment-containing histiocytes is highly increased. The histological picture does not appear to deviate from that encountered in full-grown young animals (3—4 months), as described on page 9.

Increasing age is now associated with a shift in the histological picture as the number of blood-carrying sinuses increases progressively, and this also applies to the pigment contents of the histiocytes (cf. Fig. 3). In animals 6 months old, histiocytes contain red blood cells in various phases of disintegration; the pigment contents may now be further increased so that the cells in unstained sections appear as markedly yellowish-green or brownish, the protoplasm being filled with close-packed golden granules or drops of varying size. With the increasing accumulation of pigment (Plate 1, fig. 6), which is observed especially in animals of 1 and 2 years, the nuclei become pycnotic, eccen-

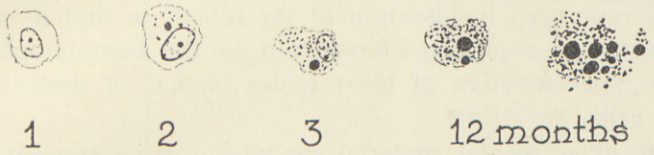

Fig. 3. Diagrammatic sketches of pigment cells showing the progressing accumulation of pigment with increasing age.

tric, and the protoplasm is increased markedly, giving the cell body an irregular outline. Now the cell is often surrounded by extracellular pigment, undoubtedly given off by the pigment-containing cells. In the pigment cells the nuclei seem finally to disappear, the cells being represented merely by a large aggregate of pigment. In sections stained by the method of Turnbull the pigment gives a positive reaction for iron; the blue colour may vary from light blue to bluish-black. Exceptionally a few phagocytes are seen to contain large granules of pigment that gives no positive reaction for iron. In contrast to SELYE and FOGLIA (1939) we always found red blood cells in he pigment-storing phagocytes.

In general structure and arrangement of lymphoid tissue the hemolymph nodes resemble the ordinary lymph nodes and they are only characterized by the presence of erythrocytes and erythrophages in the sinuses. With increasing age, then, the number of sinuses containing blood increases, and this applies also to the amount of pigment taken up by the erythrophages, so that hematogeneous pigment may be found extracellularly after the 6th month of life.

The Hemolymph Nodes during Inanition.

Apparently the changes of the hemolymph nodes during inanition have not been investigated previously. Still, a paper by RETTERER (1902) is of some interest in this connection, as he mentions that the sinuses of the ordinary lymph nodes in the cat under normal conditions are filled with erythrocytes; after starvation, however, very few erythrocytes are seen in the sinuses, and then these erythrocytes are small and deformed. Further, we were led to take up this question as we have observed that the hemolymph nodes of animals died of starvation were often found to be quite pale. Besides, inanition is associated with pronounced inspissation of the blood, so that it might be reasonable to expect some reaction in the hemolymph nodes, as the chief function of these nodes is that of destroying red blood cells.

Our experimental material includes three age-groups (1, 3 and 12 months old) so that the hemolymph nodes were examined at 3 different stages: (1) at a time when the rate of growth of the nodes is very great, (2) when the lymphoid tissue has reached its maximum development, and (3) when the tissue has already long been undergoing involution due to age.

In all three age groups we found the hemolymph nodes atrophied in the same degrees as seen in ordinary lymph nodes; after food supply regeneration takes place apparently at the same rate as in the ordinary lymph nodes. In the infantile animals the hemolymph nodes have become completely white after extreme inanition (3 days), while in the older animals the red colour usually was visible even though the atropic nodes did not show the lively red colour seen in the control animals.

The microscopic examination includes serial sections of hemolymph nodes from a group of animals, 3 months old, which had been submitted to inanition of varying duration. A survey of this material, with notes on the blood contents of the sinuses is given in Table 2.

From this survey it is evident that the amount of erythrocytes in the sinuses decreases during starvation so that after extreme starvation the red blood cells have disappeared, only a remnant of them often persisting in the form of eosinophil

Table 2.

Survey of Blood Admixture in Sinuses during Inanition and Restitution Periods.

Animal No.	Inanition period in days	Restitution period in days	Erythrocytes in sinuses
III, 31	5	..	Numerous
III, 32	7	..	Numerous
III, 33	7	..	Few
III, 34	9	..	None, but very scanty eosinophil aggregates
III, 35	10	..	None, but eosinophil aggregates
III, 36	7	2	None, but eosinophil aggregates
III, 37	10	3	Numerous
III, 38	8	6	Numerous
III, 39	8	8	Numerous
III, 40	10	10	Numerous

aggregates; during the restitution period the erythrocyte supply to the sinuses soon appears to be established again. In all phases of atrophy and regeneration the sinuses present numerous pigment-containing phagocytes (cf. Plate 2).

During starvation, then, the hemolymph nodes undergo atrophy—just like the ordinary lymph nodes. The erythrocyte supply to the sinuses decreases gradually and finally it ceases completely, and the hemolymph nodes come to look like ordinary lymph nodes—except for the presence of pigment-containing histiocytes in the formerly blood-filled sinuses.

How does Blood Seep into the Sinus?

The question as to how the blood gets into the sinuses of the hemolymph nodes in the rat has been the subject of several investigations. These works were based on injection into blood vessels—which will always imply some risk that the injection pressure may cause ruptures of the vessels.

Lewis (1902) states that arterial injection fills the sinuses of the hemolymph nodes with injection fluid, whereas they are filled but partly or not at all by venous injection; and he claims that small arteries open directly into the sinuses. He further states that the injection mass may be found also in the sinuses of ordinary lymph nodes, but he is unable to decide whether there is any communication between arterial capillaries and the sinuses, or whether the delicate capillary walls may have ruptured.

In contrast, Helly (1902) stated that by injection into the nodes he had been able to ascertain a complete separation between the blood vessel system and the lymph vessel system, and that the former is not regularly in direct connection with the sinuses of the hemolymph nodes.

On arterial injection through the aorta, Keller (1922) observed that certain parts of the hemolymph nodes were constantly filled with the injection mass which entered the sinus from a thin-walled vessel resembling an afferent lymph vessel. According to Keller, this vessel was no vein that had been ruptured by the injection pressure and thus put in communication with the sinus; for also after vital injection India ink was sometimes found free in the sinuses. On the basis of his experiments Keller concludes that the blood is brought to the hemolymph nodes by way of lymph vessels that are in rather intimate connection with the blood vessel system; but he does not mention how this connection is established.

To us it seems more obvious to associate the admixture of blood in the sinuses with the lymphaticovenous communications which in many mammals and in man can be demonstrated retroperitoneally in the lumbar region. Such communications have been described also in the rat by Job (1915), who says (p. 452): "On the left side the number of lymph vessels leading from the lumbar node may vary from one to four, or form a network, depending somewhat on the mode of attachment with the right lymph vessel. However, all the vessels lead along the left side of the vena cava, in any case. If there is only one vessel, it will open into a single node, just anterior to the left renal vein, from which a branch is given to the renal vein, and one to the group of single nodes lying to the left of the

cisterna chyli. If there be more than one lymph vessel leaving the lumbar node, some one of them will enter the renal node, the rest may join the cisterna group, the cisterna directly, the renal vein directly, or any combination thereof. The latter conditions are fewer than the single vessel method."

In a subsequent paper (1918) Job says (p. 469) that "barely 8 per cent of the material showed renal-vein communications that could be satisfactorily demonstrated. In a large number of cases a lymphatic vessel branched off the main system about 1 cm. posterior to left renal vein and outward toward the hilus of the kidney, but only occasionally was it possible to demonstrate its connection with the vein." Strange to say, Job does not mention that the lymph nodes he designates as the renal nodes show a red colouring and are hemolymph nodes, and hence he does not associate this red colouring with the special vascular communications applying to the lymphatics of these nodes.

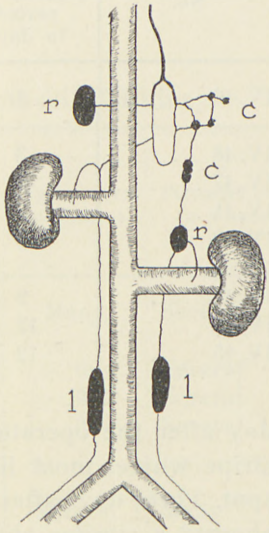

Fig. 4. The lymphatico-venous communications in the renal region (after Job, 1915). *c*: Cisternal lymph nodes, *l*: Lumbar lymph nodes, *r*: Renal hemolymph nodes.

From Job's reports it seemed obvious to assume that the blood supply to the sinuses of the hemolymph nodes might take place through reflux from the lymphatic that connects the lymph node with the renal vein (cf. Fig. 4).

This connection may be severed by excision of the kidney after binding its stalk. We therefore performed left-sided nephrectomy on several rats, about 4 months old, and examined the homolateral hemolymph node a varying number of days after the operation. After inspection *in situ*, the hemolymph node was dissected out, fixed and later examined microscopically in serial sections. A survey of the material and finding is given in Table 3.

From Table 3 it is evident that the hemolymph node on the operated side loses its characteristic red spots. Already one

Table 3.
Left Renal Hemolymph Nodes after Removal of Kidney.

Animal No.	Duration of post-operative period in days	Macroscopic appearance of the hemolymph node	Erythrocytes in sinuses	Pigment cells in sinuses
IV, 46	1	Amber-coloured stripe	Few	Many
IV, 48	3	» »	0	Many
IV, 47	3	Quite pale	0	Many
IV, 41	7	» »	0	Very few
IV, 42	9	Narrow reddish-yellow ring	0	Several
IV, 43	9	Quite pale	0	Few
IV, 44	12	» »	0	Few
IV, 45	12	» »	0	Very few

day after the operation it presented merely an amber-coloured stripe where, most likely, prior to the operation it had a red spot. Three days after the operation one of the examined animals showed a similar change in colour, while in the other animal (IV, 47) the node was now quite pale, looking exactly like the ordinary lumbar lymph nodes (Plate 3, fig. 9). In the animals examined a longer time after the operation (7, 9, 12 days) the red colour likewise had disappeared (Plate 3, fig. 10). The hemolymph nodes on the operated side seemed normal in size, form, and consistence, so that the operation appeared to have had no effect on the node except for the change in colour. The contralateral hemolymph node was mottled and the red areas did not appear to be more extensive than usual in animals of this age-class.

Examination of the serial sections of the left node showed the sinuses to be empty of erythrocytes; only in one animal, killed on the day after the operation, a few erythrocytes were still left. Otherwise the histological picture did not deviate from the normal. Mitotic figures were seen in the lymphoid tissue, and there was no suggestion whatever of any damage to the node from the operation. The contralateral hemolymph nodes showed blood-filled sinuses and neither qualitative nor quantita-

tive evidence of changes could be demonstrated on microscopic examination.

This experimental series was later supplemented by another —this time with employment of younger animals (about 2 months old). The results are presented schematically in Table 4.

Table 4.
Left Renal Lymph Node after Removal of Kidney.

Animal No.	Duration of post-operative period in days	Macroscopic appearance of hemolymph node	Erythrocytes in sinuses	Pigment cells in sinuses
II, 100	2	Pink stripe	Many	Several
II, 103	3	Quite pale	..	..
II, 105	5	» »	0	Several
II, 107	6	» »	0	Several
II, 101	6	Yellow marmorate spot	0	Several
II, 106	7	Quite pale	..	..
II, 102	7	Rust-coloured spots	Several	Many
II, 108	8	Quite pale	0	Few
II, 104	9	Rust-coloured spot	A few	Many
II, 109	10	Quite pale	0	Very few

On the whole, the results obtained in this series agreed very well with those obtained in the first series though here they were hardly as clear-cut as in the first series. Two of the animals, killed respectively 7 and 9 days after the operation, still contained erythrocytes in the sinuses; the number of erythrocytes, however, had decreased considerably.

So the outcome of these experiments has been that the supply of blood to the sinuses can be interrupted by nephrectomy, probably because a reflux from the renal vein through an efferent lymph vessel is rendered impossible. The correctness of this view is further rendered probable by the fact that we have been able in infantile animals with perfectly pale hemolymph nodes by cautious compression of the renal vein in the

living animal (under light ether anesthesia) to produce a red colour of some parts of the node. Subsequent microscopic examination of the node showed the sinus to be partly filled with erythrocytes, and the appearance of the node corresponded to that of the hemolymph node in somewhat older infantile animals. As the venous pressure rises on compression of the renal vein, the supply of blood to the sinuses may conceivably result from a rupture of one of the veins of the hemolymph node. So, naturally, we do not consider this compression as an *experimentum crucis*.

For that matter, certain objections may be raised to the interpretation of our experiments with nephrectomy. Thus one might conceive that the lymphatics of the hemolymph nodes drain the kidneys, and that red cells are present in the renal lymph; *a priori*, this possibility cannot be excluded, but it is still improbable that lymph from the kidneys should contain such a large amount of blood cells, for in the sinuses the erythrocytes are so closepacked that their mixing with lymph has to be regarded as out of the question. Previous authors who have investigated the renal lymph have not mentioned that it was mixed with blood cells (SCHMIDT and HAYMAN, 1929—30; DRINKER and FIELD, 1931).

Further, one might think that ligation of the stalk of the kidney would involve also the small arteries which, according to LEWIS, carry blood to the sinuses of the node; but this is rather a theoretical proposition. For HELLY, as mentioned, has shown that such a connection between the sinuses and the circulatory system does not exist, and the studies reported by KELLER show that blood seeps into the sinus through a thin-walled vessel that cannot be an artery.

A universal vascular effect produced by unilateral nephrectomy that would prevent a possible diapedesis to the sinuses is also improbable, as the contralateral hemolymph node shows normal red colour and a normal amount of erythrocytes in the sinuses.

The question whether the blood is conveyed to the sinuses jerkily or as a continuous stream is closely connected with that of the mechanism of the supply of blood to the sinuses. A cyclic function has been attributed to the hemolymph nodes by several

observers (e. g., LEWIS, 1902; KELLER, 1922). This view has been based on the fact that different appearances have been noticed in nodes taken from the same positions suggesting different phases of activity. KELLER further states that the varying ratio between macrophages and free blood cells from the convexity of the node to the hilus might lend support to such a view. Thus, according to KELLER, the macrophages usually fill the intermediary sinus completely, whereas the free erythrocytes are predominant in the marginal sinus.

In contrast to previous assumptions, we think that our observations plainly show that the blood is conveyed to the node continuously. Macrophages are always rather scarce in the marginal sinus, it is true, but this is only natural as the phagocytic potency of the reticulo-endothelium under all conditions has proved to be lower in the marginal sinus than in the intermediary one. In the intermediary sinus the density of macrophages is the same peripherally and centrally, and on the whole the content of blood pigment in the cells is also the same peripherally and centrally in the same node. The plasma cell reaction of the lymphoid tissue is strongly suggestive of a continuous flow of blood through the same parts of the node; and only in exceptional instances have we found pigment cells in other parts of the node signifying that the sinus has previously contained erythrocytes.

Thus it seems safe to sum up the outcome of these experiments as follows:

By unilateral nephrectomy it is practicable to stop the supply of blood to the sinuses of the homolateral hemolymph node, thus making it impossible macroscopically and microscopically to distinguish this node from an ordinary lymph node. It seems most likely that the blood is conveyed to the renal hemolymph node by reflux through an efferent lymph vessel that opens into the renal vein. Microscopic findings seem to indicate that the blood is conveyed to the sinuses of the hemolymph node continuously.

How Long do Erythrophages Stay in the Hemolymph Nodes?

Whether the erythrophages are cells with a short lifetime that are carried away by the lymph stream immediately after phagocytosis and destruction of the red blood cells, or whether they remain and keep functioning in the hemolymph node for a considerable length of time—perhaps throughout the lifetime of the individual—is a question about which it is difficult *a priori* to form any opinion. On the basis of the afore-mentioned (p. 11) age changes in the pigment content of the erythrophages, one might perhaps be inclined to think that here we meet with a progressive development of pigment within the same cell, commencing intracellularly and terminating extracellularly.

The question as to how long the erythrophages stay in the sinuses of the hemolymph nodes has been elucidated, however, through the above examination of the hemolymph node after nephrectomy. For here we found that when the supply of blood to the sinuses ceased a disappearance of the pigment cells might be observed at the same time (Plate 3). In Tables 3 and 4, in one of the columns a rough estimate is made of the number of pigment cells in the sinus. About one week after the nephrectomy, most of the pigment cells had disappeared, and 10—12 days after the operation the sinuses are wide, empty and almost completely rid of erythrophages. In a couple of the animals (II, 102 and II, 104) the sinuses still contained many pigment cells as late as 7 and 9 days after the nephrectomy. Thus individual variations may assert themselves—above all, probably with regard to the rate of flow of the lymph and thus the liberation of the pigment cells.

The increasing pigment content of the erythrophages with advancing age therefore indicates an increasing erythrophagy in the hemolymph nodes, even though it cannot be excluded that the slower lymph flow with advancing age may contribute to prolonging the stay of the erythrophages in the sinuses, which implies the possibility of a more prolonged erythrophagic function.

After unilateral nephrectomy, which stops the supply of blood to the sinuses in the homolateral hemolymph node, the number of pigment cells in the sinuses decreases rapidly, indicating that the function of the

erythrophages in hemolymph nodes and other lymph nodes is of brief duration.

Are the Hemolymph Nodes of the Rat Organs "*sui generis*"?

In conclusion, on the basis of our own studies and the reports in the literature we shall now try to place the hemolymph nodes of the rat systematically. The statements made below apply only to the hemolymph nodes of the rat and we take no stand on the systematics of the hemolymph nodes in other species beyond emphasizing that the term "hemolymph nodes" can hardly be said to denote an anatomical unity and that generalization from one species to another is not justifiable till thorough studies have been carried out on a fairly large number of species.

The following facts show how closely the hemolymph nodes are related to the ordinary lymph nodes. Morphologically the hemolymph node of the rat thus does not differ from the ordinary lymph nodes except for a characteristic red colour and the presence of erythrocytes and erythrophages in the sinuses. Like the other lymph nodes, the afferent lymphatics belonging to the hemolymph nodes drain a skin area, the lumbar region. This was stated already by KELLER (1922); and by subcutaneous injection of India ink in this region we have been able to ascertain that the India ink was found distributed in the sinuses of the hemolymph nodes just as in the other lymph nodes. After subcutaneous injection of India ink in the hind legs we have likewise been able to demonstrate this substance in the hemolymph nodes (as well as in the popliteal and lumbar nodes)—which indeed was to be expected after JOB's establishment of the lymphatic trunks. Consequently, also the hemolymph nodes are situated in the lymph stream passing from the hind leg to the cisterna chyli, and thus they do not differ in this respect either from the ordinary lymph nodes located on the posterior abdominal wall (lumbar lymph nodes).

The hemolymph nodes appear to be closely related to the lumbar lymph nodes also in other respects. Thus changes with advancing age, variability and changes in weight during inanition show a close relationship between the two groups of lymph

nodes. On the other hand, on comparison of the hemolymph nodes with the spleen, to which an erythrocyte-destroying function is generally attributed, we find no such conspicuous relationships, and the variability of the two organs within various age-groups is even quite deviating in several respects.

The close relationship with the ordinary lymph nodes manifests itself also in other respects. Thus the hemolymph nodes develop from quite ordinary lymph nodes, only that blood is conveyed to their sinuses in the latter half of the first month of life; before that time the hemolymph nodes do not differ in any respect from ordinary lymph nodes. Later in life the red colour of the hemolymph nodes is constant, it is true, but it is practicable in young animals by starvation to transform hemolymph nodes into ordinary lymph nodes in so far as the admixture of blood to the sinuses may be checked completely by extreme inanition, so that only the presence of pigment cells in the sinuses indicates the previous function of erythrophagy. Further, it is possible also by unilateral nephrectomy to transform the homolateral hemolymph node to an ordinary lymph node as erythrocytes and pigment cells after the operation may disappear so completely that the node cannot be distinguished from the adjacent lumbar lymph nodes—neither macroscopically nor microscopically. Conversely, SELYE and FOGLIA (1939) found that hemolymph nodes may be produced experimentally in the rat following exposure to damaging agents capable of eliciting an "alarm reaction" (excessive muscular exercise, exposure to cold, and toxic doses of formaldehyde).

Considering the question on the systematics of the hemolymph nodes, it will further be of importance to settle whether there exists a continuous series of intermediate forms between hemolymph nodes and ordinary lymph nodes. If such types really exist, it will only be reasonable to hold that the hemolymph nodes are not entitled to a special classification, as the possibility for transition from one form to another under normal conditions then would be present continually. For the elucidation of this question we are able to state that the ordinary lymph nodes in the normal animals never show any red colour like the hemolymph nodes. The presence of free erythrocytes and erythrophages in the sinuses of ordinary lymph nodes is

a frequent finding—but never in such massive accumulation as in the hemolymph nodes. In our opinion, the constant location of the hemolymph nodes, their characteristic changes with advancing age and the pronounced cellular erythrocyte destruction render it justifiable to maintain the term "hemolymph node".

We shall not here enter into the capacity of the erythrophagic function of the hemolymph nodes as compared to that of the other blood-destroying organs, but merely state that undoubtedly large amounts of erythrocytes are decomposed daily in the hemolymph nodes of the rat. This is suggested strongly by the rapid disappearance of the numerous pigment cells from the sinuses of the hemolymph nodes after unilateral nephrectomy. Studies reported by DRINKER, FIELD, and WARD (1934) lend support to this view. By perfusion of an ordinary popliteal lymph node from a dog with an autogeneous erythrocyte suspension in heparinized plasma and examination of the lymph collected from the efferent lymphatics, these investigators found that the filtration had been fairly complete. The morphological features observed in the course of the blood destruction in this experiment appear to correspond completely to the normal picture of the activity of the hemolymph nodes in the rat. It is evident also from papers by MÜLLER (1879) and KELLER (1922) that the phagocytic activity of the lymph nodes is very great during resorption of blood extravasates.

In our opinion the term hemolymph nodes should be used for the renal lymph nodes of the rat notwithstanding the fact that they are derived from ordinary lymph nodes and that it is possible to reverse them into the ordinary lymph node type. We think this view is justified by the constancy of the occurrence of the hemolymph nodes, the characteristic changes with advancing age and the special function of the nodes: a pronounced cellular destruction of erythrocytes.

Summary.

The hemolymph nodes of the rat belong to the group of hemolymph nodes, which have afferent and efferent lymphatics, and are situated in the lymph stream as ordinary lymph nodes. They are not mottled or red until the latter part of the first month of life, and as a rule the coloured areas increase in extent with increasing age. Apart from the red colour the appearance of the nodes does not differ from that of ordinary lumbar lymph nodes, and the nodes present the same characteristic weight changes and variability with increasing age as the ordinary lymph nodes. In the last mentioned respects the hemolymph nodes differ from the spleen.

In general structure and arrangement of lymphoid tissue the hemolymph nodes resemble the ordinary lymph nodes and they are only characterized by the presence of erythrocytes and erythrophages in the sinuses. With increasing age the number of sinuses containing blood increases and this also applies to the amount of pigment ingested by the erythrophages, so that hematogeneous pigment may be found extracellularly after the 6th month of life.

During inanition the hemolymph nodes undergo atrophy—just like ordinary lymph nodes. The erythrocyte supply to the sinuses decreases gradually and finally it ceases completely, and the hemolymph nodes come to look like ordinary lymph nodes—except for the presence of pigment-containing histiocytes in the formerly blood-filled sinuses. By unilateral nephrectomy it is also practicable to stop the supply of blood to the sinuses of the homolateral hemolymph node, thus making it impossible macroscopically and microscopically to distinguish this node from an ordinary lymph node.

It seems most likely that the blood is conveyed to the renal hemolymph node by reflux through an efferent lymph vessel opening into the renal vein. Microscopic findings seem to indicate that the blood is conveyed to the sinuses of the hemolymph node continuously.

After unilateral nephrectomy the number of pigment cells in the sinuses of the homolateral hemolymph nodes decreases rapidly, which indicates that the function of the erythrophages in these nodes and probably also in ordinary lymph nodes is of brief duration.

On the basis of these studies and reports in the literature we feel justified in maintaining the term hemolymph nodes for the renal lymph nodes of the rat notwithstanding the fact that they are derived from ordinary lymph nodes and that it is possible to reverse them into the ordinary lymph node type.

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Only after this paper went to press we have had an opportunity to go through the English and American literature, published during the war, on the subject dealt with here. Selye and Schenker (J. Anat. **73**: 413–415, 1939) have succeeded in reverting hemolymph nodes to the ordinary lymph node type by homolateral nephrectomy, especially when combined with adrenalectomy. If these operations were performed in the immature rat before the transformation of the renal lymph node into an "iron pigment lymph node", they prevented the occurrence of such a transformation. It appears, therefore, that in the absence of these glands, the node discontinues its blood-destroying activity but remains different from other lymph nodes in that it contains no germinal centres. Lasnitzki and Woodhouse (J. Anat. **78**: 121–129, 1944) after subcutaneously administrations of 1:2:5:6: dibenzanthracene found that many of the lymph nodes of the animals (rats) were transformed into more or less pronounced hemolymph nodes.

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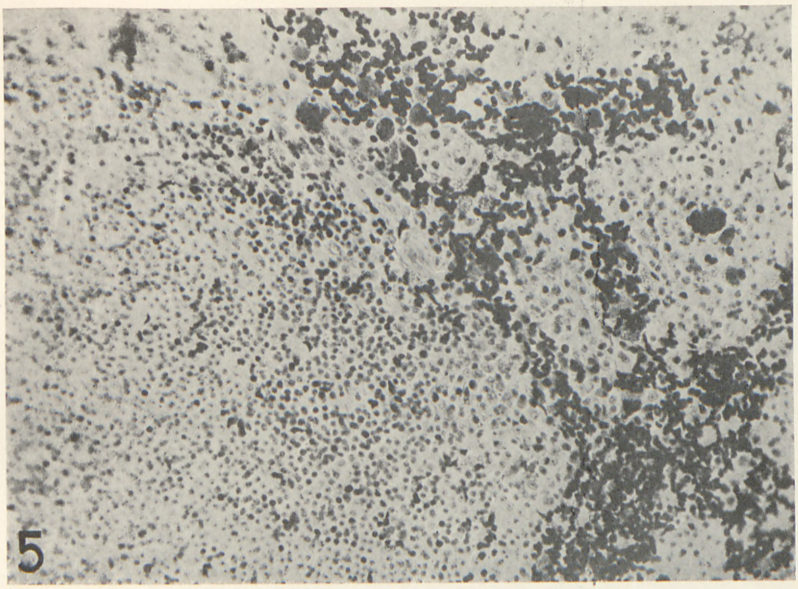

Fig. 5. Microphotograph. Cortical substance and blood-filled intermediary sinuses of a hemolymph node. The histiocytes are regularly distributed among the erythrocytes. From a normal animal, 1 year old. Heidenhain's iron hematoxylin. × 250.

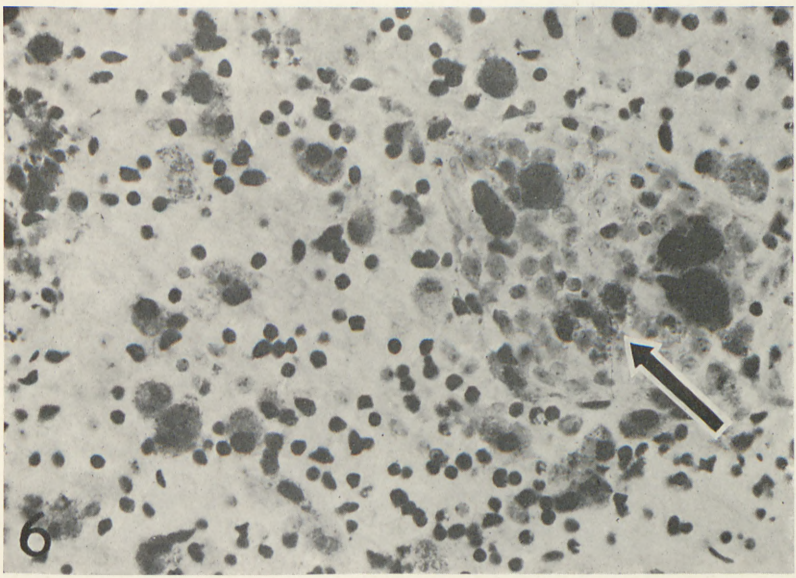

Fig. 6. Microphotograph. Intermediary sinus from the same node as in Fig. 5. The histiocytes are filled with hematogenous pigment; extracellular pigment is seen in the medullary cord on the right Heidenhain's iron hematoxylin. Hom. imm. 1/12. Oc. 5. Zeiss. × 450.

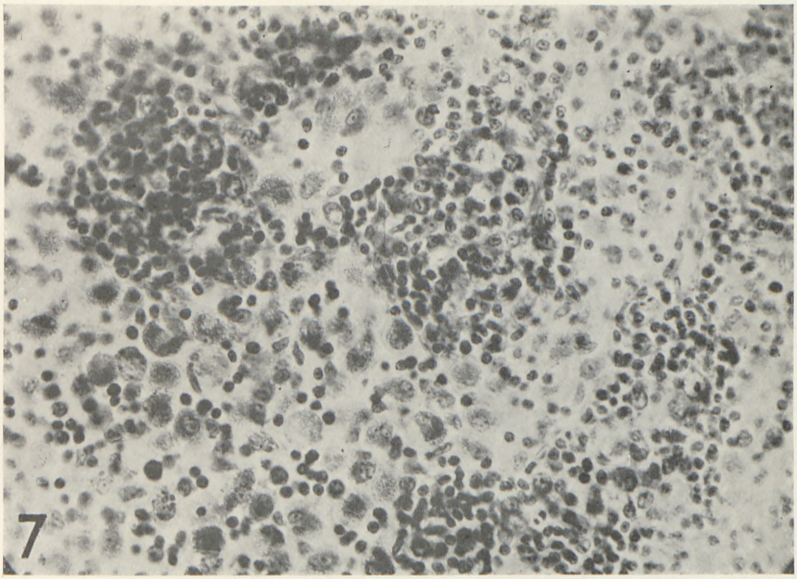

Fig. 7. Microphotograph. Intermediary sinus from an animal which had been submitted to inanition for nine days (III, 34). The red blood cells have disappeared, the pigment-containing histiocytes persist. Maximow's azure-eosin. Hom. imm. 1/7. Oc. 5. Zeiss. × 450.

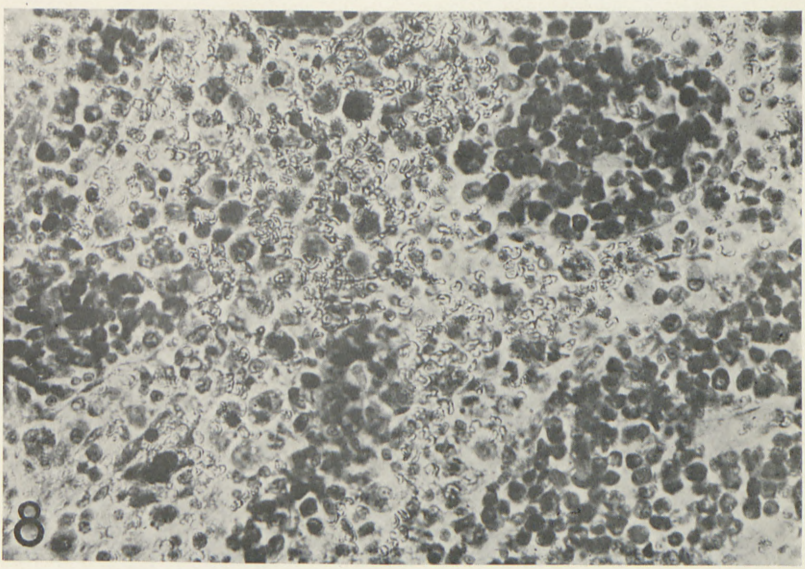

Fig. 8. Microphotograph. Intermediary sinus from the control animal. The sinus packed with erythrocytes and pigment cells. Maximow's azure-eosin. Hom. imm. 1/7. Oc. 5. Zeiss. × 450.

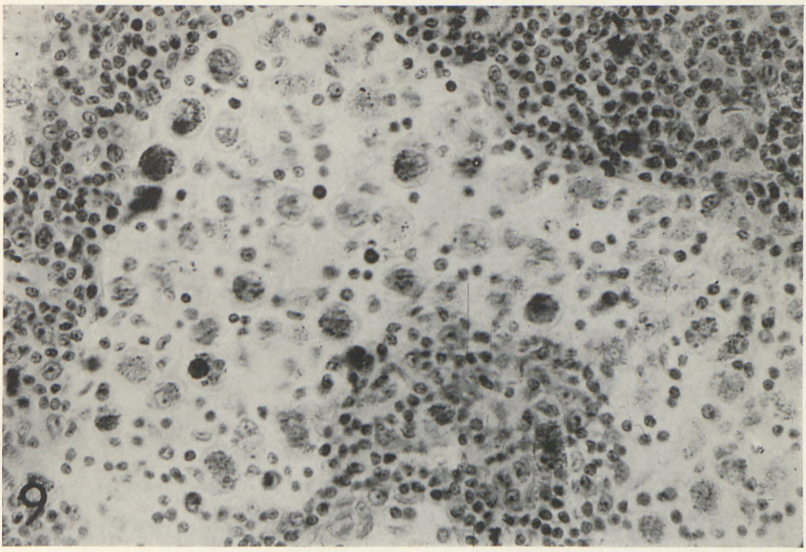

Fig. 9. Microphotograph. Intermediary sinus of a hemolymph node from an animal three days after unilateral nephrectomy (IV, 47). The sinuses are empty of erythrocytes, but they still contain many pigment cells. Maximow's azure-eosin. Hom. imm. 1/7. Oc. 5. Zeiss. × 450.

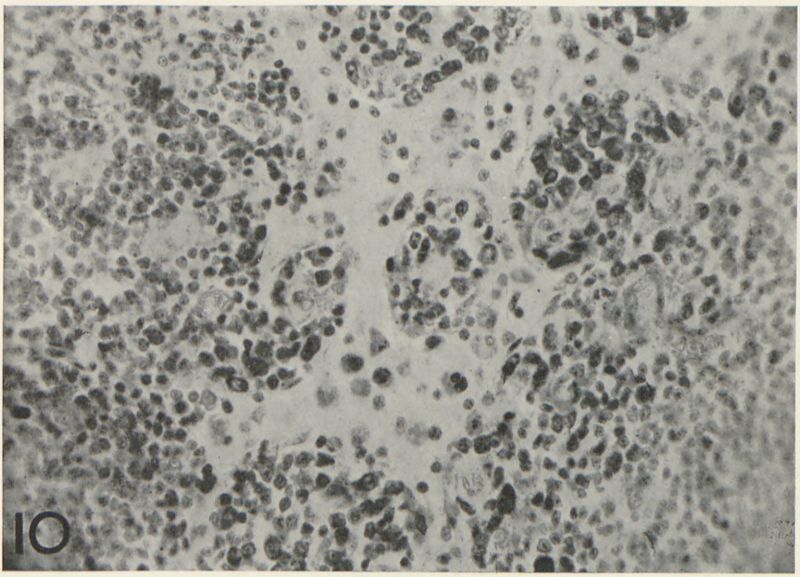

Fig. 10. Microphotograph. Intermediary sinuses of a hemolymph node from an animal twelve days after the operation (IV, 45). The sinuses are completely rid of erythrocytes and pigment cells. Maximow's azure-oesin. Hom. imm. 1/7, Oc. 5. Zeiss. × 450.