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RECHERCHES SUR LA MÉTAMORPHOSE D'UN MYCÉTOPHILE *DELOPSIS ATERRIMA* (ZETT.) (*DIPTERA NEMATOCERA*)

PAR

C. M. STEENBERG

AVEC 8 PLANCHES

KØBENHAVN
LEVIN & MUNKSGAARD
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Au mois d'août 1923, au cours d'une excursion à «Skäralid» (Söderåsen) en Scanie, j'ai découvert quelques formations particulières, noires, attachées à la face inférieure d'une planche. L'endroit de ma trouvaille était un petit étang, formé par une source dont l'écoulement avait été barré par la construction d'un chemin. L'une des extrémités de la planche était plongée dans l'eau, tandis que l'autre en émergeait, supportée par des touffes de laîche. Le dessous de la planche était donc très humide et c'est là que se trouvaient deux coques noires, collées par l'un des côtés. Leur forme était celle d'une bouteille couchée ou plutôt d'un urinal semblable à ceux qu'on emploie dans les hôpitaux. Le goulot des bouteilles était très court et l'ouverture fermée par un couvercle construit d'un joli tissu épais (pl. V, fig. 5, 6).

Il était évident que ces formations étaient des cocons d'insectes; elles rappelaient tout à fait les urnes noires d'un Mycétophile (Fungivore), décrites pour la première fois en 1846, par Bremi, de Zürich (litt. 3) comme appartenant au *Sciophila cellaria*, et plus tard, en 1849, par E. Perris, de France, sous le nom nouveau de *Mycetophila scatophora* (litt. 14).

La même année, j'ai réussi à trouver un grand nombre de ces cocons à «Timsfors»; en 1936, je les ai rencontrés à plusieurs endroits de Blekinge (p. ex. près de «Ronneby»),

et en 1937, à «Malen» (Hallandsås), qui sont toutes des localités de Suède; enfin, j'en ai également trouvé au Danemark: au bord du lac nommé «Furesø», dans la forêt de «Jelling», en Jutland, et dans la partie Nord de Seeland, mais dans ces derniers cas, en très peu d'exemplaires. Monsieur E. Nielsen et Mademoiselle A. Jøker m'ont indiqué une localité nouvelle pour le Danemark, à savoir un marais entre les deux petits lacs nommés «Store Gribsø» et «Lille Gribsø» dans le «Gribskov» en Seeland.

Deux questions se sont alors présentées: 1) Le cocon de Mycétophile que j'ai découvert, est-il de la même espèce que celle qui a été décrite par Perris? Car il y a une grande probabilité que les espèces de Bremi et de Perris sont identiques (litt. 7, 9). 2) Quel est l'aspect de la larve qui avait construit la coque remarquable?

A la première question il a été facile de trouver une réponse: J'ai réussi à découvrir quelques cocons dans la gorge de «Skäralid» en Suède, au bord du ruisseau nommé «Skäråen»; ils étaient posés sur des branches écorcées de hêtre, parfois à moitié immergées dans l'eau, ou bien sur de petits rameaux à demi cachés dans la mousse et le feuillage humide au pied des éboulis de pierres.

Ils ont été emportés à Copenhague, et au bout de 11 jours environ (le nombre de jours variant selon la température), il en est sorti un moustique noir (pl. VI, fig. 1). Il avait les particularités typiques d'un Mycétophile: de longues antennes articulées, attachées à une petite tête, qui est fortement infléchie sous le thorax très bombé, de longues pattes, qui présentent des hanches exceptionnellement robustes; mais sur la face inférieure du second segment abdominal on pouvait apercevoir deux longs poils raides, ce qui est très remarquable dans cette famille. D'après les indications

du diptérologue anglais M. F. W. EDWARDS, ce fait (avec des particularités du thorax) prouve qu'il appartient au genre *Delopsis*, dans lequel est également rangée la forme décrite antérieurement par PERRIS. De même, l'examen fit constater que mon cocon n'appartenait pas à l'espèce que les deux savants avaient trouvée en Suisse et en France: *Delopsis scatophora* (PERRIS), mais que c'était un cocon de l'espèce nordique *Delopsis aterrima* (ZETT.), connue comme imago, notamment en Suède (litt. 19, 21).

Il ne restait donc que de résoudre la seconde question: quel est l'aspect de la larve qui construit ce cocon singulier? C'était une question bien plus difficile, car, en dehors des deux larves connues de *Phronia*, j'avais trouvé, au cours de mes excursions, 2 à 3 larves de Mycétophiles qui se couvrent le dos de leurs excréments; chez quelques formes, les excréments étaient très foncés, de sorte qu'on pouvait bien s'imaginer que cette masse pouvait durcir et former le cocon de *Delopsis*. L'observation de ces larves n'a donné aucune réponse définitive.

J'avais soupçonné une larve, qui n'a été trouvée qu'assez rarement, d'être celle qui faisait la coque; elle se couvrait le dos d'une masse luisante, noirâtre, pâteuse, à contours irréguliers; mais je ne pouvais avoir une sûreté absolue que si j'emportais quelques-unes de ces larves si rares pour les isoler, afin de voir si elles construisaient vraiment la coque si caractéristique, et d'essayer de les faire éclore; mais, dans ce cas, je ne pouvais pas déterminer l'espèce de la larve, car il ne serait probablement pas possible de l'identifier d'après son aspect extérieur (c'est-à-dire d'après ses excréments). Le seul moyen qui me restait, était donc de chercher à trouver une larve en train de construire sa coque; celle-ci terminée, je pourrais alors y pratiquer un

trou et enlever la larve pour l'examiner, en espérant qu'elle présenterait, dans sa structure, des traits assez caractéristiques pour me permettre d'en déterminer l'espèce et d'éviter ainsi d'avoir, dans chaque cas, à faire construire leur coque aux larves trouvées.

En 1935, j'ai réussi à observer une de ces larves en train de construire son cocon, et j'ai pu suivre tout le processus. Plus tard, j'ai eu l'occasion de faire des observations sur des larves apportées de Suède, et de les photographier dans les différentes phases de la construction du cocon.

Étant désormais sûr d'avoir devant moi la larve que je cherchais, je l'ai enlevée de sa coque avant sa transformation en nymphe, et les traits de sa structure se sont montrés si caractéristiques, qu'elle se distinguait complètement des autres larves de Mycétophiles ayant un mode de vie semblable. Cette observation a été plus tard confirmée, lorsque j'ai réussi à trouver dans la nature plusieurs larves qui présentaient nettement la structure caractéristique du *Delopsis aterrima*.

La larve.

La larve complètement développée, prête à se chrysalider, a 4—6 mm de longueur, selon qu'elle est contractée ou étendue. Son dos est couvert d'une masse noirâtre, pâteuse, formée par les excréments de l'animal (pl. V, fig. 4); par des mouvements de l'extrémité postérieure, la larve rejette les excréments de l'anus sur son dos, où les nouvelles particules s'unissent avec la couche déjà existante. La masse excrémentielle est luisante, irrégulière, avec quelques rares boules; elle est retenue sur le dos par des zones de poils. En général, les excréments couvrent l'animal complètement,

et il est rare de voir apparaître la tête, comme le montre la figure mentionnée plus haut. Pourtant, il en est ainsi quand la larve n'est pas dérangée et quand la lumière n'est pas trop forte.

Il est également rare de voir une partie du corps aussi grande que celle qui se distingue sur la figure en question. Lorsque l'animal est regardé par sa face dorsale, le corps n'est pas du tout visible, et regardé de profil, on ne voit généralement qu'un bord inférieur étroit. Les stigmates, dessinés comme de petits points sur les côtés, ne sont pas couverts de la matière excrémentielle.

Sur les figures 1 et 2 (pl. I), on voit une larve bien étendue, complètement développée et débarassée de la masse excrémentielle.

La tête est petite en comparaison avec le corps, de forme conique, fortement chitinisée et par conséquent d'une couleur sombre, brun noir, se détachant nettement de la partie claire du corps. Seule la partie du «museau» est blanche et peu chitinisée.

Lorsque l'animal est vu par sa face dorsale (pl. II, fig. 1), on distingue nettement le *clypéus* (*präfrons, cl*); la partie étroite de celui-ci n'arrive pas tout à fait jusqu'à la limite postérieure de la capsule céphalique, et, en avant, il se continue imperceptiblement dans la partie antérieure de la capsule céphalique latérale et dans le *postlabrum* (*po*). Par là, la larve de *Delopsis* se distingue nettement des espèces de larves de *Phronia* (*P. strenua* et *P. johannae*) que j'ai décrites précédemment (litt. 17), et chez lesquelles une ligne très claire sépare distinctement le clypéus du bord chitinisé du «museau», situé antérieurement. La partie latérale de la capsule céphalique («Lateralplatten des Kopfes», *pl*) est postérieurement très bombée et ressemble d'ailleurs beaucoup

à celle du *Phronia strenua*. Pourtant, le collier (*co*), qui se trouve tout à fait en arrière, est plus grand, et il est surtout remarquable que les coins, qui, sur la face inférieure, indiquent la limite de la grande échancrure ventrale de la capsule céphalique, se continuent dans une longue saillie chitineuse, foncée et pointue vers la partie médiane (pl. II, fig. 2, *avp*). Probablement la saillie du côté droit et celle du côté gauche sont réunies par un isthme très peu chitinisé, qui disparaît pendant la préparation.

Le pont qui se trouve entre l'orifice buccal (*fb*) et l'échancrure ventrale de la capsule céphalique est entier chez les espèces de *Phronia*, et toute la limite de la capsule céphalique est garnie d'une bordure ininterrompue de chitine épaisse et noire (litt. 17, pl. III, fig. 2). Chez la larve de *Delopsis* (pl. II, fig. 2), le pont est entrecoupé, étant donné que les côtés latéraux de la capsule céphalique, qui constituent deux triangles (*ava*) se rétrécissant vers la ligne médiane, s'étendent à cet endroit l'un vers l'autre, sans pourtant se réunir complètement. Chez les *Phronia*, ces triangles sont plus courtes, mais la plaque maxillaire est large et occupe toute la place restante.

La plaque maxillaire (*pm*) est aussi pointue vers la partie postérieure médiane, mais les deux plaques ne se réunissent pas et n'arrivent pas jusqu'à la limite postérieure du pont. La capsule céphalique ouverte du côté ventral est une caractéristique très marquée de cette larve.

Les antennes (pl. II, fig. 1; pl. III, fig. 1, 2, *an*), qui ne se composent que d'un seul article, sont grandes, claires, bombées et pourvues d'un bord de chitine bien net; postérieurement, entre celui-ci et la capsule céphalique, se trouve un certain nombre d'organes sensoriels; un plus petit nombre est placé antérieurement et latéralement.

Les yeux (ocelles, *oc*) se voient au-dessous des antennes; ils sont très peu marqués.

Un peu plus loin, en avant des antennes, se termine la capsule céphalique, cependant que du bord antérieur dorsal (*postlabrum*, *po*) part, de chaque côté, un isthme chitineux formant un cadre de support pour le museau (les *prémandibules*, pl. II, fig. 1; pl. III, fig. 1, *pr*). Ce cadre, qui décrit une courbe autour de la région du museau, est pourvu d'une petite branche dorsale (*bd*) qui supporte la partie basale et dorsale de celui-ci; au milieu de la région latérale du cadre, part une pièce antérieure en forme de demi-lune (*bl*), qui, avec l'extrémité distale (ventrale) élargie du cadre même (*pd*), sert d'appui à une rangée de poils placés en éventail et ayant la forme de poignards ou de feuilles (*ép*). Sur la figure 1, pl. III, on aperçoit 26 poils, dont 19 sont placés sur la branche en demi-lune et les autres 7 en-dessous, sur la partie distale du prémandibule.

Le «museau» (pl. II, fig. 1, *la*, fig. 2 *mu*) est, en ce qui concerne sa forme, sujet à des changements au cours de la préparation; chez les animaux conservés dans l'alcool, il se peut que le liquide soit plus ou moins résorbé et que, par conséquent, le museau apparaît plus court que chez la larve vivante. Il est, en avant, coupé net, et, sur sa face supérieure, un peu aplatie; on voit 4 paires d'organes sensoriels, dont les deux premiers sont placés de chaque côté, tout près l'un de l'autre. La face ventrale (pl. III, fig. 1) est graduellement courbée vers le bas et se continue insensiblement dans la partie dorsale de la cavité buccale. Cette pente possède, en avant du cadre du museau, une dizaine de rangées transversales de spinules chitineuses très fines (*sc*), suivies — vers la bouche — d'une quantité de poils courts (*pi*), dont les pointes sont dirigées en dedans.

Pièces buccales.

L'ouverture de la bouche (pl. II, fig. 2, *fb*) forme une fente longitudinale étroite, qui s'élargit un peu postérieurement. Les pièces buccales extérieures les plus apparentes sont les mâchoires (*mâ*). Les mandibules sont invisibles du dehors, à part une petite partie libre de leur bord tranchant.

Les mandibules (pl. IV, fig. 1 et 2), qui ressemblent à la moitié d'une scie circulaire, sont pareilles à celles de la larve de *Phronia strenua* que j'ai décrites précédemment. Les condyles (fig. 1, *cd*) se trouvent plus près du point d'attache du tendon supérieur (*ts*) que de celui du tendon inférieur (*ti*), qui est très développé. Le bord libre est fourni de 12 grandes dents et se termine vers la base inférieure par un crochet recourbé en haut.

La face externe (pl. IV, fig. 2) est légèrement bombée. La face interne (pl. IV, fig. 1) porte, dans sa partie principale (*mala*), une rangée de petites protubérances situées parallèlement au bord tranchant dentelé, et, en dedans de celui-ci, vers le bas, une mince plaque en saillie, placée de biais et garnie d'une dizaine de longs poils clairs en forme de couteau (*praemala, prostheca, pr*).

Chacune des mâchoires (pl. V, fig. 1) repose sur une plaque de chitine carrée («Maxillarplatte» Holmgren, *cardo maxillae* De Meijere, *pm*), de forme irrégulière et séparée des côtés latéraux de la capsule céphalique par un «carreau» clair, allongé, en forme de virgule et très faiblement chitinisé (pl. II, fig. 2, *fv*). Dans la ligne médiane, elles sont également séparées par un «carreau» clair (*fm*). La plaque maxillaire est renforcée sur les côtés et au milieu.

La mâchoire proprement dite (pl. V, fig. 1, *mâ*) consiste en une plaque double, dont le bord médial est pourvu d'une série de grandes protubérances dentiformes, qui

servent probablement à retenir la nourriture pendant que les dents des mandibules, extrêmement mobiles, la coupent. Le bord latéral est droit, lisse et sans arêtes; l'extrémité se continue dans la palpe maxillaire courte et épaisse (*mp*), qui possède une partie de chitine fine et claire, garnie de différents organes sensoriels. La plaque externe, saillante et fortement chitinisée, se termine, du côté proximal et médial, par un manche assez long (*mc*), qui se prolonge à l'intérieur de la plaque maxillaire. La plaque interne est épaissie à quelques endroits seulement; une bande de renforcement, en forme de demi-lune, supporte les dents du bord médial; une seconde bande se continue le long de la partie proximale du bord médial et se prolonge jusqu'au manche de la plaque externe, auquel elle est soudée.

Ces processus en forme de manches servent à l'insertion des muscles moteurs des mâchoires. L'attache des muscles des deux paires de pièces buccales occupe presque toute la face interne de la capsule céphalique. Sur la face dorsale de celle-ci, on aperçoit nettement les cavités où s'insèrent les muscles, et à ces endroits la chitine est plus faible et par conséquent plus claire (pl. II, fig. 1, 2).

La troisième paire de pièces buccales, la lèvre inférieure, est tout à fait rudimentaire; une petite plaque ventrale (pl. II, fig. 4, *pc*) en est peut-être un reste, étant donné que l'orifice des glandes salivaires (*ds*) se trouve juste au-dessus, sur une papille. Sur la pl. II, fig. 4, on voit le liquide glandulaire coagulé (*lc*) dans l'ouverture.

A l'intérieur de la cavité buccale et sous l'entrée du pharynx se trouve l'hypopharynx (pl. II, fig. 3—5), placé presque parallèlement aux plans des mandibules et des mâchoires. C'est une pièce de chitine en forme de traîneau dont les deux patins sont constitués par deux barres de

chitine épaisses, se terminant en haut et en bas par deux apophyses. La partie médiane liant les deux parties latérales est pourvue de deux grandes ailes arquées et claires, séparées par deux dents triangulaires. Entre celles-ci on remarque une ouverture ronde ressemblant à l'orifice d'une glande, mais je n'ai jamais pu découvrir à cet endroit de sécrétion glandulaire. La partie externe de l'œsophage est supportée, du côté ventral, par une grande plaque courbée en U (pl. V, fig. 2), comme c'est aussi le cas chez les espèces de Phronia (litt. 17, pl. VI, fig. 1).

Le corps de la larve est divisé en deux parties: le dos et la sole à ramper; le premier, qui est large et fortement bombé, surplombe la partie inférieure comme un appentis (pl. I, fig. 1). C'est le bord de celui-ci qui porte les stigmates.

La sole à ramper (pl. I, fig. 2) nous montre de plusieurs manières une segmentation très nette. Dans la partie thoracique, elle se manifeste entre autres par les trois paires de disques imaginaux des pattes, qui se dessinent comme six taches circulaires de couleur blanche.

Immédiatement derrière la dernière paire, commence toute une série de rangées transversales de fines spinules noires, qui ressemblent à des épines de roses. Il y a en tout huit rangs transversaux, mais seul le premier est simple, les autres sont doubles. Pourtant, ces derniers se trouvent très souvent si près l'un de l'autre, que, vus à l'aide d'un microscope faible et l'animal étant en position contractée, ils ont l'air d'être simples comme le premier rang. Les pointes des spinules de celui-ci (au nombre de 52 environ dans la rangée transversale) sont dirigées en arrière. Aux rangs suivants (2 à 5), qui sont doubles, les spinules de la rangée de derrière sont les plus fortes, et elles sont égale-

ment dirigées en arrière, tandis que celles de la rangée de devant, qui sont plus faibles, sont dirigées en avant. Dans les trois derniers rangs doubles, les spinules de la rangée antérieure sont au contraire les plus fortes. Dans un des rangs transversaux du milieu du corps, j'ai compté environ 70 crochets.

La figure 3, pl. V, montre deux segments de la sole à ramper de la larve, vue du côté ventral et avec un fort grossissement. Ils portent le 4e et le 5e des doubles rangs de spinules. Devant et derrière chaque double rang, se trouvent de fines lignes de chitine foncée, généralement au nombre de 5 en avant et 5 en arrière.

La fonction des spinules et de leurs muscles respectifs a été décrite par Regine Schulze (litt. 15), qui mentionne aussi le mode de locomotion de la larve de Mycétophile.

La face dorsale de l'animal, fortement bombée (pl. I, fig. 1), est garnie de nombreux poils qui supportent et retiennent la matière excrémentielle. Cette garniture de poils est nettement divisée en segments, avec une large zone de soies plus fortes au milieu et des poils plus fins sur les pentes antérieure et postérieure.

En avant, derrière la première paire de stigmates, les individus préparés à l'alcool sont toujours quelque peu resserrés, et comme la sole à ramper n'y est pas pourvue de crochets, cette partie apparaît chez la larve vivante (vue de côté) comme un peu relevée du sol (pl. I, fig. 1; pl. V, fig. 4).

La région postérieure du corps est aplatie, surtout la face ventrale. En état de complète extension, l'anus est entouré de larges papilles, deux du côté dorsal et deux du côté ventral, situées tout près l'une de l'autre et très courtes. Vu d'en haut, le vaisseau dorsal apparaît par

transparence, et si l'on regarde par le dessous, on aperçoit nettement, chez quelques individus, la partie ventrale du système nerveux. Sans préparation, on peut distinguer du dehors le ganglion sous-œsophagien et neuf ganglions de la chaîne nerveuse ventrale. La région postérieure n'est pas tout à fait translucide, et les ganglions ne se voient pas. Les connectives sont nettement doubles. La partie postérieure de la tête est couverte par la partie antérieure de la peau du corps.

Les stigmates (pl. VIII, fig. 4—6) sont placés sur des saillies et ressemblent à des boutons. La première paire, qui est la plus grande (fig. 6), sort juste devant les disques imaginaux des premières pattes (pl. I, fig. 2). Les sept paires suivantes sont toutes pareilles, mais plus petites que la première; elles commencent un peu en arrière des disques imaginaux de la troisième paire de pattes, un peu en avant du premier rang de spinules.

Il n'y a pas grande différence entre la structure des stigmates. Sur chacun se trouvent 2 fentes stigmatiques, dirigées en biais vers le bas et vers le devant; le bord qui entoure chacun d'eux (*péritrème*, *pc*) n'est pas partout pareil. Dans la partie dorsale postérieure, il est plus haut et plus fortement chitinisé. La cicatrice trachéenne (*ci*) est nettement distincte sur la première plaque stigmatique (pl. VIII, fig. 6), où elle est placée excentriquement devant les fentes, mais sur les petits stigmates, elle se trouve entre les pointes de ces dernières (pl. VIII, fig. 4, 5).

La construction de la coque.

Voyons maintenant comment la larve construit sa coque de chrysalide. On trouvera ci-après la description de ce que j'ai vu le 6 août 1935. Vers 5 h. de l'après-midi, j'étais

en train d'examiner une grande et lourde larve, posée sur une branche écorcée de hêtre, que j'avais enfermée dans un tube de verre.

La larve ne bougeait pas, contrairement à son habitude. Quand elle est en mouvement, on peut apercevoir, sous la couche d'excréments, le bord blanc et étroit du corps de l'animal et un peu de la tête; mais, pour le moment, on ne voyait que la carapace, que l'animal avait abaissée jusqu'à l'objet sur lequel il reposait, de sorte que le bord de la masse excrémentielle s'attachait à la branche.

L'animal avait été placé dans un endroit frais, sur le balcon de l'hôtel, dans un verre cylindrique fermé par un bouchon finement perforé, afin de maintenir l'humidité nécessaire. J'ai alors enlevé le bouchon de crainte que le durcissement de la masse vaseuse ne pût se faire.

Bien que l'animal ne changeât pas de place, on apercevait des mouvements constants dans la masse excrémentielle noire. Les parois molles s'effondraient continuellement à différents endroits, formant des creux, mais la larve était toujours prête à les débosseler; elle travaillait probablement avec la tête. Visiblement, l'animal remuait beaucoup et poussait sans arrêt de la tête contre la coque. Plus tard, lorsque j'ai ouvert celle-ci, j'ai pu constater ce qu'il avait fait: la face interne était lisse et couverte d'un tissu extrêmement fin. Certainement, il se retournait sur lui-même, de sorte que la tête était tantôt à une extrémité de la coque, tantôt à l'autre.

Après un moment de repos, la masse vaseuse s'est abattue tout à coup à un bout de la coque ovale et allongée, et un assez grand trou est apparu. Il m'a été impossible de constater si la larve avait travaillé avec la tête ou bien avec l'extrémité postérieure; mais comme, par la suite, elle

a plusieurs fois agrandi l'ouverture avec cette dernière, pendant que la tête travaillait de l'autre côté, il est presque sûr que la perforation a été faite à l'aide de l'extrémité postérieure. L'ouverture était au commencement circulaire, mais bientôt elle a pris la forme d'un ovale allongé, la masse étant encore trop molle pour conserver la première forme. C'est seulement lorsqu'elle a commencé à durcir, que l'on a pu se rendre compte des contours définitifs. L'extrémité postérieure du corps, plate et linguiforme, était employée comme une truelle, et la face ventrale plate était spécialement active, cependant que le côté dorsal bombé servait à former le trou et à en appuyer le bord.

Le col de la coque n'a pas été construit avec de nouveaux excréments, mais avec l'ancienne paroi, pressée vers l'extérieur. Dans certains cas, les coques montrent distinctement, à des endroits assez secs, que cette opération a eu lieu au moment où la masse était déjà durcie, car le col est composé de débris irréguliers résultant de l'effondrement de la paroi de la coque.

La tête est apparue plusieurs fois dans l'ouverture, faisant des mouvements de va-et-vient dans le col comme pour en mesurer le diamètre et la longueur; puis l'animal se retournait, et l'extrémité postérieure recommençait son travail d'agrandissement et de polissage. La tête aussi était occupée à polir et à tapisser la paroi de la chambre principale. La larve travaillait donc simultanément avec les deux extrémités.

Lorsque le col de la coque a été maçonné, la larve s'est retournée et l'a couvert d'un tissu pour se mettre ensuite à filer la couche qui sert de couvercle. Cette couche (pl. IV, fig. 3, 4) ressemble aux disques de carton qui servent à boucher les bouteilles à lait, et est placée de la

même manière, un peu en dedans du col de la bouteille. Il était alors sept heures moins le quart, et il y avait deux heures que j'observais l'animal. C'était le moment du dîner, mais j'ai renoncé volontiers à mon repas, car je voulais suivre jusqu'à la fin ce phénomène si intéressant.

La larve a commencé par tisser le bord extérieur du couvercle (pl. IV, fig. 3), qui est massif et assez épais. Puis elle a continué par tisser le couvercle proprement dit. Le fil employé précédemment était très fin et à peine visible au microscope binoculaire grossissant de 20 à 30 fois, tandis que celui qui a servi pour la charpente du couvercle formait des bandes larges et bien visibles. Ces bandes étaient parallèlles au bord, mais pas tout autour. Elles couvraient $^1/_4$, $^1/_2$ ou bien $^3/_4$ du tour de l'ouverture; à chaque moment, la larve appuyait la tête contre les parois ou contre le bord de la bande déjà sécrétée, et à chaque fois la bande y collait et laissait un fil de fixation très fin et très court. Après avoir filé, par exemple un demi-tour, la larve s'arrêtait et se tournait pour recommencer à un autre endroit. Toute la partie périphérique du tissu est donc formée de larges bandes concentriques. Lorsque l'ouverture n'a plus mesuré qu'un tiers ou un quart du diamètre du col, la larve a cessé de tisser régulièrement et concentriquement, et elle s'est mise à filer irrégulièrement à travers l'ouverture centrale, formant ainsi de nombreux trous, petits, mais distincts (pl. IV, fig. 3). Ces mailles ont été ensuite remplies d'une manière spéciale: la larve a recommencé à sécréter des fils très fins, comme ceux de la couche interne de la coque; elle travaillait en jetant très rapidement la tête en avant et en arrière, comme si elle employait une brosse à blanchir ou bien un pinceau. La vitesse avec laquelle elle remuait la tête était de 3 à 4 fois

par seconde (j'ai compté en une demi minute 100 mouvements, dont 50 en avant et 50 en arrière). Au bout d'une heure à peu près, le couvercle était fermé; il avait une couleur blanchâtre et n'était pas transparent; mais pourtant on devinait le châssis (pl. IV, fig. 4).

En rentrant chez moi après les vacances, j'ai rapporté une autre larve, que j'ai observée un soir, alors qu'elle était en train de se bâtir une maison de la manière que je viens de décrire.

Pour étudier ce qui se passait dans l'intérieur, j'ai fait un trou dans la coque, et j'ai trouvé la larve en train de se fixer à la paroi interne à l'aide de fils.

Sur la fig. 3, pl. I, on aperçoit la nymphe à travers la grande ouverture que j'ai pratiquée. On distingue nettement les traits de l'imago. La nymphe est couchée sur le dos, la tête tournée vers le couvercle de la coque, et, en arrière, on remarque les restes de la peau larvaire rejetée, parmi lesquelles la capsule céphalique, fortement chitinisée, apparaît prédominante. Les ailes très irisées et les pattes longues et fortes, ainsi que les longues antennes, sont les plus remarquables des organes.

Les fils très fins qui fixent la larve à la face intérieure de l'enveloppe et la maintiennent sans doute en place, sont très caractéristiques. Malheureusement il ne m'a pas été possible d'en constater l'origine et la nature; ils avaient été vraisemblablement filés dans une période antérieure et collés au corps après la mue. Ils ne semblaient pas être des hyphes.

La nymphe s'est transformée plus tard en adulte. La durée de la métamorphose dépend de la température; mais en général elle est à peu près de 11 jours.

J'ai réussi plusieurs fois à observer comment fait l'imago

pour sortir de la coque. L'insecte parfait pousse avec le côté dorsal du thorax, courbé en avant et employé comme une sorte de bélier, le couvercle qui cède au bord, et l'imago en sort. Souvent le couvercle se referme, ce qui, au cours de mes excursions, m'a causé plusieurs fois des déceptions, lorsque l'examen d'un cocon trouvé m'a montré qu'il était vide: le moustique s'était envolé!

L'imago.

L'insecte qui vient d'éclore (pl. VI, fig. 1) présente un thorax très bombé et une petite tête fortement infléchie sous celui-ci et emboîtée dans sa partie antérieure comme un condyle dans sa cavité cotyloïde. Les yeux composés (*oe*) sont assez grands et possèdent chacun une petite ocelle (*oc*) située sur le bord antérieur. La longueur des antennes (*an*) correspond à peu près à celle du thorax; elles prennent naissance entre les yeux, tout près l'une de l'autre, et se composent de 16 articles, dont les deux proximaux sont les plus grands.

La partie distale de ces derniers a la forme d'un entonnoir bas, avec les bords couverts de poils fins. Une très longue soie se trouve sur la face dorsale du second article. Tous les autres articles sont cylindriques sans échancrures profondes. La surface des antennes est noire, mais recouverte de poils fins et soyeux, de couleur blanche.

La figure 1, pl. VI, nous montre que toute la face dorsale de la tête et du thorax, ainsi que plusieurs pièces pleurales et tout l'abdomen sont striés de poils gris clair, très fins, qui se détachent sur un fond noir ou brun très foncé. La figure que je donne du thorax correspond exactement à celle, un peu schématique, de M. Edwards.

Au-dessus de la première paire de pattes, on aperçoit,

du côté dorsal, *l'antepronotum* et le *postpronotum* fusionnés (*ap* + *pp*), et, du côté ventral de ceux-ci, le *propleure* garni d'une rangée longitudinale de 4 soies.

La rangée transversale des pièces pleurales qui suit (*mesopleura*), montre, vers le haut, derrière le stigmate thoracique antérieur, une grande plaque quadrangulaire, *l'anépisternite* (*supraepisternum, aé*), dont la partie postérieure est garnie de plusieurs soies, longues et raides, rangées transversalement, tandis que la plaque inférieure, un peu plus petite, le *sternopleure* (*infraepisternum, sp*), est dépourvue de poils.

La partie postérieure du thorax se termine, de chaque côté, par les 3 pièces chitineuses suivantes, nommées, en partant du haut: *ptéropleure* (*supraepimeron, pt*), *pleurotergite* (*pl*, partie latérale du *postcutellum*) et «*hypopleure*», pièce dont la formation est très complexe et qui, sur la figure, est en partie couverte par le balancier (*ba*). Les deux premières pièces sont sétifères; le ptéropleure porte deux soies, et le pleurotergite est pourvu d'une série de soies assez petites, tandis que la troisième pièce est tout à fait nue.

Le *scutum* (*sc*) et le *scutellum* portent, sur le bord postérieur, une série de soies raides, mais il n'y a pas de soies pronotales rigides.

Comme c'est ordinairement le cas, les pattes sont extrêmement longues. Les hanches (pl. VI, fig. 1) sont fortement développées, surtout les hanches postérieures. Elles sont de couleur jaune clair, à l'exception du quart supérieur de ces dernières, qui est noir. Le bord postérieur des hanches antérieures présente, dans sa moitié inférieure, un rang de longues soies, très fortes et noires; quelques soies également noires se trouvent aussi dans la partie distale

des deux autres hanches. La face extérieure des hanches antérieures est garnie de poils soyeux, blancs, les hanches intermédiaires sont lisses, et un tiers seulement des hanches postérieures est couvert de poils blancs.

Postérieurement et dans la région proximale, les hanches postérieures forment une protubérance ressemblant à une large bosse arrondie.

Les cuisses sont jaunes, couvertes de poils clairs, et, dans la partie antérodistale, garnies d'un rang de soies noires. Les tibias sont également jaunes et couverts de poils, les uns clairs, les autres foncés; les côtés et la région postérieure portent des rangées de soies noires, rigides, dont deux surtout sont très longues, atteignant jusqu'à deux ou trois fois la largeur du tibia. La partie distale est pourvue de deux ergots très longs.

Les pieds ont cinq articulations; ils semblent assez foncés, car la couleur jaune du fond est couverte de rangées de soies noires, dont les plus longues sont celles de la partie distale de chaque articulation.

Pour la description des ailes (pl. VI, fig. 2), je me suis servi du système de COMSTOCK et NEEDHAM, un peu modifié par PIERRE et HENDEL. *La nervure costale* (*c*), très prononcée, s'avance jusqu'au *radius 5* (r_5). Tout près de sa base, elle est réunie, par *la nervure transversale humérale*, à *la nervure sous-costale* (*subcosta*, *sc*), assez faible, qui se perd rapidement dans la direction distale. Les deux fourches, médiane (m_1, m_2) et cubitale (cu_{1a}, cu_{1b}), sont très longues, mais la bifurcation de la dernière (*cubitus*) se trouve un peu plus près de la base de l'aile que celle de la première (*media*), moins près toutefois que dans l'espèce typique (*D. flavipennis*). En dehors de la nervure transversale mentionnée plus haut, les ailes n'en présentent

qu'une seule autre, *la nervure radio-médiane* (*r-m*), qui réunit la nervure radiale (r_5 ou r_8) et la nervure médiane (*m*).

La nervure anale (a_1) est très vague; juste en avant, on remarque la trace d'une autre nervure (peut-être le *cubitus*$_2$). La partie postérieure de l'aile est consolidée par *la nervure axillaire* (a_2), mais aucune de ces trois dernières n'arrive jusqu'au bord de l'aile.

Comme le montre la figure, les nervures principales de la partie antérieure de l'aile sont garnies de soies. Toute la membrane de l'aile est recouverte de rangées longitudinales de poils fins (*microtricha*).

Les ailes sont de couleur légèrement bistrée; les parties qui, sur la figure, apparaissent comme un peu plus sombres, sont plus épaisses et d'une teinte jaunâtre. Sur un seul individu j'ai remarqué, près de la pointe et à la face inférieure de l'aile gauche (mais non sur l'aile droite), une tache sombre due à l'existence à cet endroit d'un assez grand nombre de poils, plus longs et plus foncés que les autres poils de la face inférieure.

L'extrémité des balanciers, qui sont jaunes et clairs, est un peu renflée et rappelle vaguement un cornet ou la cavité d'une oreille (pl. VI, fig. 1; pl. VII, fig. 2, *ba*).

En ce qui concerne l'abdomen, comme je n'ai réussi à faire éclore que des mâles, ma description s'applique seulement à ceux-ci. Fortement comprimé et formant, vu de côté, une ellipse allongée, il se compose de 7 segments, facilement visibles du dehors, dont le premier est très étroit et aplati, tandis que les autres augmentent de hauteur jusqu'au milieu de l'abdomen, pour diminuer ensuite dans la partie arrière. Notons, pour le septième segment, qu'on n'en aperçoit qu'une petite fraction de la partie postérieure (pl. VIII, fig. 1). Le mâle présente encore un segment rudi-

mentaire (le 8e), et l'hypopyge (pl. VIII, fig. 3), qui est très petit et presque tout à fait caché. Les individus éclos étaient presque toujours d'une couleur noire ou brun noir luisante, et couverts de fins poils clairs.

Un seul individu constituait par son aspect une exception: l'abdomen était plus large que d'ordinaire, la face inférieure était aplatie ou légèrement voûtée, et ne présentait pas la carène prononcée que l'on constate chez les autres. La figure 2, pl. VII, nous montre l'abdomen de cet individu, vu de dessous, et la figure 4 une coupe transversale de sa partie moyenne. On peut constater que les tergites (*tg*) sont très grands et ont la forme d'un fer à cheval. Leurs bords clairs, légèrement chitinisés, sont recourbés sur la face ventrale, et c'est sur la peau molle entre ces bords et les sternites, mais plus près des premiers, que se trouvent les stigmates (*sm*). Le premier sternite (fig. 2, st_1) se compose d'une seule plaque, fortement concave du côté antérieur où elle est munie de 2 pointes latérales, dirigées en avant. Le 2e segment de l'abdomen (st_2) porte 4 plaques, dont les 2 médiales, qui sont les plus grandes, sont fusionnées et offrent, sur le bord distal, les deux soies (*so*) caractéristiques du genre en question; de chaque côté de la plaque médiale se trouve une plaque plus petite. Cette quadripartition du sternite se retrouve dans les segments suivants. Sur chacun de ceux-ci (n° 3—6) se voient 4 longues plaques étroites (*stl, stm*), fortement chitinisées et réunies par des parties intermédiaires plus molles. Les deux plaques de chaque côté sont placées un peu de biais, de manière à former un toit bas, dont la crête est recouverte de poils fins dirigés vers le côté (pl. VII, fig. 4); les plaques médiales (*stm*) sont pourvues, du côté postérieur, de 4 soies,

dont les deux médiales, qui correspondent aux soies du second segment, sont les plus fortes.

Le sternite du 7e segment (pl. VII, fig. 2, *st. 7*) possède, dans la partie médiale, 2 renforcements de chitine, qui font saillie dans la région postérieure comme un lobe divisé en deux; sur les parties latérales se trouve de légères traces de plaques latérales. Le sternite du 8e segment (pl. VII, fig. 2; pl. VIII, fig. 3, *st. 8*), qui a la forme d'une pelle, est dirigé en arrière et ses bords sont garnis de soies, tandis que le tergite (*t 8*) est rudimentaire et forme une petite plaque de chaque côté. Le 9e segment, l'hypopyge (pl. VIII, fig. 2), est faiblement chitinisé.

Chez tous les autres spécimens que j'ai observés, les segments abdominaux 3, 4, 5 et 6 différaient de ceux de l'individu décrit ci-dessus, le grand tergite de chaque anneau étant complètement fermé du côté ventral, de sorte que les parties du bord, claires et molles, se touchaient presque (pl. VII, fig. 3, *tg*); les sternites étaient par conséquent arqués vers le haut de manière à former un canal ventral (*cv*), dont les ouvertures antérieure et postérieure se trouvent respectivement au 3e et au 6e segment abdominal. La figure 1, pl. VII, représente l'entrée postérieure de ce canal. Par les ouvertures distale et proximale, l'air y pénètre jusqu'aux stigmates abdominaux de la région fermée.

On comprend maintenant l'importance de la division des sternites en quatre plaques longues et étroites, séparées par des parties molles, car cette structure leur permet de se plier facilement (pl. VII, fig. 3).

Les grandes soies de la face ventrale du 2e segment sont placées à la surface, et non pas dans un creux, mais, lorsque l'animal se replie, la face inférieure du 2e segment

se trouve naturellement plus en retrait que la carène ventrale du 3e segment, ce qui explique que, dans les descriptions antérieures, les soies aient été représentées comme étant dans un creux.

Je ne peux pas expliquer pourquoi la partie ventrale de cet unique individu se trouvait «déployée»: c'est peut-être qu'il venait d'éclore, ou bien que des champignons parasites lui avaient dilaté l'abdomen; peut-être aussi cette particularité est-elle due à la préparation.

L'hypopyge (pl. VIII, fig. 2; voir aussi pl. VII, fig. 1, 2 et pl. VIII, fig. 1, 3) a l'aspect d'une coupe assez basse (*lamina basalis, lamelle basilaire, lb*), dont le bord ventral s'étire au milieu en une saillie linguiforme (*pv*). Le bord du creux porte, de chaque côté, 3 lamelles, dont la dorsale (*ls*) et la ventrale (*pi*) sont en forme d'ellipses, tandis que l'intermédiaire (*ps*) est plus étroite, pointue et a l'apparence d'un pivot. Du côté interne de la lamelle ventrale se trouve une toute petite lamelle garnie de soies (*ai*). Les trois grandes lamelles correspondent probablement — en allant du côté dorsal jusqu'au côté ventral — aux lamelles dénommées par Dziedzicki: «lamelle supérieure», «pince supérieure» et «pince inférieure». A l'intérieur du creux, entre les lamelles, on aperçoit l'appendice interne (*l'adminiculum, ad*); celui-ci ressemble à un demi-cône, ou encore à une langue creuse; il porte, sur la face ventrale, 2 soies longues et fortes, au-dessous desquelles se trouve une paire de plaques chitineuses posées en biais et délimitant une sorte de coussin, muni de 3 à 4 soies très courtes.

Contrairement aux 2 autres lamelles, l'extrémité de la «pince supérieure» (*ps*) ne possède pas de soies. Sur la face intérieure de la partie distale, qui est lisse, on aperçoit de petites saillies courtes et dentiformes.

Généralement, l'hypopyge est, chez les Mycétophiles, assez grand et dégagé, mais chez les individus examinés de l'espèce en question, il est, comme le montre la fig. 1, pl. VIII, presque complètement caché, de telle sorte que seules les pointes des lamelles sont visibles; si l'on ne procède donc pas à un examen microscopique très minutieux, on risque facilement de prendre ces individus pour des femelles.

Je ne connais qu'une seule reproduction de l'hypopyge de l'espèce examinée, celle de F. W. Edwards (litt. 7, pl. 56, fig. 157). Elle s'accorde bien avec mes propres observations en ce qui concerne cet organe. Il convient de remarquer expressément que l'hypopyge est tout à fait pareil dans les deux formes ci-dessus décrites.

Antérieurement, on a confondu les deux espèces, *Delopsis scatophora* (Perris) et *D. aterrima* (Zett.). M. Edwards prétend que la première est facilement reconnaissable par la couleur des côtés de l'abdomen, qui sont «extensively orange», tandis que l'abdomen de *D. aterrima* (Zett.) est complètement noir. Perris donne de la couleur de l'abdomen de *D. scatophora* une description un peu différente: «Vu de côté, on le dirait d'un brun cendré, parce que la couleur des poils, très visibles dans ce cas, se combine avec celle du fond; le ventre est toujours fauve, et, à certain jour, les segments de l'abdomen paraissent en dessous finement bordés de brun fauve; l'extrémité du dernier segment est toujours de cette dernière couleur, ainsi que l'organe copulateur tant mâle que femelle».

Cette description pourrait — en ce qui concerne la couleur — s'appliquer à l'individu aberrant que j'ai trouvé. Tant qu'il n'existe pas une reproduction exacte de l'hypopyge de *D. scatophora* (Perris), la distinction entre les

espèces ne pourra être faite avec certitude; mais M. EDWARDS a raison lorsqu'il dit que la description donnée par PERRIS de l'hypopyge de l'espèce désignée par son nom ne s'accorde pas avec la structure de cet organe chez les individus qu'il a, lui-même, classés sous *D. aterrima*. Je me crois donc en droit d'affirmer que mes individus appartiennent à l'espèce *Delopsis aterrima* (ZETT.).

Les deux espèces étaient antérieurement classées dans le genre *Epicypta* (comme je l'ai fait moi-même dans mon travail sur le *Phronia*, litt. 17); mais aujourd'hui on les rattache au genre *Delopsis*. C'est le mérite de M. F. JENKINSON et M. F. W. EDWARDS d'avoir signalé un caractère spécifique de ce genre, c'est-à-dire les 2 soies proéminentes qui partent d'un creux situé sur le côté ventral du second segment abdominal. Les deux espèces mentionnées plus haut possédant cette caractéristique, il faut donc les attribuer au genre *Delopsis*. D'après M. EDWARDS, elles sont les seules qui se rencontrent en Europe, le genre n'étant représenté, par ailleurs, qu'en Orient, en Afrique, en Australie et peut-être en Amérique.

Avant de conclure cette étude, je tiens à exprimer mes meilleurs remerciements à la Direction de LA FONDATION CARLSBERG, qui, de plusieurs manières, m'a accordé son appui, entre autres en m'aidant à acquérir un microscope binoculaire excellent, qui se prête particulièrement bien à mes analyses morphologiques spéciaux.

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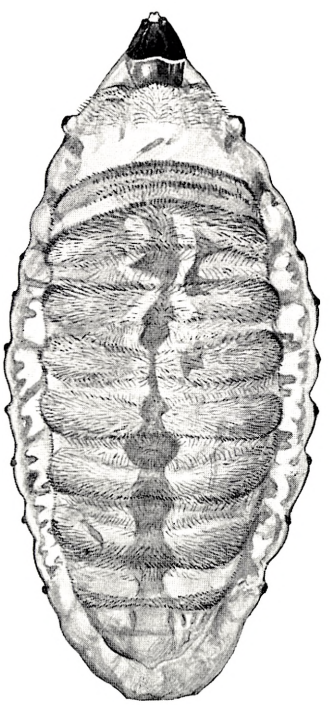
PLANCHES

Planche I.

Fig. 1. Larve du *Delopsis aterrima* (ZETT.), vue par la face dorsale et dépourvue de sa couche protectrice d'excréments.
Fig. 2. La même larve, vue par la face ventrale.
Fig. 3. Un cocon ouvert latéralement, de manière qu'on distingue la nymphe. Près de l'extrémité postérieure de celle-ci se trouve la peau larvaire. On aperçoit nettement la tête de la larve.



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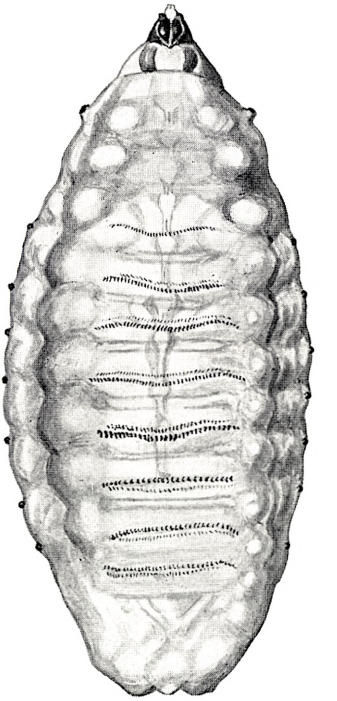

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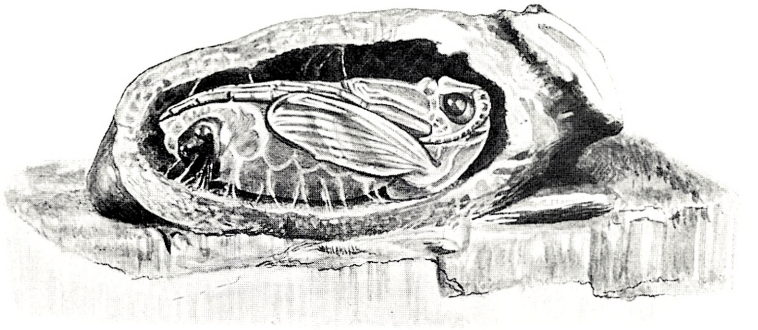

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Planche II.

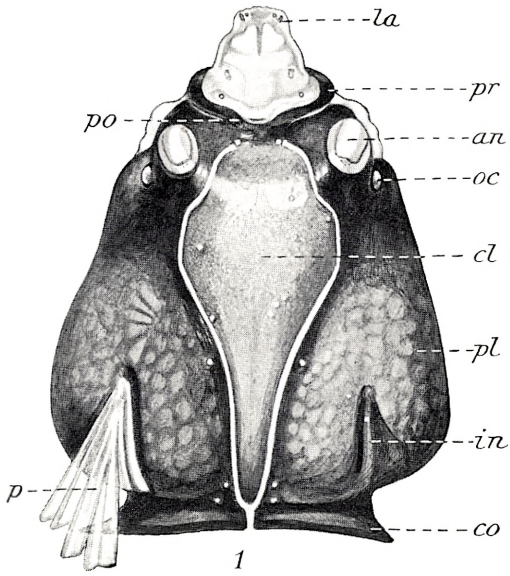
Fig. 1. Tête de la larve de *Delopsis aterrima* (ZETT.), vue par la face dorsale.
an, antenne; *cl*, clypéus; *co*, collier; *la*, labre avec des organes sensoriels; *oc*, ocelle; *p*, partie de la peau dorsale du premier segment thoracique, insérée dans une incision (*in*) de la capsule céphalique; *pl*, partie latérale de la capsule céphalique; *po*, postlabrum; *pr*, prémandibule.

Fig. 2. La même tête, vue par la face ventrale.
ava, angle ventral antérieur de la capsule céphalique; *avp*, apophyse ventrale postérieure de la capsule céphalique; *cl*, clypéus; *fb*, fente buccale; *fm*, «carreau» médial; *fv*, «carreau» en forme de virgule; *mâ*, mâchoire; *mu*, «museau»; *pl*, partie latérale de la capsule céphalique; *pm*, plaque maxillaire; *pr*, prémandibule avec l'éventail de poils.

Fig. 3. *Hypopharynx*, tourné de manière qu'on aperçoit les deux parties latérales en forme d'ailes et les deux protubérances dentiformes de la partie médiale (vu d'en haut).

Fig. 4. *Hypopharynx* de la larve, vu latéralement.
ds, conduit commun des glandes salivaires (séricigènes); *hy*, hypopharynx; *lc*, liquide glandulaire coagulé dans l'ouverture; *pc*, plaque chitinisée.

Fig. 5. *Hypopharynx*, vu ventralement et du côté antérieur.



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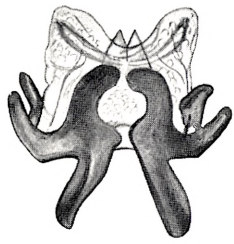

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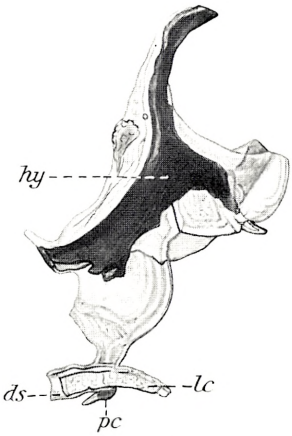

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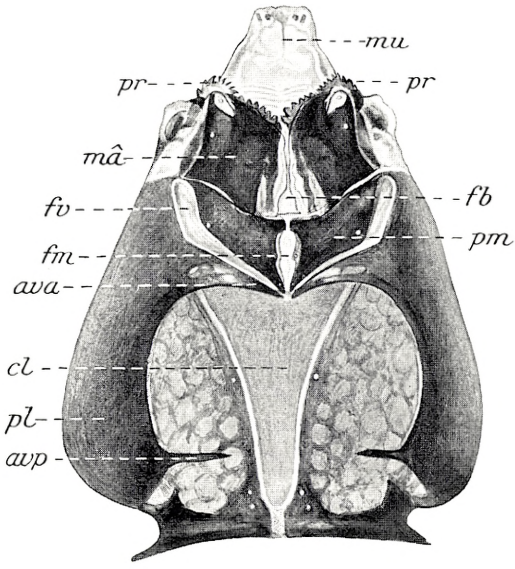

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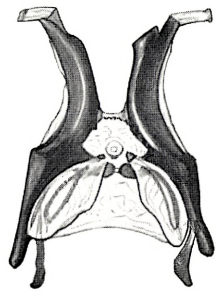

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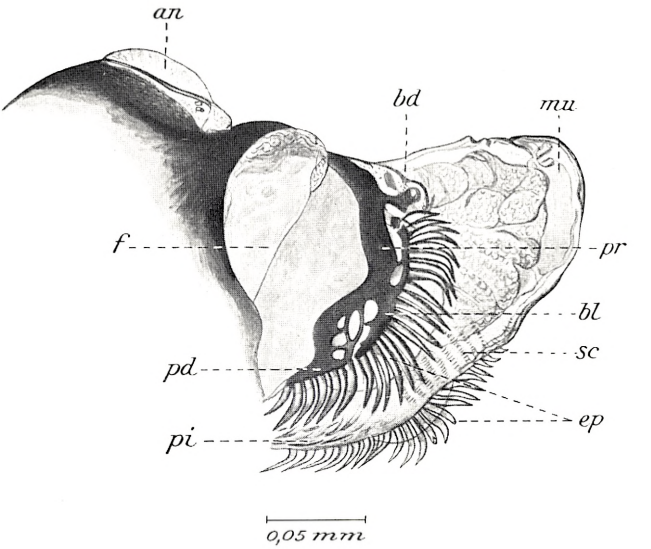
Julie Tesch del., Steenberg dir.

F. Hendriksens Repr.-Atelier

Planche III.

Fig. 1. Partie antérieure de la tête de la larve.
an, antenne; *bd*, branche dorsale du cadre de support; *bl*, branche en forme de demi-lune, qui sert d'appui à la partie supérieure de l'éventail; *ép*, éventail de poils; *f*, «carreau» latéral, séparant le cadre labial de la partie antérieure de la capsule céphalique, située en avant des antennes; *mu*, «museau»; *pd*, partie distale de la prémandibule; *pi*, poils internes du labre; *pr*, prémandibule; *sc*, spinules chitineuses.

Fig. 2. Partie antérieure de la tête de la larve, vue de côté et un peu de biais.
an, antenne; *bd*, branche dorsale du cadre de support; *ép*, éventail de poils; *f*, «carreau» latéral, séparant le cadre labial et la prémandibule de la partie antérieure de la capsule céphalique; *ma*, manche de la mâchoire; *mâ*, mâchoire; *md*, mandibule; *mp*, palpe maxillaire; *mu*, «museau»; *oc*, ocelle; *pm*, plaque maxillaire.



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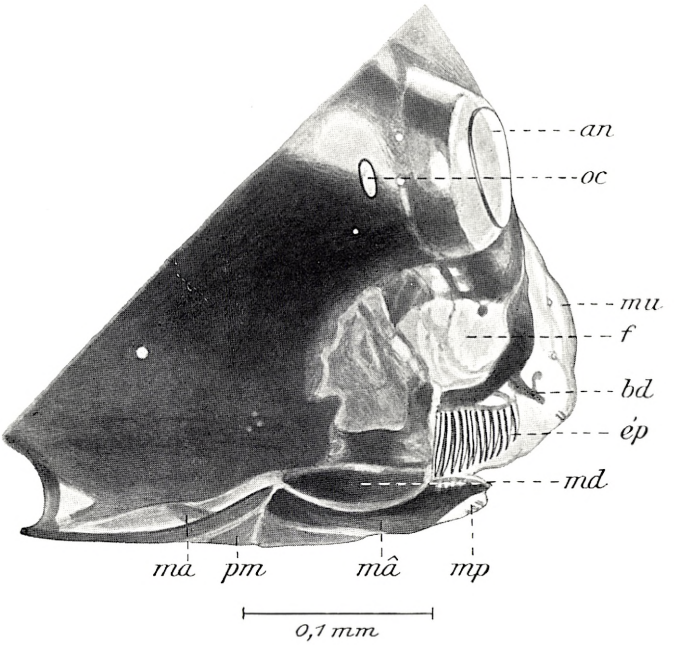

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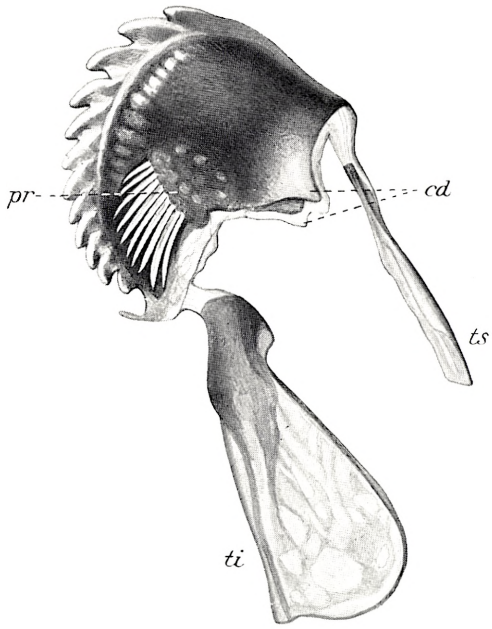
Planche IV.

Fig. 1. Mandibule droite, vue par la face interne.
cd, les deux condyles; *pr*, *prostheca* (*praemala*, *lacinia*) avec sa garniture de poils; *ti*, tendon inférieur; *ts*, tendon supérieur.

Fig. 2. Mandibule gauche, face externe.
ti, tendon inférieur; *ts*, tendon supérieur.

Fig. 3. Charpente du couvercle du cocon formée de gros fils.

Fig. 4. Couvercle achevé du cocon.



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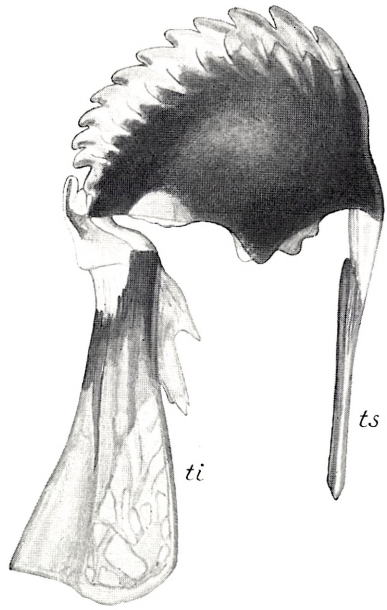

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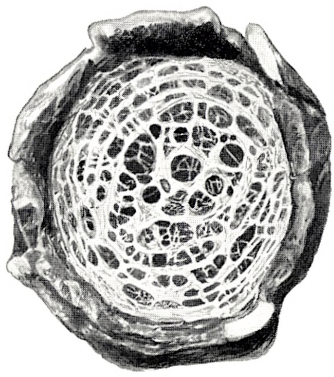

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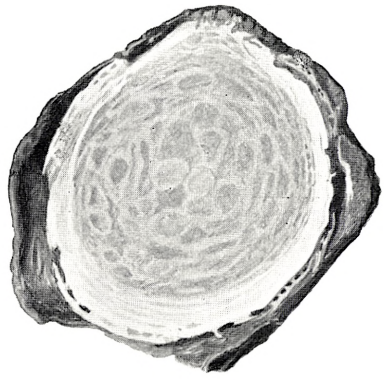

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Planche V.

Fig. 1. Mâchoire et plaque maxillaire droite, vues par leur face externe.
ava, angle ventral antérieur de la capsule céphalique; *fb*, fente buccale; *fm*, «carreau» médial; *mà*, mâchoire; *mc*, manche de la mâchoire; *mp*, palpe maxillaire; *pm*, plaque maxillaire.

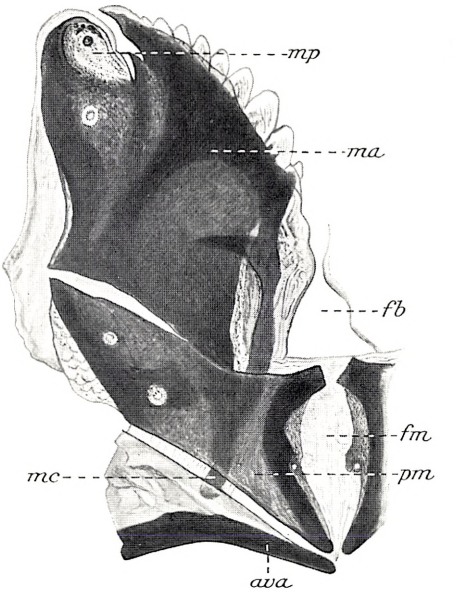
Fig. 2. Plaque en forme de U qui supporte l'entrée du pharynx.

Fig. 3. Deux segments de la sole à ramper de la larve, montrant les rangées de spinules et les fines lignes de chitine.

Fig. 4. Larve du *Delopsis aterrima* (ZETT.), vue de côté et dessinée d'après un individu vivant.

Fig. 5. Coque du *Delopsis aterrima*, vue du côté supérieur.

Fig. 6. La même, vue de profil.



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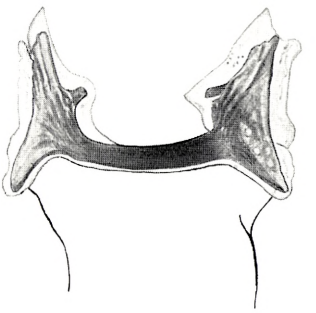

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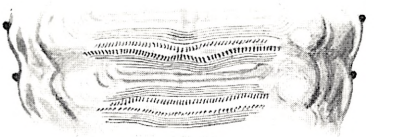

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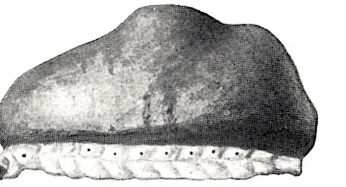

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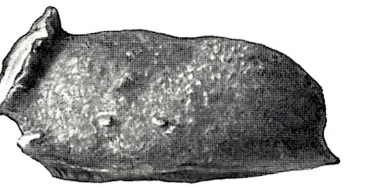

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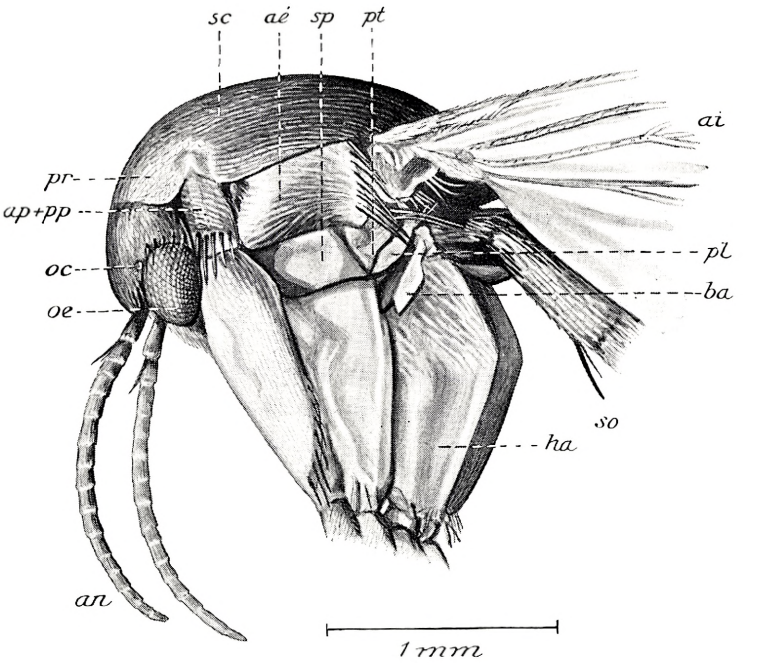
Planche VI.

Fig. 1. Tête, thorax et partie proximale des pattes et des ailes de l'imago.

aé, anépisternite; *ai*, aile; *an*, antenne; *ap* + *pp*, l'antepronotum et le postpronotum fusionnés; au-dessous de ceux-ci, le propleure avec les 4 soies; *ba*, balancier; au-dessous et au-devant de celui-ci, l'hypopleure; *ha*, hanche; *oc*, ocelle; *oe*, œil composé; *pl*, pleurotergite; *pr*, praescutum; *pt*, ptéropleure; *sc*, scutum; *so*, deux soies du deuxième segment abdominal; *sp*, sternopleure.

Fig. 2. Aile droite.

a_1, nervure anale; a_2, nervure axillaire; *c*, nervure costale (*costa*); cu_{1a}, cu_{1b}, nervure cubitale (*cubitus* 1); m_1, m_2, nervure médiane (*media* 1 et 2); r_{1+2}, r_5 (r_s), nervure radiale (*radius* 1 + 2 et 5); *r-m*, nervure transversale radio-médiane (*radio-medialis*); *sc*, nervure sous-costale (*subcosta*).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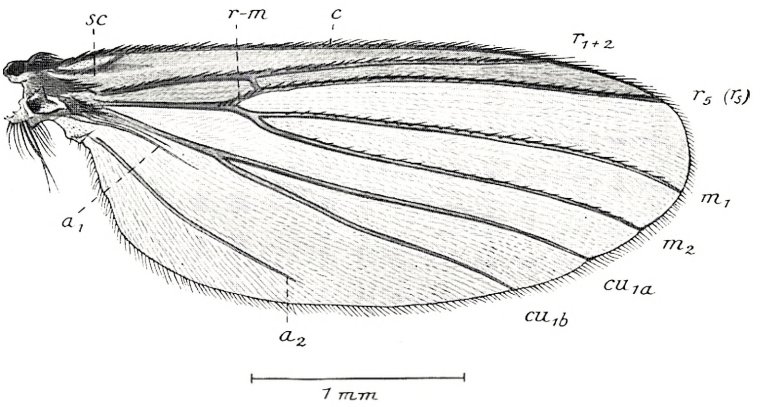
sc
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a_1
a_2
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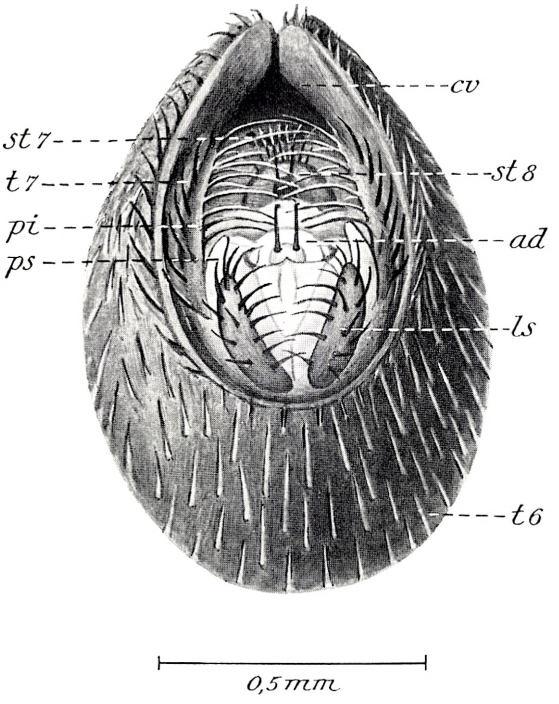
2

Planche VII.

Fig. 1. Extrémité postérieure d'un individu normal, vu par derrière pour montrer le canal ventral et l'hypopyge.
ad, adminiculum ou appendice interne; *cv*, canal ventral; *ls*, lamelle supérieure; *pi*, pince inférieure; *ps*, pince supérieure; st_7 et st_8, les sternites 7 et 8; t_6 et t_7, les tergites 6 et 7.

Fig. 2. Abdomen d'un individu aberrant, vu du côté ventral. Sur le côté gauche se distingue une petite partie du 3e segment thoracique avec le balancier, réuni avec le 1er segment abdominal. La figure montre la structure des sternites.
pv, partie ventrale claire des tergites; *sm*, stigmate; *so*, les deux soies caractéristiques; $st._1$, $st._2$, $st._7$, $st._8$, les sternites 1, 2, 7 et 8; *stl*, plaque latérale des sternites; *stm*, plaque médiale des sternites.

Fig. 3 et 4. Deux coupes transversales, un peu schématisées, de la partie moyenne de l'abdomen de l'imago. Fig. 3: coupe d'un individu normal; Fig. 4: coupe de l'individu aberrant. *cv*, canal ventral; *sm*, stigmate; *stl*, plaque latérale du sternite; *stm*, plaque médiale du sternite; *tg*, tergite.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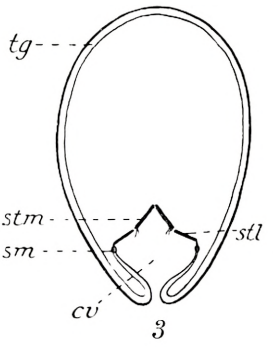
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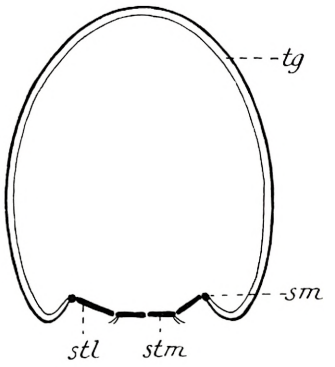
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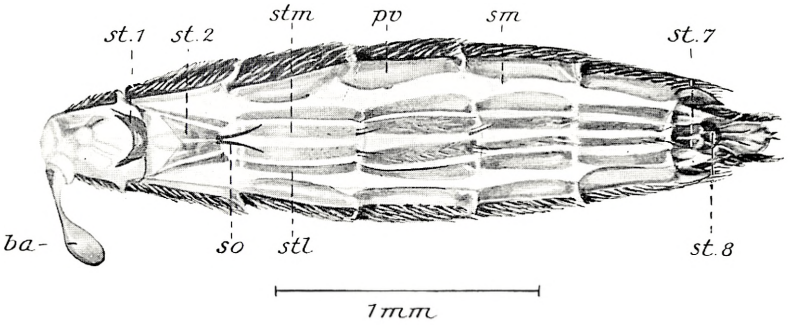
st.1
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2

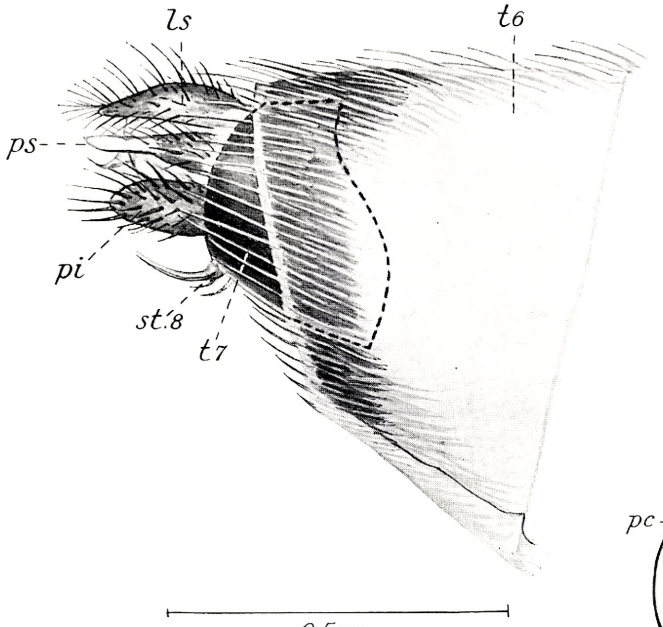
Planche VIII.

Fig. 1. Partie postérieure de l'abdomen de l'individu aberrant avec les sternites visibles du dehors; vue du côté droit. Le 6e segment n'est que partiellement dessiné; la limite antérieure du 7e segment est marquée par une ligne brisée. On aperçoit l'extrémité postérieure du 8e sternite et la partie libre de l'hypopyge.
ls, lamelle supérieure; *pi*, pince inférieure; *ps*, pince supérieure; *st.8* sternite du 8e segment; t_6, t_7, 6e et 7e tergite de l'abdomen.

Fig. 2. Hypopyge de l'individu aberrant, vu du côté ventral.
ad, adminiculum; *ai*, appendice interne ventral; *lb*, lamelle basilaire; *ls*, lamelle supérieure; *pi*, pince inférieure; *ps*, pince supérieure; *pv*, saillie ventrale sur le bord du creux de l'hypopyge.

Fig. 3. Hypopyge et avant-dernier segment d'un individu normal, vu de côté, après que le 7e segment est enlevé.
lb, partie basale creuse de l'hypopyge, lamelle basilaire; *ls*, lamelle supérieure; *pi*, pince inférieure; *ps*, pince supérieure; *st.8*, 8e sternite; t_8, 8e tergite.

Fig. 4—6. Stigmates de la larve.
Fig. 4, 5, un des stigmates postérieurs dans deux positions différentes; fig. 6, stigmate antérieur.
ci, cicatrice trachéenne externe; *pc*, péritrème de la fente stigmatique.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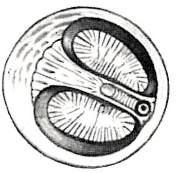

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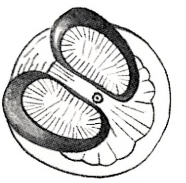

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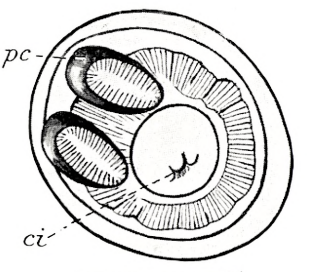
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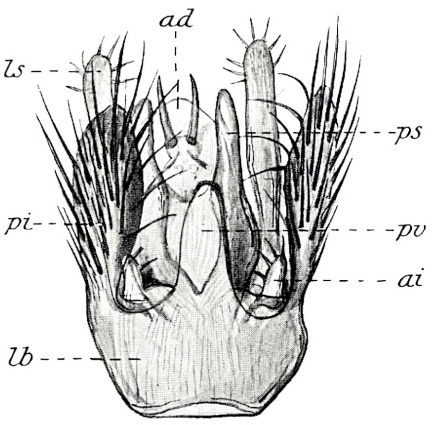
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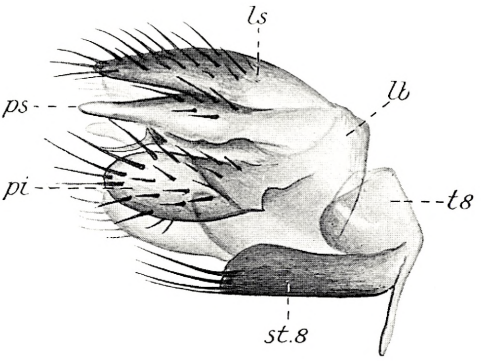
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In this paper we discuss the origin of eggs phosphorus by making use of labelled (radioactive) phosphate as indicator. As the presence of labelled phosphorus in organic compounds proves that these compounds were synthetised since the administration of the labelled inorganic phosphate we can draw conclusions as to the place and time of formation of the lecithin and other compounds containing phosphorus and present in the egg, by making use of the above mentioned method. In the hope of finding which phosphorus compounds of the blood are responsible for the formation of the lecithin and possibly other phosphorus compounds of the egg we administered labelled sodium phosphate to hens by subcutaneous injection and investigated after some time the yolks removed from the ovary and further the composition of blood and of some of the organs. In other experiments the eggs, layed at different times, were investigated. Finally we carried out also a few experiments in vitro.

Several of the compounds building up the egg contain phosphorus. Lecithin and other phosphatides form about one tenth of the yolk of the hens egg, the ratio of P to that of the other elements present in these compounds being about 1:25. From the phosphoprotein of the yolk vitellin is the most abundant, it contains on the average 0.54 % P. The total of phosphoprotein P present in the yolk is some-

what less than half of the phosphatide P present, while only small amounts of nucleoprotein P are found, as seen in table 1[1].

Table 1.

Phosphorus present in the yolk in percent of the total phosphorus.

Phosphatide P	61.4
Water soluble P	9.5
Phosphoprotein P	27.5
Nucleoprotein P	1.6

The phosphatide P content of the average yolk amounts to 60 mgm and its total P content to about 94 mgm. The total P content of the yolk is thus about 0.6 % of its total fresh weight, while that of the white of the egg is much smaller, amounting to about 0.01 %. The P content per gm of the small yolks found in the ovary is appreciably lower as seen from table 2 and increases with the increasing size of the yolk.

Table 2.

Phosphorus content of yolks.

Weight of yolk in gm	Lecithin P[2] in yolk in mgm	Total P in yolk in mgm	Lecithin P in 1 gm yolk	Total P in 1 gm yolk
0.03	0.03	0.049	1.00 mgm	1.63 mgm
0.1	0.20	0.26	2.00 —	2.67 —
0.694	1.72	3.57	2.49 —	5.18 —
2.51	6.25	13.0	2.49 —	5.2 —
4.63		40.0		8.7 —
7.68		93.75		12.2 —
13.6		125.0		9.2 —

[1] R. H. A. Plimmer and F. H. Scott, Journ. of Physiol. **38**, 247, 1909.

[2] Lecithin plus other phosphatides.

The phosphorus content of the shell of hens eggs is very variable, fluctuating between 0.1 and 0.3 % of the shell weight. It may be of interest to recall that on the average 59 % of the weight of the hens eggs is due to albumin, 30 % to yolk and 11 % to the shell.

According to general experience the yolk is formed while the growing egg is located in the ovary, about half of the white of the egg is formed by the albumin secreting portion of the oviduct, the shell membrane is deposited directly on this; and the more fluid portion of the albumin, constituting the second half of its entire bulk, enters through the shell membrane while the egg is in the isthmus and uterus. It has been found that the egg spends three hours in the glandular portion of the oviduct, one hour in the isthmus, sixteen to seventeen hours in the uterus including the time of laying.

Phosphorus compounds in hens blood.

The concentration of inorganic phosphorus, acid soluble phosphorus, lipoid (phosphatide) phosphorus and also of the total phosphorus present in blood, plasma and cells of chickens determined by HELLER, PAUL and THOMPSON[1] is shown in Fig. 1. The curves seen in the figure were obtained by analysing the blood of a large number of white Leghorn chickens. The analyses were repeated once a month or oftener beginning at the time when the chickens were 1 month of age and continuing through the periods of growth, egg production, and subsequent molting. The results present very instructing data, they show that the phosphatide phosphorus alone, especially that of the plasma, changes very markedly with the age of the chicken, a rapid rise in

[1] V. G. HELLER, H. PAUL and R. B. THOMPSEN, J. Biol. Chem. **106**, 357, 1934.

the latter taking place after the lapse of 5 months at the time of production, this high level being held under the

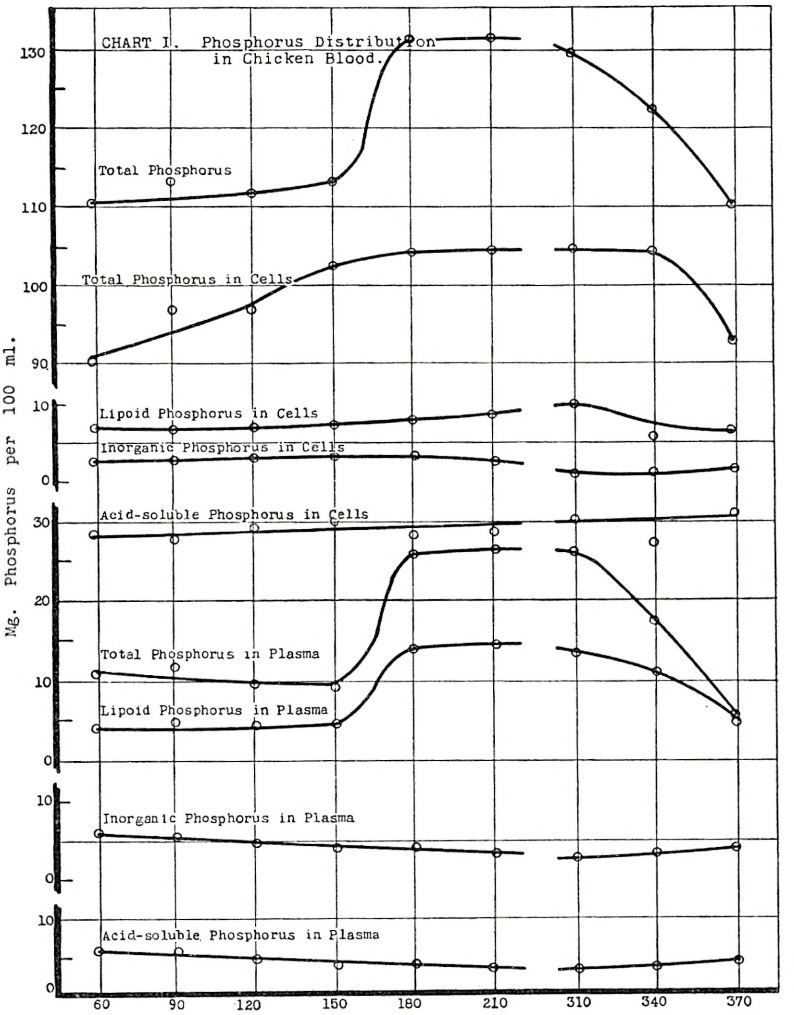

Fig. 1.

entire production period with some fluctuations and dropping quickly as production ceases and molting season approaches.

We determined the blood phosphorus of the laying hen

denoted as I, the result being seen from table 3. The blood phosphorus of another hen is discussed on p. 29.

Table 3.

P-content of hens blood.

	mgm % in plasma	mgm % in corpuscles	mgm % in blood
Phosphatide P	20.0	22.6	20.7
Inorganic P	5.4	—	—
Total acid soluble P	—	53.1	21.3
Rest (Protein) P	9.4	31.8	16.8

In the blood of non-laying hens[1] after 24 hours fasting an average phosphatide P content of 16.8 mg% was found the total plasma P amounting to 13 mgm%, the plasma inorganic P to 4.6 mgm%.

Experimental methods.

The yolk was dryed by adding ice cold aceton, the dry yolk was carefully pulverised and the powder obtained shaken for 15 min with 150 cc ether, the last mentioned procedure being repeated four times using fresh ether. The ether was than carefully evaporated, the residue taken up with dry ether, the latter removed by evaporation, this time in a Kjeldahl flask, and the residue ashed.

The phosphatides of plasma, corpuscles and total blood were extracted by an ether-alcohol mixture after Bloor. The extract was several times carefully evaporated to dryness and taken up with ether or petrol ether. The residue of the first extraction was treated with trichloracetic acid (10 cc of 10 % solution for each cc of blood) and from the filtrate obtained the inorganic P precipitated as ammonium mag-

[1] H. M. Dyer and I. H. Roe, J. of Nutrition, 7, 623, 1934.

nesium phosphate; the esters present in the filtrate were hydrolised and the phosphate produced by the hydrolysis of the esters precipitated as ammonium magnesium phosphate. Though the extraction and the neutralisation of the acid solution were both carried out at —9°, some of the inorganic phosphorus present may be due to decomposition of the esters and we therefore in table gave only the total acid soluble phosphours present in the corpuscles which includes both the inorganic and the ester phosphorus.

The liver was minced, dryed in vacuo at room temperature, pulverised, dryed again in vacuo and extracted with ether-alcohol (1:3), the latter being left to boil for 15 sec. In one case we extracted with ether alone to compare the active P content of the ether soluble phosphatides such as lecithin with that of the total phosphatides. The acid soluble P was extracted from the dryed liver powder by treatment with cold (—10° to —15°) solution of trichloracetic acid, first with a 10 % solution for 10 min and than twice with a 5 % solution each for 5 min. The inorganic and organic constituents of the acid soluble phosphorus were separated as stated above. The P content and activity of the residue obtained after extraction of the phosphatides and the acid soluble P was also investigated.

We determined the phosphorus content of a known fraction of the inorganic phosphate solution obtained in the above described procedures by the colorimetric method of Fiske and Subbarow. The phosphate content of another fraction of the phosphate solution was precipitated in the form of ammonium magnesium phosphate and its activity determined by making use of a Geiger tube counter. Let us say we have administered a hen a labelled phosphate solution containing 1 mg P and showing an activity of 10^6 kicks

per minute. We want to know what percentage of this labelled phosphorus will be found in the yolk lecithin. To arrive at this figure we take from our solution containing the labelled phosphorus as much as corresponds to $^1/_{10000}$ of the amount administered to the hen and precipitate the phosphate, denoting the precipitate obtained as our standard preparation, while we will call the precipitate obtained from the yolk lecithin as lecithin preparation. Before precipitating both the standard and the lecithin preparation we add to the solution a known amount, usually about 80 mgm, of inactive sodium phosphate, by so doing we diminish the amount of labelled phosphate possibly remaining in solution after precipitating with the magnesium citrate reagents and furthermore we obtain a standard and a lecithin preparation of equal weight. The β-rays emitted by the active phosphorus being to an equal extent absorbed in the two preparations the activity of which is to be compared, their weight and thus the thickness of the layers investigated being the same, there is no need to pay attention to the absorbtion of the β-rays in the samples investigated. Nor need the decay of the radioactive P be considered, as both the preparations to be compared, the lecithin and the standard preparation, decay at the same rate. The yolk residue obtained after removal of the lecithin was treated in similar way and also the white of the egg, while the shell was ignited and dissolved after ignition in hydrochloric acid, the solution being treated in the way described above. The samples were placed in small aluminium dishes having a surface area of 1.1 cm^2 and were placed immediately below the aluminium window of the Geiger-counter used.

Before discussing the results obtained we recall some facts about the circulation of labelled phosphorus in the blood.

Sensitivity of labelling.

Let us start from labelled sodium phosphate preparation of such activity that when the later was first put into the blood, 1 mgm. P will show 10000 activity units. As a result of a rapid exchange going on chiefly between bone phosphate and the inorganic phosphate of the blood 1 mgm will soon correspond to less than 10000 activity units. The total inorganic phosphate content of the blood remains constant, except in the case which we will not consider at present where a comparatively large amount is injected, while the individual phosphate ions will very soon be replaced to a large extent by other phosphate ions which were hitherto located in the skeleton or in other organs. After some time we shall find a large part of the labelled phosphate in the organs and the probability that the labelled phosphate leaves the organs and gets back again into the blood will increase, the effect of this reentrance into the blood will be that with increasing time the net rate of decrease of the inorganic labelled phosphate content of the blood will be less and less. Loss of phosphate by excretion and by the formation of organic phosphorus compounds in the blood and in the organs will further complicate the curve representing the labelled P content of the blood as a function of time. We determined the latter experimentally for the blood of different animals and also of human subjects, but not for the hen, (Compare, however, the results given on page 20.) The conclusions drawn in this paper do not necessitate the knowledge of the change of the labelled phosphate content of the hens blood with time, it is for our present purpose sufficient to bear in mind that an initial rapid decrease of the labelled inorganic P content of the plasma occur and becomes slower.

In the first experiments described in this paper, in contrast to most of our experiments, we administered large amounts of P, of the order of magnitude of 100 mgm. The very strongly active phosphorus preparation (of a strength of about 10^6 counts) used in these experiments was a generous gift of Professor LAWRENCE and was prepared by the bombardment of few grams of red phosphorus by high speed deuterium ions generated in Professor LAWRENCE's powerful cyclotron. The active P was thus mixed with a comparatively large amount of inactive phosphorus. In the experiments to be described, in contrast to some other experiments, the comparatively large amounts of phosphorus did not interfere, their presence in the active preparation has even the advantage that we can fix exactly the limit within which the sensitivity of our indicator, the number of mgm of total inorganic P indicated by 1 count activity, varied throughout the experiment. The 100 mgm P administered had an activity of 10^6 kicks. If the labelled P had not been diluted by non-labelled P of the organs we should have found after the lapse of 28 hours, the time of the experiment discussed on page 7, a specific activity of the plasma blood inorg. P — activity per mgm P — amounting to about 1 % of the total activity administered. (The amount of inorg. P present in the total plasma is only about 5 mgm and thus much smaller than the 100 mgm P administered.) As seen from Table 9, however, only 0.01 % was found, showing that from the inorganic P atoms present in the blood of the hen after the lapse of 28 hours only 1 % were those actually administered, the rest being ones originating from different organs and partly also from the food taken within that time.

We carried out three types of experiments:

a) Administration of labelled sodium phosphate to a hen and investigation of the eggs layed at different dates.
b) Administration of labelled sodium phosphate, killing the animal, removal of the yolks and investigation of these yolks the blood the liver and other organs.
c) Experiments in vitro in which eggs were placed in labelled sodium phosphate solutions for few days and investigated as to what extent the labelled P penetrated into the egg.

We will first discuss experiments of the type a).

a) Investigation of the labelled phosphorus content of eggs layed at different dates.

We injected radioactive phosphorus as sodium phosphate subcutaneously to hens and investigated the radioactive phosphorus content of the different parts of the eggs layed at different times. The first egg was layed $4^1/_2$ hours after administering the radioactive (labelled) phosphorus. We found the albumin to contain 0.0015 % of the 40 mgm of phosphorus injected, a similar amount 0.0014 % being present in the yolk. As the total phosphorus content of the yolk was found to be 100 mgm and that of the albumin only 4 mgm, the specific activity (active phosphorus per mgm normal phosphorus) was twentyfive times larger in the albumin than in the yolk. We found the lecithin phosphorus to be 53 % of that of the total phosphorus of the yolk and to be entirely inactive. No synthesis of lecithin molecules took place in the yolk therefore within the $4^1/_2$ hours preceding the laying of the egg, as in that case some active

lecithin molecules should have been formed; taking this fact into account the specific activity of the other than lecithin phosphorus present in the yolk works out to be thirteen times smaller than that of the albumin P. As 40 mgm active phosphorus were injected and only 0.0006 mgm are found in the albumen we can conclude that the formation of albumen from inorganic blood phosphorus in the course of the last $4^1/_2$ hours which the egg spent in the oviduct is a very moderate one, even when we take into account that the 0.0006 mgm active phosphorus found in the yolk passed through the albumen into the yolk bringing the amount of labelled phosphorus present at least temporarily in the albumen to 0.0012 mgm and that a large part of the active phosphorus injected gets rapidly replaced in the blood by non active phosphorus present in the skeleton and other organs as discussed on p. 7.

In the shell of the egg we find 10 mgm phosphorus by chemical analysis (colorimetric method of FISKE and SUBBAROW) and 0.1 mgm of the labelled phosphorus administred by radioactive determination (measurements with a Geiger-counter). 1 % of the shell phosphorus originates thus from the labelled phosphorus administred, which got into the shell in the course of the last $4^1/_2$ hours before laying the egg.

The labelled phosphorus content of eggs layed at different time is shown by the figures of the tables 4 to 6.

In what follows we discuss the significance of these figures. That the specific activity of the shell is very much higher after 0,17 days than at a latter date is due to the fact that shortly after the administration of the labelled P the activity of the inorganic P of the plasma is very high and it is the latter which is incorporated into the shell. As found by us

in numerous cases the active P content of the plasma decreases first rapidly and later at a decreasing rate the difference between the specific activity of the plasma and that of the tissues becoming less and less. The specific activity of the shell phosphorus is a measure of that of the inorganic plasma P at the time of formation of the shell and vice versa. The low specific activity of the albumin P in the egg layed after 0.17 days comes possibly for the following reasons. The white of the egg was already to an appreciable extent formed before the administration of the labelled P, the phosphorus compound of the plasma, presumably the plasma protein which mainly enters into the white was at such on early date after the administration of the labelled P active only to a small extent. The synthesis of labelled organic compounds takes some time and therefore shortly after the administration of labelled P the specific activity of the inorganic plasma P is much higher than that of the organic P. On the other hand the labelled organic P disappears at a slower, usually even much slower, rate from the plasma than the labelled P, the latter having an unique opportunity to exchange with the inactive tissue, especially bone tissue P. This fact explains also why except in the already discussed 0.17 day experiment in which special conditions prevailed, the albumin P has a greater specific activity than the shell P, in spite of the fact that albumin and shell are formed at about the same time. An other possible explanation of the low specific activity of the white P few hours after administration of labelled sodium phosphate is discussed on pg. 31.

When comparing the yolk figures with those of the albumin we have to bear into mind that contrary to the albumin which is formed within the day preceeding the

laying of the egg the greater part of the yolk was already present when the active phosphorus was injected and therefore the labelled phosphorus of the yolk was diluted by the unlabelled phosphorus already present in the yolk. With increasing time we should expect the amount of active phosphorus in the yolk to increase.

Labelled P administered at different dates.

In another set of experiments we were interested in producing strongly active egg lecithin to find out whether after feeding the latter as dry yolk to rats, the presence of active

Table 4.

Active phosphorus content of eggs. Hen I.

Egg layed after administration of active phosphorus	Percentage of active phosphorus administered found in: Shell	Albumin	Total yolk	Yolk lecithin
0.17 days	0.24	0.0015	0.0014	0.000
1.0 —	0.052	0.032	0.109	0.014
3.0 —	0.036	0.030	0.42	0.17
4.5 —	0.026	0.027	0.95	0.34
6.5 —	0.022	0.020	0.85	0.35

lecithin in the blood of the rats can be ascertained. This was found not to be the case. In these experiments we administered to the hen on several days active phosphorus which made the interpretation of the activity measurement of the yolk removed from the ovary rather difficult. A comparison of the activity of the shell of the yolk with its fluid interior revealed large differences. The semi-solid yolk shell formed from very active blood was found in one case to be

Table 5.

Hen I.

Percentage active phosphorus administered found in 1 mgm egg phosphorus x 10^3. (Specific activity x 10^3).

Egg layed after administration of active phosphorus	Shell	Albumin	Yolk after removal of lecithin	Yolk lecithin
0.17 days	24.0	0.38	0.03	0
1.0 —	5.2	8.0	2.0	0.26
3.0 —	3.6	7.5	5.1	3.3
4.5 —	2.6	6.8	12.6	6.4
6.5 —	2.2	5.0	10.4	7.0

Table 6.

Distribution of the active phosphorus administered between different parts of the egg.

Egg layed after administration of active phosphorus	Shell	Albumin	Yolk after extraction of lecithin	Yolk lecithin
		Hen I.		
0.17 days	98.9 %	0.6 %	0.5 %	0.0 %
1.0 —	27.0 -	17.0 -	48.8 -	7.2 -
3.0 —	7.0 -	6.0 -	50.9 -	36.1 -
4.5 —	2.6 -	2.7 -	60.5 -	34.2 -
6.5 —	2.5 -	2.2 -	56.0 -	39.3 -
		Hen II.		
0.5 days	46.1 %	35.0 %	18.9 %	
3 —	30.5 -	6.8 -	38.2	24.5
4 —	15.0 -	6.2 -	56.4	32.4
6 —	7.2 -	2.8 -	58.5	31.5

seven times more active than the fluid interior of the yolk, and five times in another case. With decreasing size of the yolk the difference between the specific activity of the yolk

phosphorus originating from the inner and the outer part of the yolk diminished and finally vanished.

b) Specific activity of yolk phosphorus.

We administered to a hen 100 mgm P as sodium-phosphate showing an activity of 10^6 kicks and killed the hen after the lapse of 28 hours. From the ovary 34 yolks were removed and from the ovidicut one egg. The weights of these are recorded in Table 7.

Table 7.

Weight of yolk	Specimens present
About 30 mgm	20
— 100 —	9
— 700 —	1
— 2500 —	1
— 5000 —	1
— 7500 —	1
— 13000 —	1
(Egg) 18000 —	1

The specimens of 700 mgm and more were treated separately while averages of the 30 mgm and the 100 mgm yolks were taken. The lecithin was extracted by ether and the residue brought into solution as described above. The results obtained are seen in Table 8.

The specific activity of the total P shows a maximum in the case of the 2500 mgm yolk. This result, puzzling at first sight, can be easily understood after considering Fig. 2 taken from a paper of H. GERHARTZ[1], in which the daily increase in weight of the yolks of a hen is recorded. The yolk grown from active blood and thus active will be diluted by the

[1] H. GERHARTZ, Arch. f. d. gesamt. Physiol., 156, 215, 1914.

Table 9.

Specific Activity of yolk phosphorus.

(Percentage of the activity administered present in 1 mgm P)

Weight of yolk	Lecithin P	Non Lecithin P	Total P
30—100 mgm	0.00055 %	0.018 %	0.0073
700 —	0.00814 -	0.0173 -	0.0129
2500 —	0.0147 -	0.0186 -	0.0164
4600 —	—	—	0.0090
7700 —	—	—	0.0055
13600 —	—	—	0.0044

non active yolk already present and this dilution will lead to a decrease of the specific activity of its P content. The dilution being least in the case of the 2500 mgm yolk, (comp. Fig. 2) its specific activity is bound to be highest. It takes some time after administration of the labelled sodium phosphate until labelled lecithin is transported into the plasma whereas inorganic P of very high activity is present almost at once after injecting the active sodium phosphate. The non lecithin P of the yolk is partly inorganic P which gets into the yolk in the early stage of the experiment when its specific activity is very high (comp. pg. 10,); we must therefore consider the lecithin P and not the non lecithin or total P content as a proper measure ot the growth of the yolk. From the fact that the lecithin P of the 30—100 mgm yolks became active only to a very slight degree we must conclude for example that they had hardly grown within the last 28 hours. When discussing the labelled lecithin P present in blood and in some of the organs we shall find definite evidence that the yolk lecithin is drawn from the plasma lecithin.

It is of interest to remark that the ratio of the total active

lecithin content of small yolks, such as would require 10 days or more to attain completion, is a quantitative measure of their relative growth since the administration of the labelled P. When, however, comparing the lecithin P activity of a small yolk which increases its weight in the course of

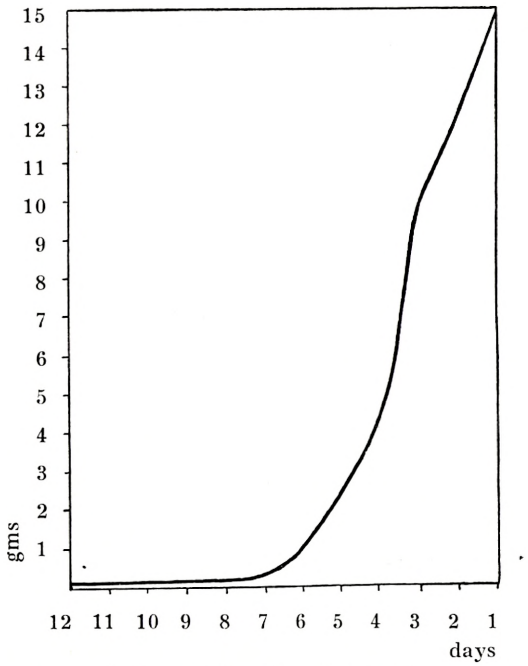

Fig. 2. Increament of the weight of yolks in the course of 12 days before completion of the yolk according to Gerhartz.

a day only to a slight extent with that of a large yolk growing at a rate of few gms per day the ratio of the total activities will not always be a correct measure of the growth since the administration of the labelled P. It may happen (comp. Fig. 2) that the growth of yolk per hour is larger at the end than in the beginning of the experiment the latter process determining thus to a larger extent the total growth within the time of experiment. From which it follows, that if at

the beginning of the experiment the specific activity of blood lecithin happens to be greater than at the end, we underestimate the growth of the large yolk.

It is, however, the determination of the slow rate of growth of the small and tiny yolks, often present in a very large number in the ovary, which can be of special interest and which can hardly be determined by any method other than that outlined above.

Investigation of blood phosphorus.

Plasma and corpuscles of the hens blood were separately investigated using the experimental method described on page 7. The results obtained are seen from Table 9 which contains data on the specific activity (activity per mgm P in percent of that injected) and also the total phosphorus present in the hens blood under the assumption that the volume of blood of the hen amounted to 150 cc and the volume of the blood plasma to 100 cc.

Table 9.

Specific activity and total phosphorus content of the hens blood.

Fraction	Specific activity	Total phosphorus content
Plasma inorganic	0.0104	5.4
Plasma phosphatide	0.0125	20.0
Corpuscles phosphatide	0.0046	11.3
Corpuscles acid soluble	0.0036	26.5
Corpuscles protein	0.0031	15.9

That the specific activity of the plasma phosphatide P is greater after 28 hours than that of the inorganic P is due, as discussed on page 14, to the rapid disappearance of the individual inorganic P atoms from the plasma. In the

experiment discussed on page 29, in which the hen was killed only 5 hours after the administration of the labelled P, the specific activity of the phosphatide P was found to be only 42 % of that of the inorganic P.

It is of great interest that the specific activity of the plasma phosphatide P is several times larger than that of the corpuscle phosphatide which shows that a much smaller percentage of the corpuscle phosphatide than of the plasma phosphatide is renewed in the course of the experiment. This is an interesting result as it definitely disposes of the often discussed possibility that the blood phosphatide is synthetised in the corpuscles. Some of the corpuscles being formed during the experiment from labelled plasma are bound to contain labelled phosphatides; labelled phosphatides can furthermore easily get into the shell of the corpuscles which are partly composed of lecithin.

A very suggesting change in the phosphatide content of hens blood at the time of production was ascertained by HELLER, PAUL, and THOMPSON (comp Fig. 1). The most interesting feature of the curves recorded by them is a gradual increase in the total P of the blood at the time of production, this high level being held during the entire production period with some fluctuations and dropping quickly as production ceases and molting season approaches. The increase is due to that of the lipoid P and is much more conspicious in the case of the plasma than in that of the corpuscles; the lipoid P content of the plasma is higher all through than that of the corpuscles, at the peak of production the former value being nearly three times higher than the last mentioned one. As about $^2/_3$ of the blood volume is composed of plasma it follows, that from the total lipoid P present in the blood $^5/_6$ are to be found in the plasma. The predominance of

phosphatide P in the plasma found for laying hens is entirely unique as seen from the figures of Table 10, but understandable if we envisage the great strain put on the organism of a hen as to lecithin supply. A hen laying daily has to produce about 60 mgm lecithin[1] P a day; taking a total plasma volume amounting to 100 cc the total lecithin P of the plasma works out to be 20 mgm. If the lecithin found in the yolk is, as suggested from our results, taken from the plasma lecithin then the plasma has to give off three

Table 10.
Phosphatide P in plasma and cells of different animals.

	mgm % P in		
	Plasma	Cell	Ratio $\frac{\text{cell}}{\text{plasma}}$
Rat	2.6	10	3.8
Rabbit	3.3	12	3.6
Man	9	19	2.1
Dog	14	14	1
Laying hen	14—20	8—23	0.87

times its total lecithin content in the course of a day thus putting an appreciable strain on the lecithin circulation. A strain which would be still more pronounced in the case of a lower plasma lecithin content.

Protein phosphorus in the hens blood.

After removal of the phosphatides and the acid soluble phosphorus, the remaining P is generally assumed to be

[1] Lecithin plus other phosphatides.

present as protein P. The protein P content of 31.8 mgm$^0/_0$ found in the corpuscles of the hen in the 28 hours experiment is much higher than in the corpuscles of the blood of other animals, the corpuscles of the rabbit containing for example, as found by Mr. ATEN, 4.4 mgm$^0/_0$. The same considerations apply to the protein P content of the plasma, which was found to amount to 9.4 mgm$^0/_0$ for the blood of the hen in question and of 7 mgm$^0/_0$ in the case of the hen discussed on page 29 while the plasma of a rabbit, for example, was found to contain only 0.03 mgm$^0/_0$ protein P. From the high value of the specific activity of the protein P in the 5 hours experiment it follows that the protein phosphorus compounds present in the plasma were renewed even at a higher rate than those of the phosphatides. This result suggests a great participation of the plasma phosphorus protein in the formation of the egg. To arrive at a final conclusion as to the relation between the phosphorus protein compounds of the plasma and those of the yolk and white is very difficult because of the fact that we lack simple methods of separation of the protein compounds. Vitelin, for example, can only be isolated by very tedious and lengthy processes and the isolation and separation of the blood protein phosphorus compounds are still more difficult, partly because only small quantities of these substances can be secured in the experiment. The simple fact that we have to base our conclusions on the amount of phosphorus present in the residue, remaining after extraction of the phosphatides and the acid soluble phosphorus compounds makes the result obtained less trustworthy than those arrived at when investigating the phosphatides, for example. The high value for the protein phosphorus of the corpuscles found by us, which may to some extent be due to an incomplete separation of the

phosphatides and acid soluble P, is supported by the data obtained by Heller, Paul, and Thompson. They find for the total P present in the cells of laying hens about 100 mgm $^0/_0$, but only about 40 mgm $^0/_0$ for the sum of inorganic acid soluble and lecithin P. The discrepancy suggests the presence of a further not investigated P fraction, which might be protein P. In the case of the plasma phosphorus the curves of Heller and his colleagues show the anomaly mentioned only to a smaller degree; the total phosphorus found by them is not very much larger than the sum of the acid soluble and phosphatide P.

The high protein P content of the blood plasma of a laying hen has presumably the same biological significance as the high phosphatide P content, namely a reduction of the strain put on the protein resp. phosphatide producing and carrying system in the organism of laying hens.

Phosphatide content of the liver.

We extracted the total phosphatide content of the liver of a hen 28 hours after the administration of the labelled P, using the method described on page 7. Since we were interested in seeing whether lecithin soluble in ether shows the same specific activity as the total phosphatides we extracted another part of the liver tissue with ether alone. We found no marked difference, as seen from Tables 11 and 12, which also contain data on the specific activity of inorganic and acid soluble (other than inorganic) P of the liver.

As seen from Table 11 the specific activity of the liver phosphatide P is 56 $^0/_0$ of that of the inorganic P, from which it follows that about one half of the phosphatide

Table 11.
Specific activity of the liver P.
(Activity per mgm P)

Fraction	Specific activity
Total phosphatides (ether-alcohol extract)	0.0152
Lecithin (ether extract)	0.0158
Inorganic	0.0272
Acid soluble, other than inorganic	0.0224

molecules are labelled and thus formed after administration of the labelled sodium phosphate. This result must, however, be interpreted with great caution. As already mentioned on page 10 in the early stages of the experiment the specific activity of P of the plasma is much higher than in the latter stages and the inorganic P of the liver was also more active at the early stage. This change of the specific activity with time would not affect our results if the specific activity of the phosphatide P should decrease with time at the same rate as does that of the inorganic P. That is, however, not the case. The phosphatide molecules can only escape from the circulation at an appreciable rate into the yolk, while

Table 12.
Percentage of labelled P administered found in plasma, corpuscles and liver.

Fraction	Total Plasma (100 gm)	Total Corpuscles (50 gm)	Total Liver (44 gm)
Phosphatide P	0.25 %	0.052 %	0.608 %
Inorganic P	0.056 -	— -	
Total acid soluble P	— -	0.100 -	1.643 -
Protein P	0.176 -	0.050 -	
Total P	0.482 -	0.202 -	2.251 -

the individual inorganic P atoms present in the plasma can rapidly exchange with such present in the skeleton, the latter being a much faster process in view of the huge extent of the skeleton. Therefore, when drawing conclusions from the comparison of the specific activities of the phosphatide P and the inorganic P as to what extent the phosphatide molecules got labelled we are apt to get values which are possibly too high. A trustworthy value could be obtained by keeping the specific activity of the inorganic P of the plasma constant by continuous injection of labelled phosphate of varying concentration and by thus avoiding a decrease in the specific activity of the inorganic P of the plasma, which is used for the synthesis of the phosphatide molecules in the liver and elsewhere. In the above case we can, however, conclude that a very appreciable part of the liver phosphatide molecules must have been renewed within the 28 hours of the experiment. In experiments on isolated livers in which the skeleton and other organs are not present it is easy to calculate from the ratio of the specific activities of inorganic P and phosphatide P the amount of newly formed phosphatides. In an isolated liver of a cat in the course of 2.5 hours about 1 % of the phosphatide molecules present are newly formed. If in the course of 2.5 hours in an isolated liver of a cat about 1 % of the phosphatide content is renewed there can be hardly any doubt that in the liver of a living hen in the course of 28 hours a large part of the phosphatide found is synthetised during that interval; in the liver of a living animal the enzymatic and other actions necessary for the synthesis of phosphatides are certainly as abundant as in an isolated liver and the phosphatide formation in the liver of a laying hen could hardly be less than in that of a cat. We are led to the same conclusion by the following

consideration. The daily amount of phosphatide P transfered from the plasma into the ovary is, in the case of the hen in question, which was laying one egg every other day, about 50 mgm. The main source of phosphatide production is, as we will see, the liver, and an amount not very far from 60 mgm must therefore have been produced daily in the liver of the hen. Since the latter containing 38 mgm of phosphatide P, a large amount of the liver phosphatide must have been renewed during the experiment. A similar conclusion applies to the plasma phosphatides, the 50 mgm phosphatide P being carried by the plasma, the total content of which is 20 mgm, the plasma phosphatide molecules must have been renewed to a large extent.

We are thus led to the result that the main source of phosphatides in a laying hen is the liver and that more than one half of the phosphatide molecules present in the hens liver were newly formed during the 28 hours preceeding the administration of labelled phosphate. That the greatest part of the phosphatides is formed in the liver of a laying hen and reach the ovary through the plasma is very clearly shown in an experiment in which the hen was killed only 5 hours after administration of the labelled phosphate. Before discussing the result of this experiment we want to mention the difference between the specific activity of the inorganic P extracted from the liver and that extracted from the plasma.

As seen from Table 6 the specific activity of the inorganic P isolated from the liver is found to be nearly three times as high as that found in the plasma. The low value of the latter at the end of the experiment was explained mainly by the exchange interaction between the skeleton and the plasma inorganic phosphate. Now one would expect the

liver inorganic phosphate to be in exchange equilibrium with the plasma phosphate and the fact that such a marked difference in the specific activities is actually found, shows that the interaction takes sometime. The above mentioned difference could also be wholly or partly explained by the assumption that what we isolated as inorganic phosphate was not wholly present as such in the liver but is freed through decomposition after the animal is killed either before or during the extraction process.

The acid soluble P of the liver, other than inorganic, mainly derived from P ester, shows, as seen in Table 11, a higher specific activity than the phosphatide P present in the liver.

Experiment on a hen killed after five hours.

3.8 cc of physiological sodium chloride solution containing 10 mgm labelled sodium phosphate were injected subcutaneously into a hen which weighed 1800 gms. The hen, which layed previously one egg daily weighing about 45 gm was killed after the lapse of 5 hours. The hen was then dissected by Dr. Marie Krogh, whom we wish to thank. Mr. A. H. W. Aten jun. extracted with great care the phosphatides of the liver, plasma, ovary and yolk. The procedure used will be described by him together with some other experiments. The results obtained are seen in Table 13 and 14. Two separate determinations were carried out, the values found and also their average are given.

As seen from Table 13 the specific activity of the phosphatide P, which is a measure of newly formed phosphatides, is by far the greatest in the liver and markedly higher than that of the plasma phosphatide P. Contrary to the 28 hours

experiment, where the percentage of newly formed phosphatide molecules in the plasma nearly reached that found in the liver, in the 5 hours experiment the concentration

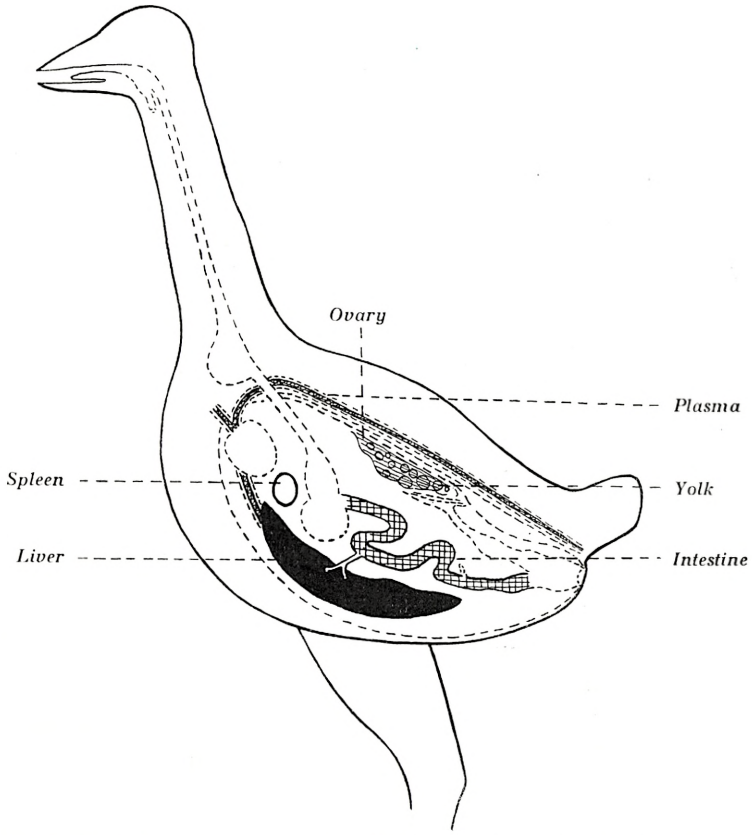

Fig. 3. The heaviness of the shading indicates the specific activity of the lecithin P and thus the percentage of the phosphatide molecules formed within the last five hours in the total phosphatides of the organ in question.

gradient in the flow of labelled phosphatides directed from the liver into the plasma is very clearly shown (comp. Fig. 3). The percentage of labelled molecules in the ovary phosphatide is, on the other hand, much smaller than in

Table 13.

Specific Activity of Phosphatide P.

Organ	Specific activity (% of activity given, found in 1 mgm P)		Relative specific activity; that of the inorganic plasma P taken = 1
	Single values	Average	
Liver	0.094 0.082	0.088	0.54
Plasma	0.069 0.069	0.069	0.43
Ovary	0.0064 0.0064	0.0064	0.039
Yolk	0.0053 0.0075	0.0064	0.035
Intestine	0.018 0.018	0.018	0.11
Spleen	< 0.02	< 0.02	< 0.1

the plasma phosphatides. From this it follows that the labelled phosphatide molecules present in the ovary were within 5 hours only partly replaced by ones present in the plasma. We investigated a yolk weighing 1.0 gms. The figures obtained are given in Table 13. A second yolk investigated weighed 2.7 gms. and its lecithin P had a specific activity of 0.0050. The specific activity of the yolk lecithin of the first mentioned yolk was found to be about $^1/_{11}$ of that of the plasma lecithin. From these figures it follows that about $^1/_{11}$ of the 1.0 gm, i. e., 0.09 gm, of yolk were grown within 5 hours. The actual growth was, however, presumably greater than 0.09 gm, since in the early stages of the experiment the plasma phosphatide was only very slightly active and so was the yolk tissue formed in this phase of its development. The fact that the specific activity of the ovary phosphatide was found to be low, as low as that of the yolk, proves definitely that the role of

the ovary is not production of phosphatides but their extraction from the blood plasma together with other suitable constituents. The combination of phosphatides with proteins giving the characteristic composition and consistency of the yolk, is evidently one of its principal functions. In the experiment described above the specific activity of the P of the yolk soluble in trichloracetic acid was found to be 0.035, thus $^1/_{4.5}$ part of that of the inorganic P of the plasma, the latter being 0.16. Making the assumption that most of the acid soluble P originates from the inorganic P of the plasma we find a growth of the yolk amounting to $^1/_{4.5}$ part of its weight of 1.0 gm during the experiment. While the above mentioned figure of $^1/_{11}$ was, as already mentioned, a lower limit of the part of the yolk newly formed within 5 hours, the figure of $^1/_{4.5}$ is a higher limit. A part of the acid soluble yolk phosphorus was formed at an earlier stage when the specific activity of the plasma inorganic P was appreciably higher than at the end of the experiment, and as our calculation is based on the specific activity of the plasma inorganic P at the end of the experiment it gives too high a value for the amount of yolk formed during the experiment.

The phosphorus of the white of the egg removed from the oviduct had a low specific activity, namely 0.0013. This is an interesting result in view of the strong activity shown by the phosphorus compounds present in the plasma (comp. Table 14). A possible explanation of this result is that some of the phosphorus present in the protein or other compounds of the oviduct tissue is utilised to produce the phosphorus compounds present in the white of the egg. In the course of five hours perhaps the compounds present in the tissue of the oviduct get labelled only to a slight extent. An other explanation is that while the average

plasma protein P has a high specific activity 0.15 after the lapse of five hours, the specific activity of the phosphorus of one of the components of the protein mixture might be low. If the P of the white originates from the protein or acid soluble fraction of the blood it must come from a less abundant component of the latter (comp. also pg. 14). We are now engaged in the investigation of the origin of the phosphorus present in the white of the egg.

Table 14.
Specific Activity of Plasma Phosporus.

Fraction	Specificactivity
Inorganic P	0.16
Lecithin P	0.069
Protein P	0.14

c) Experiments in vitro.

We placed eggs in a neutral physiological sodiumphosphate solution containing 30 mgm P for 24 hours and investigated the activity of the different parts of the eggs, the results being seen in Tables 15 and 16.

The comparatively high labelled P content of the shell is due to phosphate exchange processes between the large

Table 15.
Ratio of the specific activity of egg P and solution P.

	Shell	Albumin	Yolk
Egg I (Total P)	1.8×10^{-1}	1.4×10^{-3}	1.9×10^{-5}
Egg II (Total P)	2.0×10^{-1}	1.5×10^{-3}	4.0×10^{-5}

Table 16.

Distribution of the active phosphorus taken up between the different parts of the egg.

	Shell	Albumin	Yolk
Egg I	99.46 %	0.44 %	0.10 %
Egg II	99.40 -	0.41 -	0.19 -

shell surface and the solution and possible also to the formation slight amounts of calcium phosphate from the carbonate of the shell. An investigation of the activity of the lecithin extracted from the yolk gave an entirely negative result, this in agreement with the observation recorded on p. 13 that after the egg left the ovary no more lecithin formation takes place.

Discussion.

We saw that by investigating the labelled phosphorus content of eggs or yolks we could draw conclusions as to the growth of the egg or yolk since the date of administration of the labelled P. It is, for example, possible to show that while the egg is in the oviduct not only is no more yolk formed but also no new lecithin molecules are synthetised. Should suitable enzymes be present, new and thus labelled lecithin molecules could be formed without any growth of the yolk. The ovary of a laying hen contains numerous tiny yolks growing at a slow rate; by comparing the incorporation of labelled P by such yolks we get a quantitative measure of their relative growth since the administration of the labelled P. When comparing the growth of small yolks with large ones we can usually not obtain strictly quantitative results as to the relative growth because of the much more

rapid relative growth of large yolks compared with that of small ones.

Placing eggs in a solution containing labelled P for some days we find the shell to contain an appreciable part of labelled P, while the amount shown by the white and especially by the yolk is very small, though easily measureable, even in the case of the yolk. No formation of labelled lecithin is, however, found in the yolk.

As to the formation of lecithin in the growing yolk, we arrive at the following result: The phosphatides found in the yolk are synthetised at least to a large extent in the liver and are transported through the plasma to the ovary which extracts the phosphatides. This is most clearly seen in the experiment in which the hen was killed only 5 hours after the administration of the labelled sodium phosphate. In this experiment the specific activity of the liver phosphatide P reached 54 % of that of the plasma inorganic P, while the specific activity of the plasma phosphatide P was appreciably smaller, amounting to only 43 %; that of the ovary was very much smaller, namely 3.9 %, and about as large as that of the strongest yolk phosphatide P. In the 28 hours experiment, as to be expected, the difference in the specific activities was much smaller, the specific activity of the liver phosphatides being only somewhat higher than that of the plasma phosphatides. In the 28 hours experiment on the hen which used to lay one egg every other day the amount of phosphatides passing through the plasma on the way into the ovary was, in the course of the experiment, about twice the amount of phosphatides present in the plasma. In the 5 hours experiment, in which the hen experimented on was laying one egg daily, the amount of phosphatide passing the plasma on the way into the ovary

was about half the amount present in the plasma. From the low specific activity of the phosphatide P, that is from the low percentage of newly formed phosphatide, in the ovary it follows that in this organ only an insignificant amount of phosphatide can be formed. We have also to consider that a part of the labelled phosphatides found in the ovary is due to the presence of blood containing the latter. The specific activity of the plasma phosphatide P being appreciably smaller than that of the liver the labelled phosphatides must have come from the liver into the blood and not vice versa. By carrying out experiments in vitro with blood containing labelled sodium phosphate we found only a slight formation of labelled phosphatides, which is in accordance with the above conclusion.

The formation of phosphatides in the intestinal mucose by using radioactive phosphorus as indicator was first shown by ARTOM, PERRIER, SANTAGELO, SARZANA and SEGRE[1]. They found in an experiment carried out on a rat, that after injecting labelled sodium phosphate the phosphatides extracted from the gut after a few days showed a specific activity only exceeded by that of the liver phosphatide P, the ratio of the specific activities being 1.2. The phosphatide production in a laying hen is larger than in any other animal of similar size, as the amount produced daily to be incorporated in the yolk is as much as about 2 times that present in the liver which contains more phosphatide than any other organ. The laying hen is, therefore, a very suitable animal for studing phosphatide formation. In our 5 hours experiment the specific activity of the intestinal phosphatide P is much smaller than that of the liver phosphatide P and also than the plasma phosphatide P. The bulk of the labelled

[1] Nature, 139, 836, 1937.

P present in the plasma can, therefore, not originate from the intestinal phosphatide and the latter can not be the chief source of the yolk phosphatide. The phosphatides formed in the intestine can, however, have and presumably actually do have a role in the supply of the plasma phosphatides. The presence of phosphatides in the intestinal lymph was repeatedly shown[1] in experiments on dogs. The amount of phosphatides reaching the hens circulation by the influx of intestinal lymph could be ascertained by measuring the amount of intestinal lymph produced and also its phosphatide content. In figure 3 we show the specific activities of the phosphatide P in the organs of the hen killed 5 hours after the administration of the labelled sodium phosphate. The heaviness of the shading indicates the specific activity.

A hen laying daily deposits about 60 mgm phosphatide P in the yolk or about 3 times as much phosphatide as present in the plasma. In the course of a day the phosphatide content of the plasma of a laying hen must therefore be replenished three times. In view of this great strain on the phosphatide circulation in the plasma it is very significant that the plasma phosphatide content of a laying hen is higher than in most other animals. If the laying hens plasma should show such a low phosphatide content as does a rabbit or a rat (per cc) the plasma lecithin would have to be replenished as much as 17—22 times a day. It is significant that the high phosphatide content is maintained only during the laying period and that the cells contain less phosphatide than the plasma, a behaviour not shown by the blood of any other animal investigated. We find furthermore that in the course of 28 hours taken by the experiment a much

[1] H. E. Hamerich, Americ. J. of Physiol. 114, 342. 1934; S. Freeman and A. C. Joy l. c. 110, 132, 1935.

greater part of the phosphatides found in the plasma is labelled than of that contained in the corpuscles. This is a significant result as it demonstrates clearly that lecithin is carried to the ovary by the blood plasma and not the blood cells which obtain their lecithin in various ways. Labelled phosphatide could be taken up by the cell membrane, possibly diffuse through the cell membrane; labelled inorganic phosphorus which was found by us to diffuse at a moderate speed into the corpuscle could lead to the formation of labelled phosphatide phosphorus inside the latter, finally the lecithin could get into the corpuscles at their birth. If they are formed from labelled plasma the newly formed corpuscles should become labelled as well. As to the formation of labelled phosphatide from labelled inorganic P in blood, we found in experiments in vitro that such a formation actually takes place, though only on very minute scale. As to the rate of formation of blood corpuscles, some information on this point could be obtained by injecting labelled plasma and investigating the radioactivity of the phosphorus compounds isolated from the corpuscles after the lapse of some time. If after the lapse of a day, for example, only 1 % of the corpuscle phosphatides were found to be labelled we could conclude that the rate of formation of the corpuscles per day is less than 1 % of the total corpuscles present.

As to the white of the egg, we find that at least a large part of its phosphorus content is drawn from organic phosphorus compounds, possibly from protein phosphorus. We arrived at this result by comparing the specific activity of the phosphorus of the white of the egg with that extracted from the shell. The latter derives its phosphate content from the inorganic P of the blood plasma and is accordingly a

convenient measure of the activity of the latter. The shell is formed at about the same time as the white of the egg, the great discrepancy between the specific activity of the shell P and albumin P exclude the possibility that they are of common origin.

Summary.

By administering labelled sodium phosphate to laying hens the share of the labelled phosphorus administered in the formation of the yolk, albumin and shell of the egg can be followed by aid of radioactive measurements. The comparison of the specific activity (activity per mgm P) of the phosphorus extracted from blood plasma phosphatides with that extracted from the liver, the ovary, and the yolk phosphatides leads to the result that the bulk of the phosphatides of the yolk originate in the liver. It gets from the liver into the plasma and is then taken by the latter to the ovary.

No formation of phosphatides takes place in the ovary. After the egg leaves the ovary no more active phosphatide is formed. No formation of labelled phosphatide in the yolk can be ascertained in experiments in which an egg is placed for a day in a labelled sodium phosphate solution. In the last mentioned experiment in vitro slight amounts of labelled phosphorus are found in the yolk, appreciable quantities in the white, and large amounts in the shell.

The specific activity of the phosphatides extracted from the blood corpuscles was found to be only $^1/_3$ of that extracted from the plasma. Therefore, we conclude that the

phosphatides formed in the liver and other organs are carried to the ovary by the plasma rather than by the corpuscles. The latter apparently play no important role in this process.

Some of the labelled phosphorus used in our experiments was prepared by us from sulphur under the action of neutrons emitted from a radium-beryllium mixture most kindly put at our disposal by Prof. NIELS BOHR, and some of it was a generous gift from Prof. LAWRENCE of the University of California. The hens and yolks were kindly given us by Prof. A. KROGH and Dr. DAM. We wish to express our sincere thanks to those named above and also to Mr. A. H. W. ATEN and Miss HILDE LEVI for their assistance in this work.

November 1937. Institut for teoretisk Fysik. København.

Færdig fra Trykkeriet den 23. Marts 1938.

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EXCRETION OF PHOSPHORUS

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KØBENHAVN
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Because of the great importance of phosphorus in the formation of bones and the functional significance of a great variety of phosphorus compounds the balance of phosphorus intake and excretion has been investigated in numerous cases. A vast literature on this subject is available in which often also the route of secretion is considered, that is the ratio in which the excreted phosphorus is to be found in the urine and faeces of the human subject or animal investigated. What percentage of the phosphorus excreted in the faeces is due to non-absorbed material and how much to phosphorus originating from the body proper is, however, not yet known. Neither is any statement to be found on the fate of the individual phosphorus atoms, for example the phosphorus taken up with the food on one certain day. By using radioactive phosphorus as indicator we can follow the circulation of the phosphorus taken up at a certain date with food, the route it takes, and the rate at which it leaves the body. Some information on this subject has already been given[1]). In this paper we are communicating the results of investigations in which the excreta of human subjects, produced in the course of few months, were investigated both by radioactive and by chemical methods. Data are also given on the phosphorus excretion of rats.

[1] O. Chiewitz and G. Hevesy, Nature **136**, 754, 1935; Kgl. Danske Vidensk. Selsk. Biol. Medd. XIII, 9, 1937; L. Hahn, G. Hevesy and E. Lundsgaard, Biochem. J. **31**, 1706, 1937. W. E. Cohn and O. M. Greenberg, J. Biol. Chem. **123**, 185, 1938.

General experience as to phosphorus excretion.

Ingested phosphates are excreted partly in the faeces and partly in the urine, the ordinary distribution in adult man being about two thirds in the urine and one third in the faeces. Conditions that diminish the solubility or promote the precipitation of phosphorus in the intestinal canal tend to reduce the amount excreted in the urine and to increase that in the faeces. Vice versa, anything that favours solubility of phosphate in the alimentary tract augments absorption and increases urinary phosphorus at the expense of the faeces. Thus, diets high in calcium and low in phosphorus lead to high fecal output and phosphorus deficiency, probably because the phosphate forms an insoluble precipitate of calcium phosphate in the intestine. It has often been observed that fatty acids, by diverting calcium from phosphoric acid, may release the latter for absorption. Anything which tends to produce a more acid medium in the intestine exerts a favourable influence on the phosphorus absorption. Thus the ingestion of hydrochloric acid increases the urinary phosphorus at the expense of the faeces. The daily excretion of phosphate in the urine of an adult in normal conditions varies from 0.3 to 2 gm. of P. A careful determination of the average daily phosphorus intake[1] of 25 college women has shown an intake of 1.40 gm., which is thus somewhat higher than required by the Sherman Standard (1.32 gm.). In experiments[2], in which the subjects used were students and an acid forming diet containing 780 gm. milk was administered, the daily phosphorus intake was found to be 1.98 gm. When as large an amount as 10.8 gm. P was ad-

[1] R. E. Havard and G. A. Reay, Biochem. J. **20**, 99, 1926.

[2] M. M. Kramer, M. T. Potter and J. Gillum, J. of Nutrition **4**. 105, 1931.

ministered to a human subject, an output of 8.9 gm. was found, 79 % of the latter being present in the urine and 21 % in the faeces. About one fourth of the phosphate fed was stored[1]. In the early hours of the day the rate of excretion in the urine is minimal[2], and then it rises during the course of the day, to reach a maximum at 4 or 5 in the afternoon. The level of excretion is then maintained for the remainder of the day and throughout the night. Within wide limits there is no relationship between the amount of urinary phosphate and urinary volume. The rate of phosphate excretion is independent of the rate of water elimination even when, owing to copious diuresis, the urinary phosphate is below the level of the plasma phosphate[3]. As to the phosphorus excretion in animals we wish only to mention the following data collected by us. Rats weighing about 230 gm. excreted daily 28.7 mgm. P; within 7 weeks the ratio urine P: faeces P varied between 1.3 and 2.4, the average being 1.6.

Excretion experiments.

The most suitable method of analysis of urine was found to be the following: Evaporate to dryness an aliquote preliminarily treated with fuming nitric acid and determine its activity. Another smaller known fraction is digested in a Kjeldahl flask and its P content determined by the method of Fiske and Subbarow. The method tried first, based on the precipitation of ammonium magnesium phosphate from the urine, was found to be unsatisfactory, as activity

[1] W. T. Salter, R. F. Farquharson and D. M. Tibbets, J. Clin. Inv. **11**, 395, 1932.

[2] comp. C. H. Fiske, J. Biol. Ch. **49**, 171, 1921. S. Bellac, J. Chaussin, H. Langier and T. Ranson, C. R. **207**, 90, 1938.

[3] R. E. Havard and G. A. Reay, Biochem. J. **20**, 99, 1926.

measurements have shown that a part of the inorganic P present in the urine remains in solution after precipitation with magnesia or the magnesium citrate reagents. In view of the low activity of many of the faeces samples, we had to work up several gms. of dry faeces. It was too troublesome to dissolve such a comparatively large amount; we have therefore chosen the following procedure: The sample was first treated with nitric acid and then dried on a sand bath below 300°, to avoid loss of phosphorus. The activity of the substance was then determined. Another known part of the faeces sample, in most cases weighing only 30 mgm., was digested in a Kjeldahl flask and its P content determined by the colorimetric method.

Table I.

Time after administration of labelled P	Volume of urine in cc.	Specific activity of urine P (% of the labelled P administered found in 1 mgm. P)
3 hours	130	0.0051
5 —	80	0.015
7 —	110	0.0109
10 —	156	0.0059
11 —	88	0.0052
22 —	348	0.0033
27 —	170	0.0038
34 —	140	0.0024
44 —	570	0.0017
3 days		0.0013
6 —		0.00056
8 —		0.00064
13 —	Daily average	0.00052
16 —	950	0.0005
26 —		0.0006
32 —		0.00027
43 —		0.00016

Excretion of labelled phosphorus through the kidneys.

In the experiment described first the urine of a 40 years old female patient suffering from diabetes was investigated. The labelled sodium phosphate of neglegible weight was given with a glass of milk. We intended originally to investigate the excretion of the above mentioned patient after treatment with insulin as well; however, because the patient was soon discharged from the hospital, this investigation could not be carried out.

The average daily P excretion in the urine was found to be 950 mgm., the maximum of the specific activity of the urine P is reached after 5 hours (comp. Table I). The rapid decrease of the specific activity of the urine P, in the later stage of the experiment, is due to the rapid decrease of the plasma P activity with time; the labelled phosphate ions of the plasma being replaced by unlabelled ones already present in the body, primarily in the skeleton and the muscles. The specific activity of urine P, which is derived from the plasma inorg. P, first increases with time, due to the increased absorption of the labelled P administered into the circulation. Besides, by interaction with tissue phosphate, the plasma inorganic phosphate also becomes "diluted" by unlabelled P absorbed into the circulation from the food taken. The latter will therefore also influence the specific activity of the urine P.

In the experiment recorded in Table II the urine of a 22 years old male subject was investigated. The subject took only a minimal amount of food and drink. His daily urine excretion amounted to 610 cc. only containing 660 mgm. P.

In the course of the first day 6.8 $^0/_0$, in the course of

Table II.

Time since administration of the labelled P in hours	Volume of urine in cc.	Specific activity of urine P
3	75	0.0158
5	52	0.0205
7	59	0.0103
9	38	0.0090
11	54	0.0063
18	180	0.0049
23	150	0.0035
25	46	0.0026
27	50	0.0027
30	115	0.0032
33	80	0.0026
39	145	0.0022
48	175	0.0015
51	110	0.00097

the second day 3,3 % of the labelled P was excreted through the kidneys. Higher figures were found in the experiment on p. 10.

In the experiment recorded in Table III we wanted to ascertain the amount of labelled P excreted after a very short time. The male subject, 23 years old, excreted daily 750 cc. urine containing 825 mgm. P.

Tabel III.

Time since administration of the labelled P	Volume of urine in cc.	Specific activity of urine P
20 min.	90	0.00076
45 min.	16	0.008
18 hours	520	0.0073

After the lapse of 20 minutes an easily detectable part of the labelled phosphorus was thus found in the urine amounting to 0.1 % of the labelled P administered. From the rate of urine production by the subject in question we can conclude that only about 10 cc. urine were produced in the course of the experiment, 80 cc. being already present at the beginning of the experiment in the bladder. By taking account of the latter the specific activity of the P found in the urine formed within the first 20 minutes work out to be 0.0068, i. e. each mgm. P found in the urine, produced within the first 20 min. after drinking the labelled sodium phosphate solution, contained 0.0068 % of the phosphorus atoms present in the latter.

The fourth male subject investigated, 50 years old, excreted daily 730 cc. urine containing 790 mgm. P. The daily excretion was high, namely 21.1 % in the course of the first and 6.4 % in the course of the second day. The specific activities are seen in Table IV.

In another set of experiments 5 cc. of a physiological sodium chloride solution were injected into the veins of each

Table IV.

Time after administration of the labelled P in hours	Volume of urine in cc.	Specific activity of urine P
4	125	0.047
18.5	590	0.0109
25	100	0.0043
28	87	0.0037
30.5	70	0.0035
34	130	0.0028
35	57	0.0026
38	160	0.0032
45	150	0.0028
75	800	0.0018

Table V a.

Labelled P administered per mouth. Age of female subject 28 years, weight 65 kg.

Urine.

Time after administration of labelled P in days	Total volume in liter	Total P content in gm.	% of total activity administered, present in urine
0—15	14.98	7.787	10.3
15—39	25.25	14.400	5.3
39—52	12.98	8.239	3.6
52—76	25.58	9.093	1.5

Excretion of labelled P in the course of 76 days 20.8 %.
Total P excretion in urine 39.519 gm.
Daily P average excretion 0.520 gm.

Table V b.

Faeces.

Time after administration of labelled P in days	Amount of dry faeces in gm.	Total P content in gm.	% of total activity administered, present in faeces
0— 9	265.7	3.600	4.44
0—15	640.2	4.840	5.46
15—33	900.5	7.185	0.98
33—41	333.1	2.135	0.14
41—53	438.6	2.744	0.08

Excretion of labelled P in the course of 53 days 6.7 %.
Total P excretion in faeces 16.924 gm.
Daily average P excretion 0.320 gm.

Table VI a.

Labelled P administered per subcutaneous injection. Age of female subject 20 years, weight 65 kg. at the beginning of the experiment and 70 kg. at the end.

Urine.

Time after administration of labelled P in days	Total volume in liter	Total P content in gm.	% of total activity administered, present in urine
0—16	18.75	13.00	8.0
16—40	24.79	16.91	3.1
40—53	15.25	16.17	1.7
53—77	27.20	12.24	1.5

Excretion of labelled P in the course of 77 days 14.3 %.
Total P excretion in urine 52.32 gm.
Daily P excretion 0.680 gm.

Table VI b.

Faeces.

Time after administration of labelled P in days	Amount of dry faeces in gm.	Total P content in gm.	% of total activity administered, present in faeces
0— 6	132.6	2.585	0.60
6—17	572.6	9.360	0.84
17—38	636.5	7.070	0.12
38—45	214.7	2.815	0.10

Excretion of labelled P in the course of 45 days 1.7 %.
Total P excretion in faeces 19.245 gm.
Daily P excretion 0.447 gm.

of twelve human subjects. The solution contained 1 mgm. P as sodium phosphate, and also ^{32}P showing an activity of 10^{-5} milliCurie. We carried out these experiments to find out if the percentage of the activity, excreted within the first 24 hours through the kidneys, varies much from individual to individual. Though the human subjects were kept on the same diet, both the total P content and also the activity excreted within the first 24 hours varied markedly from individual to individual, as seen in Table VII.

Table VII.

% of labelled sodium phosphate, administered per intravenous injection to human subjects, excreted within the first 24 hours through the kidneys.

Human subject	Weight in kg.	Volume of urine in cc.	Total P in mgm.	% of labelled P recovered	Specific activity of faeces P
A	75	425	630	14.5	0.023
B	75	1515	1370	20.0	0.015
C	50	716	792	19.0	0.024
D	66	926	864	12.4	0.015
E	73	850	994	23.0	0.023
F	56	833	373	9.9	0.027
G	62	790	839	8.0	0.096
H	71	1164	1061	8.5	0.0080
I	80	1100	561	4.0	0.0071
J	61	632	707	14.4	0.020
K	64	420	518	8.9	0.017
L	71	1405	703	12.3	0.018

Excretion of labelled phosphorus through the bowels and the kidneys.

In the case of 2 female subjects we investigated the excretion in urine and faeces over a period of several

weeks. The results are seen in Tables V a and V b, resp. VI a and VI b.

In the first experiment (Table V) the labelled phosphorus found in the faeces was partly non absorbed P and partly such originating from the body proper. In the second experiment, registered in Tables VI a and VI b, the labelled P being not given by mouth, the labelled P present in the faeces must have originated solely from the body phosphorus and got through the digestive juices into the faeces. The lower total phosphorus excretion in the last mentioned case (Table VI) is presumably partly due to the remarkable increase in weight of the subject in question during the experiment.

Comparison of excretion through the bowels and the kidneys.

From the labelled P administered by mouth, in the course of two months, 6.7 % were excreted in the faeces. When given by subcutaneous injection about 1.7 % left through the bowels. The latter must have reached the intestinal tract with the digestive fluids. These carry labelled P just as well when the latter was administered by mouth; we have therefore to assume that somewhat less than $^1/_4$ of the 6.7 % labelled P found in the faeces originated from the body proper. The same ratio was found in our former experiments[1], while the absolute amount excreted in the course of the first week was in those cases 2.5 times as high as found in the present cases. The labelled phosphate which left the body unabsorbed was therefore 6.7 %—1.7 % = 5.0 %. We will now turn our attention to the result of chemical analyses which indicate the excretion of total P contained in the diet of the subjects.

[1] O. Chiewitz and G. Hevesy, l. c.

From the total P of the diet excreted 33 % and 35 % left the body through the bowels in two experiments, thus a decidedly higher figure than found for the excretion of the labelled sodium phosphate. It is also higher than found in a former case for the amount of labelled P which left the body absorbed (13 %). To account for this discrepancy two different explanations can be put forward. According to one explanation, phosphorus present in some of the organic phosphorus compounds of the food is less effectively resorbed than the labelled inorganic phosphorus added to the food. Such P is only split off in the lower region of the intestinal tract, in which place it has more chance to form insoluble calcium phosphate, for example, than in the more acid upper region. An alternative explanation is that it is not the binding of the phosphorus in the compound which matters, but the mechanical protection of the phosphorus compounds present in the food. From solid undigested particles the phosphorus particles will not be leached out properly. As to the resorption of phosphorus, in a recent work, carried out in Verzar's laboratory, Laskowski[1] has shown that the phosphate radical present in sodium glycerophosphate, introduced artificially into the upper part of the small intestine, splits off rapidly. The effect of this fast process is that the phosphate of the above mentioned compound is absorbed into the circulation as quickly as that of the sodium phosphate. When experimenting on rats an absorption of 68 % of the P administered was ascertained, after the lapse of one hour, with either compound. In the case of sodium phytin 62 %, in that of sodium diphosphoglycerinate only 42 % of the P content was resorbed. When the

[1] M. Laskowsky, Biochem. Z. **292**, 312, 1937.

phosphorus compounds were introduced into the lower part of the small intestine the percentage adsorbed into the circulation was much smaller[1] and amounted, in the case of sodium phosphate, to 38 % of that introduced. The difference observed is presumably due partly to the greater activity of phosphatases in the upper part of the intestinal tract, partly to the greater acidity prevailing there. We mentioned already that low acidity is favourable to the formation of insoluble phosphorus compounds. In so far as some of the phosphorus compounds present in the food decompose or get leached out in lower parts of the intestine, the yield will be lower and this may explain the difference observed between the absorption of labelled sodium phosphate and the total phosphorus present in the diet of the human subjects in question. We have also to consider that a part of the phosphorus may be contained in undigested fractions of the diet taken, protected by mechanical obstruction from the leaching effect of the digestive juices. We can expect more information on these points by replacing the administration of labelled sodium phosphate by that of vegetables grown on labelled soil and thus containing labelled phosphorus compounds. We can also feed labelled eggs, layed by hens to which labelled sodium phosphate was administered, or labelled meat. The tracing of to what extent the labelled P is absorbed from these foodstuffs is to be expected to supply us with important information as to their digestibility and seems to be a rational approach to the study of digestion, especially if foodstuffs containing other labelled elements beside phos-

[1] comp. also F. Verzar and H. Wirz, Biochem. Z. **292**, 174, 1937.
[2] L. Hahn, G. Hevesy and E. Lundsgaard, Biochem. J. **31**, 1705, 1937.

phorus could be administered as well. We can, however, also obtain a knowledge as to the amount of unresorbed P present in the faeces by an easier method than that sketched above, a method which we will describe in the following.

Origin of faeces phosphorus.

Let us assume that all phosphorus present in the food is absorbed into the circulation. In this case all labelled P found in the faeces must originate from the body proper. It is ultimately the plasma inorganic phosphorus which is responsible for the formation of the phosphorus compounds present in the digestive juices and, therefore, the specific activity (activity per mgm. P) of the faeces P should, in the above mentioned case, be equal to that of the plasma P. The specific activity of the inorganic plasma P being equal to that of the urine P we shall expect to find the specific activity of the faeces P to be equal to that of the urine P. If the above assumption does not hold and a part of the faeces P is unabsorbed inactive P originating from the undigested food, in that case the specific activity of the faeces P will be found to be lower than that of the urine P. The ratio $\frac{\text{specific activity of faeces P}}{\text{specific activity of urine P}} \times 100$ gives the percentage of P present in the faeces which originates from the body proper. If the food P is, for example, quantitatively absorbed, then the above ratio will work out to be 100. It is clear that different objections can be raised against the above considerations. One may object on the grounds that the specific activity of the plasma P, after the active P was added to the food, will first increase and then decrease, its variation with the time being

thus an intricate one. Another objection which can be raised is that the tissue P of the organs involved will also participate in the formation of the phosphorus compounds present in the digestive juices. These objections will not, however, be valid if we, before comparing the specific activity of urine P and faeces P, wait a considerable time, after administering the labelled P, before collecting the urine and faeces samples; preferably samples should be collected for several days. After the lapse of a considerable time, most P present in the different compounds of the organs responsible for the production of the digestive juices will be in exchange equilibrium with the plasma P, these showing thus the same specific activity.[1] In Tables Va and Vb the amount of P found in urine and faeces and also its total activity is stated, from which the specific activities could be evaluated. In view of the very long duration of the experiment in question and the comparatively low activities shown by many of the faeces samples, the accuracy of these experiments did not suffice to carry out such a calculation. To enable us to determine with sufficient accuracy the ratio of the specific activity of the urine P and the faeces P, we administered labelled sodium phosphate having an activity of about $^1/_{1000}$ milliCurie to a female subject and investigated the urine and faeces collected after the lapse of 7 and 8 days resp. As the faeces, collected after the lapse of 8 days, actually accumulated in the bowels at a somewhat earlier date it is advisable not to compare urine and faeces collected the same day, but to compare the

[1] A possible source of error may be found in the different rates of decrease of the specific activity of the inorganic P and of some forms of organic P present in the body (comp. G. HEVESY and A. H. W. ATEN, Kgl. Danske Vidensk. Selsk. Biol. Medd. XIV, 5 p. 35, 1939).

Table VIII.

Specific activity of urine P and faeces P of a female subject 7 resp. 8 days after administration of labelled sodium phosphate per intravenous injection.

Fraction	Number of counts	P content in mgm.	Specific activity (% of the activity administered per mgm. P)
Urine P	107.4	9.01	11.9
Urine P	118.9	9.80	12.1
Faeces P	53.9	18.60	2.9

faeces with the urine collected one day previously. The result of this experiment is seen in Table VIII. The specific activity of the total faeces P is only 24 % of that of the urine P, the faeces P must therefore to a large extent originate from non-absorbed food, which is the only source of non-active P. It follows from the above figure that 76 % of the P present in the faeces of the human subject in question is non-absorbed P, the rest originating from the body proper. This is, however, not to be interpreted as indicating a phosphorus absorption of the food taken amounting to only 24 %. When interpreting the above figure, we must take into account that the P excreted through the kidneys amounts to about twice of that lost through the bowels, and the sum of both values represents the total P present in the food, if we assume that the subject in question is in P balance. We then find that only 25 % of the total P present in the food was not absorbed into the circulation.

Through the courtesy of Dr. Kjerulf-Jensen, who is investigating the P metabolism of a human subject by making use of radioactive P, we could investigate the

faeces P and the urine P collected 28 days after administration of labelled P. The results are seen in Table IX.

Table IX.

Specific activity of urine P and faeces P of a female subject 28 days after administration of labelled sodium phosphate by subcutaneous injection.

Fraction	Specific activity
Urine P	8.07
Urine P	8.10
Faeces P [1]	1.77

From the ratio of the specific activities it follows that 20 % of the P precent in the faeces was of endogenous origin and that of the total P present in the food 27 % was not absorbed into the circulation.

Excretion by rats.

We determined also the ratio of the specific activities of the urine P and faeces P excreted by a rat to which labelled sodium phosphate was administered, by subcutaneous injection, 98 days previously. The results are recorded in Table X. The rate of the specific activities is 2.4, thus 59 % of the P found in the faeces originates from non-absorbed food P. The faeces P making 57 % of the total excreted P, we can conclude that, from the total food P taken

[1] 18 % of the total P found in the faeces was residual P obtained after the removal of the acid-soluble P (mostly calcium phosphate) and the traces of phosphatide P present. The specific activities of the different P fractions differed only to a minor extent.

by the rat, 33 % was unabsorbed.[1] The daily diet of the rat contained about 30 mgm. P.

Table X.

Specific activity of urine P and faeces P of a rat 98 days after administration of labelled sodium phosphate by subcutaneous injection. Weight of rat 208 gr.

Fraction	Specific activity (% of the activity administered per mgm. P)
Urine	0.39×10^{-2}
Faeces	0.16×10^{-2}

Similar values for the ratio of the specific activity of the urine P and faeces P were obtained in experiments with other rats kept on the same diet. The values obtained were 2.29, 2.47, 2.61, and 3.10 respectively. The samples were collected 30, 98, 10, and 20 days respectively after the administration of labelled sodium phosphate.

It is of interest to compare these figures with those obtained when labelled sodium phosphate is administered to the rat by mouth, the animal killed, and the activity of the total intestinal tract investigated after the lapse of 4 hours. Such determinations were carried out by several investigators. We found[2], 4 hours after administering labelled sodium phosphate (having a P content of about 1 mgm.) to a fasting

[1] K. M. Henry and S. K. Kon, (Biochem. J. **33**, 173, 1939.) emphasized recently that a large part of the P present in the gut becomes fixed by intestinal bacteria and is thus no longer available to the host. Bacterial bodies account for about 40 % of the dry weight of rat faeces and P is a more essential component of bacteria than Ca.

[2] G. Hevesy and O. Rebbe, Kgl. Danske Vidensk. Selsk. Biol. Medd. (in print).

rat, that the total digestive tract and its content contained 12.7 % of the phosphate administered; thus more than 87.3 % was absorbed. The latter figure represents the lower limit, since some of the active P present was actually absorbed and got subsequently with the digestive juices into the intestinal tract again and some active P present in the food exchanged with the tissue phosphate of the intestinal tract before the active P had an opportunity to be absorbed. Still higher figures for the labelled P absorbed into the circulation are recorded by ARTOM, SARZANA and SEGRE[1], namely 88—97.9 %; the duration of their experiments was appreciably longer, it varied from 9 hours to 4 days. A smaller absorption was found by DOLS and JANSEN[2]; after the lapse of 8 hours, the stomach and small intestine alone are stated by them to have contained 4.1—15.4 % of the labelled sodium phosphate administered. COHN and GREENBERG[3] found that, in the course of 8 hours, only 60—70 % of the labelled sodium phosphate administered got absorbed.

The above data inform us as to the lower limit of the rate of absorption of the sodium phosphate administered, which can materially differ from the rate of absorption of the phosphorus contained in the food, as discussed on p. 14. We get, however, trustworthy information on the latter point by comparing the specific activity of the urine P and the faeces P. As already mentioned, this comparison is based on the assumption that the specific activity of the P contained in the urine is equal to that of the P present in the digestive juices. We tested this

[1] C. ARTOM, G. SARZANA and E. SEGRE, Archiv Internat. Physiol. **47**, 245, 1938.

[2] J. L. DOLS and B. C. P. JANSEN, Koninklijke Akad. van Wetenschappen **40**, No. 6, 1937.

[3] W. E. COHN and O. M. GREENBERG, J. Biol. Chem. **123**, 185, 1938.

assumption in the following way: Labelled sodium phosphate was administered to a cat, the animal was sacrificed after 17 days fasting and the phosphorus contained in the last urine produced, and also in the sample removed from the small intestine, investigated. We found 1 mgm. P contained in each sample to have, within the error of the experiment (± 4 %), the same activity. The latter amounted to 0.003 % of the total activity administered.

Informations on the amount of endogenous P present in the faeces were also obtained by determining the P content of the faecal output of fasting animals.[1] The conditions prevailing in such experiments are, however, far from being physiological ones. The amount of the digestive juices, and thus also of the phosphorus secreted into the digestive tract, will much depend on the amount and quality of the food administered. When, for example, 50 gm. oil and 300 mgm. labelled P as sodiumphosphate were administered[2] to a fasting dog and the total P content of the intestinal tract investigated, after the lapse of 5 hours, the latter was composed to an extent of about 75 % of P of endogenous origin.

Summary.

After the lapse of 20 minutes a slight amount (about 0.01 %) of the radioactive phosphorus atoms taken by mouth as sodium phosphate can be recorded in the urine of a human subject. In the course of the first day 4—12 % of the amount taken was recovered. When administering the labelled phosphate by intravenous injection the figures varied between 4 and 23 %.

[1] R. Nicolaysen, Biochem. J. **31**, 107, 1937.
[2] G. Hevesy and E. Lundsgaard, Nature **140**, 275, 1937.

Phosphorus contained in the normal diet is less efficiently absorbed than sodium phosphate. As much as about 30 % of the former leaves through the bowels. To what extent the latter is unabsorbed (exogenous) P and to what extent it is endogenous, thus derived from the body proper, can be determined by comparing the specific activity of the faeces P with that of the urine P. Such a comparison leads to the result that, in the cases investigated, 70—80 % of the phosphorus present in the faeces was non-absorbed food P.

Similar determinations were also carried out on the excreta of rats.

It is emphasized that important information on the digestibility of different foodstuffs could be obtained by administering foodstuffs containing labelled phosphorus and other labelled elements, for example of vegetables grown on a soil containing labelled phosphate.

We want to express our best thanks to Prof. NIELS BOHR for the facilities kindly put at our disposal, to Professor CHIEWITZ and Dr. HAGEDORN for kindly administrating the labelled phosphorus to some of the subjects investigated, to Prof. LAWRENCE for the generous gift of radiophosphorus, and to Miss HILDE LEVI for carrying out the numerous counting experiments.

Institute of Theoretical Physics
University of Copenhagen.

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BY

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PREFACE

The material for the present work has been collected on *Salix cinerea* individuals growing wild at Brede near Copenhagen. The most important developmental stages of the flowers have been fixed in Carnoy's liquid in July—August whereupon they have been cut into continuous microtome series 6—12 μ thick. These stain well in Saphranin or Haematoxylin.

My manuscript has been read through by Professors K. JESSEN and O. PAULSEN of Copenhagen, besides by Professor J. MCLEAN THOMPSON of Liverpool, who has also revised the translation. I owe a heavy debt of gratitude to these three gentlemen and specially to Professor J. MCLEAN THOMPSON for many valuable critical hints.

To the trustees of the CARLSBERG Foundation, who have rendered possible my studies for a number of years, I tender respectful thanks.

I. The Problems.

The present work is a direct continuation of my earlier phylogenetic studies, which have aimed at a revision of the current view of the morphology of the gynaecium. For it may be considered that through enquiry in this direction an understanding can be reached of the manner in which the dominant vegetation of the present day has evolved upon our globe; on which subject little is, in fact, known.

We have already examined in earlier publications numerous gynaecia with central placentae, and have shown in each case that the placenta is the apex of the floral axis, and that the ovules seated thereon are complete independent "leaves" which may be called macrosporophylls. In addition, it has been shown that the carpels surrounding the placentae do not bear the ovules on their edges, but form merely an involucre of barren leaves.

It is among the remaining forms of gynaecium that the classical carpellary theory might be expected to find its strongest support, especially in such cases as those in which the carpels remain free from each other. But in these also the Cycadean homologies which have previously been proposed are invalid, and even in the Leguminosae J. M. Thompson has shown that the ovules arise on the apex of the floral axis, being formed like young leaves upon it. It is true that the apices of the carpels may be formed, as

in *Acacia* (NEWMAN, 1936), as lateral outgrowths immediately beneath the tips of the floral axes. But as THOMPSON has fully shown, using NEWMAN's own figures of floral development in two Australian species of *Acacia*, intercalary growth occurs so that certain parts of the apex are soon involved in carpellary organisation.

From among the remaining common forms of gynaecium we shall now consider an example of an ovary with parietal placentation, supplementing in this way our earlier enquiries. In this case also one might expect to find whatever confirmation is possible for the classical carpellary theory. For this theory would be readily understandable on the facts as they stand if, for example, an ovary such as is shown in Figs. 1 and 2 were composed of two carpels, the swollen edges of which were united and so formed the two placentae. Nevertheless, as is shown in detail below, a thorough organogenetic and teratological research renders the classical interpretation untenable.

Salix has been chosen as a subject well suited to investigation for the simple reason that there may be found in this genus diverse and unusually valuable abnormalities, occurring in such large numbers as to make possible an important teratological analyses not only of the carpels but also of the ovules. Our investigation will, however, begin with an account of the earliest ontogenetic stages of the normal gynaecium, a special attempt being made to determine whether the placentae originate and are formed upon the carpels, as is maintained on the Cycadean hypothesis, or on the apex of the stem and between the carpels.

The adult structure of the flower of *Salix* is so well known that it may here suffice to give but a brief account of it, referring the reader to the accompanying Figs. 1 and 2.

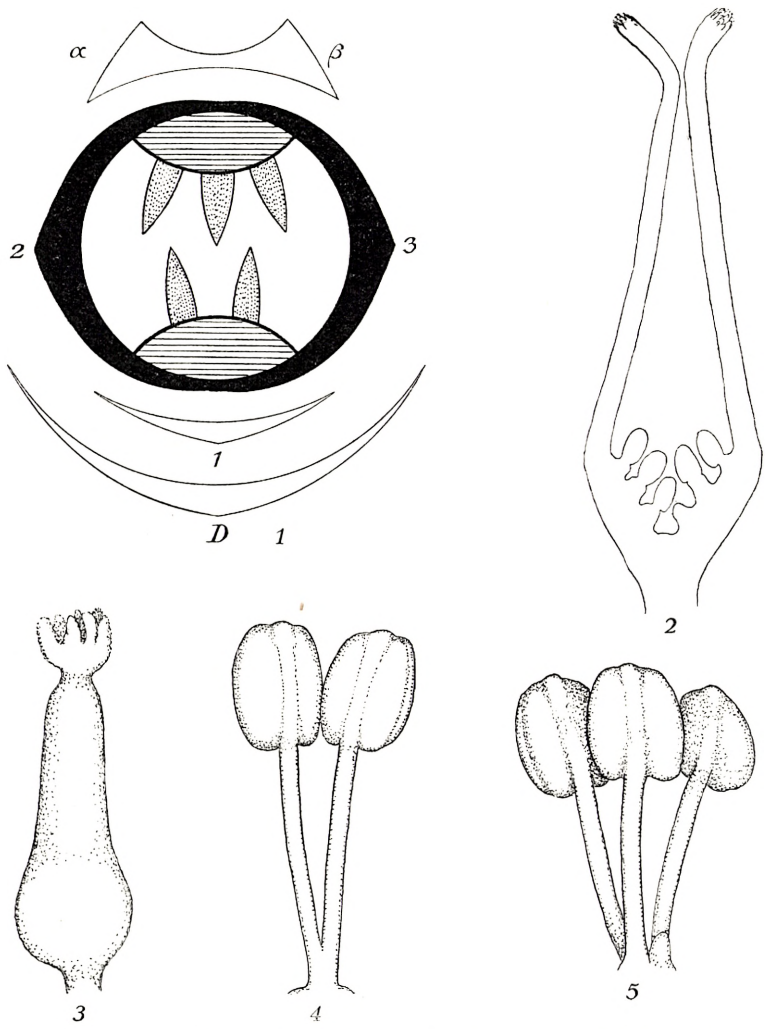


Fig. 1. Diagram of a normal female flower. *D*, bract; *α* and *β*, bracteoles; *1*, perianth; 2 and *3*, carpels; the floral axis (placenta) horizontally shaded; ovules (macrosporophylls) dotted. — Fig. 2. Longitudinal section of a normal gynaecium. × 20. — Fig. 3. Normal gynaecium. × 12. — Fig. 4. Normal male flower. × 12. — Fig. 5. Male flower with 3 stamens, corresponding to leaves *1*, 2 and *3* in Fig. 1. × 12.

VELENOVSKÝ has already shown that the gland situated behind the gynaecium is the union-product of the bracteoles (α and β). The abaxial leaf, 1, is generally rudimentary in *S. cinerea*, but occasionally and rarely it may be developed as a stamen (Fig. 5): or in other species it may mature as a gland. Leaves 2 and 3 are placed laterally: they mature as carpels in the female flower; in the male flower they mature as stamens (Fig. 4). In *S. cinerea* the placentae stand on the lines of coalescence of the carpellary margins as is required by and is in accordance with the classical theory. But the validity of the latter becomes immediately doubtful once the fact is considered that in many other species (cf. EICHLER) the placentae are developed along the mid-lines of the carpels.

II. The Organogenesis of the Gynaecium.

In a series of publications GRÉGOIRE has shown that the growing point of a floral axis may be built on quite a different plan from that of a vegetative shoot. These differences are so definite that in referring to a floral axis, GRÉGOIRE has spoken of it as an organ "sui generis". Doubtless the use of such a term in this connection may be carrying matters to too great an extreme as is shown, for instance, by such malformations as those in which the gynaecium is replaced by a leaf-bearing stem. Some of GRÉGOIRE's observations are, however, of great value; and, indeed, we shall find that they are confirmed by *Salix* in which the histological conditions furnish much fresh information and throw an unexpected light on the morphological nature of the placentae.

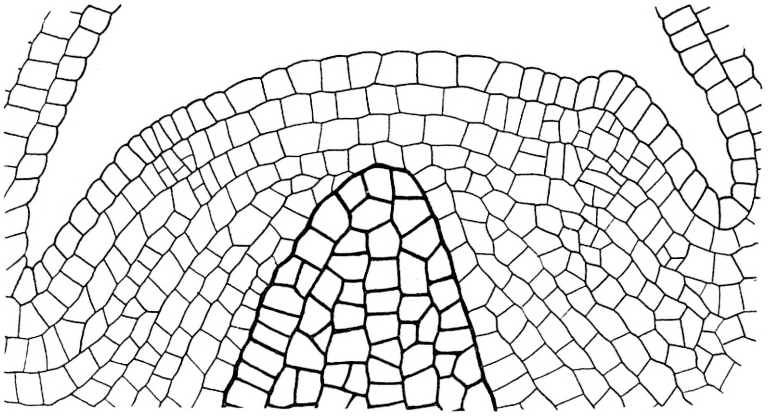
For purposes of comparison we will begin our study by

examining the apex of an ordinary vegetative shoot of *S. cinerea* (Fig. 6). In no single respect does it differ in principle of organisation from, for instance, such branch tips as are figured as typical in our common text books, such as the vegetative tip of *Hippuris*. The cells are arranged in regular and almost parallel layers. The central cell rows of the medulla—which are drawn with firm outlines in Figs. 6—13[1]— are of special interest. For it will be seen that these rows do not extend to the epidermis but are separated from the latter by several layers of cells. In addition, Fig. 6 shows that a vegetative leaf is initiated in the sub-epidermal layer, and that, at an early stage, other cells immediately beneath the dividing cells of the sub-epidermal layer are also involved in leaf-formation.

The apex of the axis of an inflorescence is constructed on a similar plan (Fig. 7); but the component cells are not arranged so regularly as in the apical cone of a vegetative shoot (Fig. 6).

On the other hand, an examination of the growing apex of a floral axis prior to carpel formation (Fig. 8) may afford some cause for surprise. For there is here a striking difference in organisation from that observed in the apex of a vegetative shoot (Fig. 6); and, in fact, the condition shown in Fig. 8 is particularly reminiscent of that which maintains in certain Algae, as for instance, *Furcellaria*. For the floral apex is traversed by a longitudinal, narrow, and almost cylindrical core of cell rows which are continued

[1] It should be emphasised that the walls of the medullary cells are all thin as are all other walls of the meristem. The thickening of the outlines of the medullary cells is therefore a subjective matter which should not escape the notice and criticism of the reader. For the choice of the outer limits whereby the medulla may be defined is a somewhat arbitrary matter.



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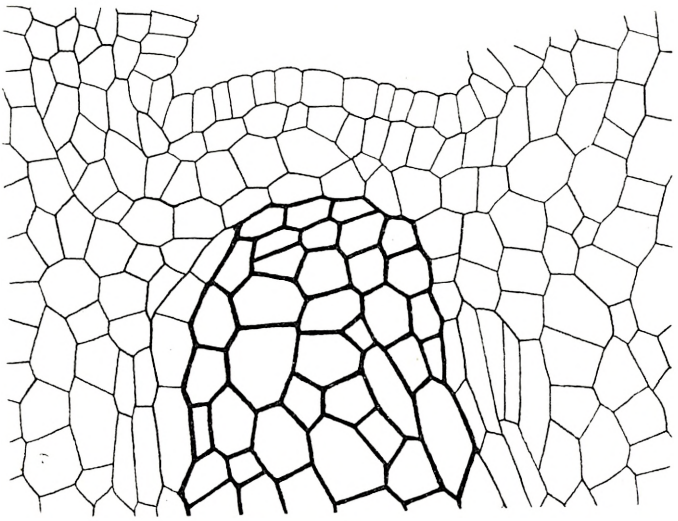

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Fig. 6. Growing point of vegetative shoot. Walls of the central medullary cells shown in firm outline. × 500. — Fig. 7. Growing point at tip of axis in a young catkin. × 500.

upwards just to the epidermis clothing the very apex. These rows are emphasised by a firm tracing of the walls in Fig. 8. Investing this core there is, in longitudinal section, a fan-like radiation of other cell rows directed almost at right

angles to the epidermis, and with divisions effected in such a way that the rows may appear to bifurcate as they are followed outwards. The number of these peripheral cell rows may be increased by new initials, arising as mother-cells for additional rows, wherever anticlinal division occurs in sub-epidermal cells. It will be noted, further, that once this state of organisation has been reached there is no longer a distinct and continuous sub-epidermal layer in the apex of the young flower stem (Fig. 8), the newly formed cells being arranged in quite another way from that which characterises the earlier stages of floral stem development (Fig. 7 a).

So that some understanding may be reached of the manner in which the remarkable condition of organisation of the cone of the young floral axis shown in Fig. 8 has been reached, we may now consider two younger stages in floral development. Thus in Fig. 7 a there is shown a longitudinal section of a floral primordium which is so young that nothing more than the base of the flower stalk is defined as a tiny cone. The latter is constructed on a plan which is almost identical with that of an ordinary vegetative stem apex, with several continuous cell layers beneath the epidermis and parallel to it (Fig. 6). When cell division occurs in these layers, the newly formed cell walls are placed as a rule at right angles to the epidermis. This arrangement of the outer tissues of the young flower stem is soon disturbed by dividing walls formed in the sub-epidermal layer, and which are parallel to the epidermis or periclinal. Two such walls may already be observed in the early stage of floral ontogeny shown in Fig. 7 a. Fig. 7 b shows a somewhat older flower stalk in longitudinal section and in the core of which the original arrangement of cells

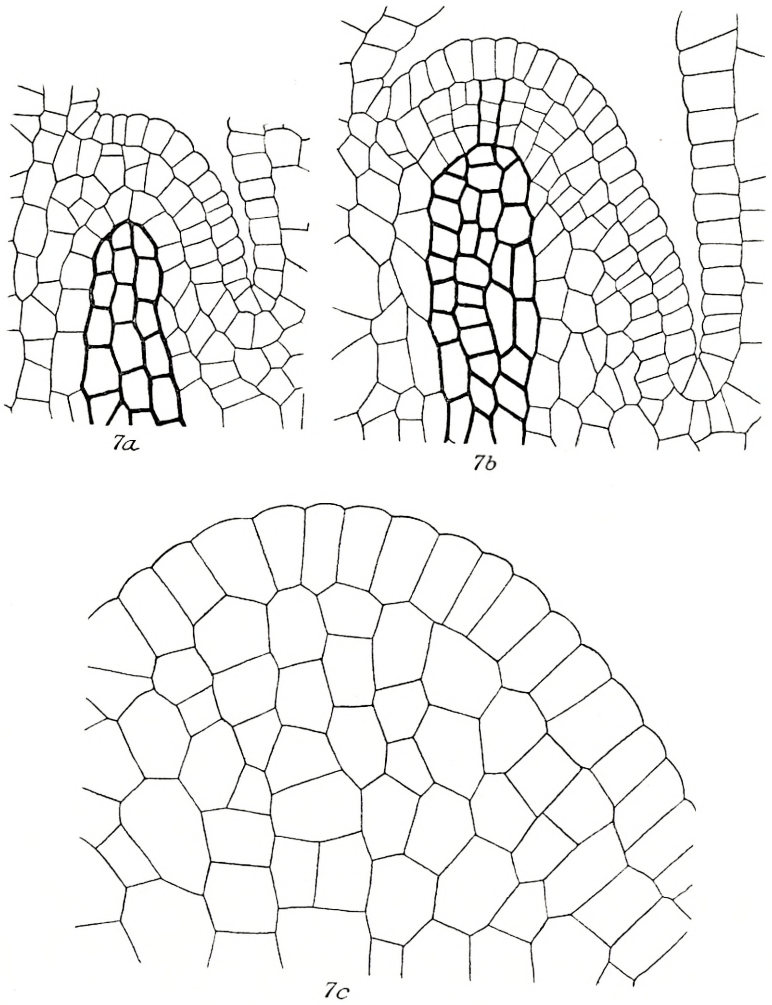


Fig. 7a. Young primordium of flower stalk with typical growing point (cp. Figs. 6—7). × 500. — Fig. 7b. Somewhat older stage with young initials of the cell rows arranged like a fan (cp. Fig. 8). × 500. — Fig. 7c. Growing point of a flower at a slightly older stage than that shown in Fig. 7b. × 1000.

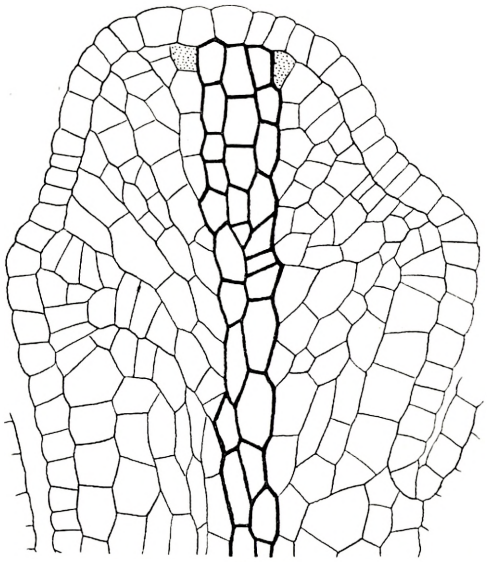
may still be faintly observed (cf. Fig. 7a). But several cells of the sub-epidermal layer have now been divided by periclinal walls. The daughter-cells thus formed divide again by periclinal walls, and division continuing in

this way, shorter or longer rows of cells are formed at right angles to the epidermis (cf. Fig. 8). The number of these rows may be increased wherever anticlinal walls are also formed. It is in this way that the young floral cone shown in section in Fig. 7c and 8 is soon produced with its distinctive features of organisation. The latter can, in large measure, be traced in origin to the balance struck between the alternative planes of division in the sub-epidermal layer of the young conical flower stalk.

There is thus in a phanerogam a unique type of generative cone (Fig. 8); and it is easily understandable that no ordinary shoot can be developed from it. And, in fact, the growth product of this stem apex is the mysterious gynaecium with its sequence of ontogenetic changes presenting still further surprises, as will be shown by the figures which follow.

But before we proceed to consider the ontogeny of the carpels, it is important to note the initial form of the floral apex (Fig. 8). For if we carry with us a picture of this form so as to be able to compare it with those given in the succeeding figures, we shall have a sufficiently accurate means for deciding the approximate limit between leaf and stem. And this despite the fact—which is not of decisive importance—that there will always be some cells which cannot be classified in this way.

It will be observed that in Fig. 9 the cells immediately beneath the epidermis are now arranged with a sufficient regularity to warrant the description of a sub-epidermal layer. It is this layer which plays an important part in giving an understanding of the further development of the gynaecium. For the carpels are initiated in this layer (Fig. 9) in a manner identical to that in which ordinary



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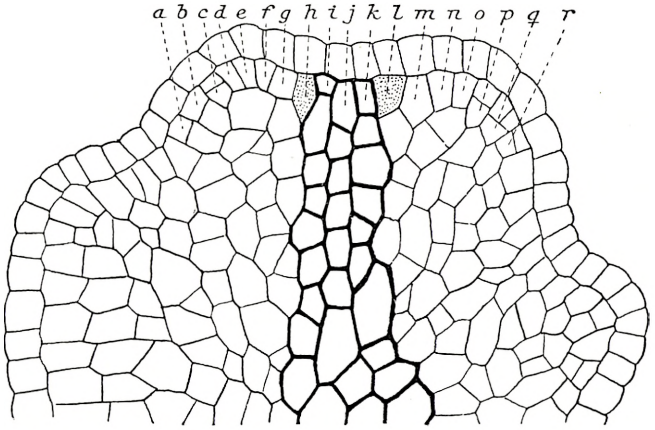

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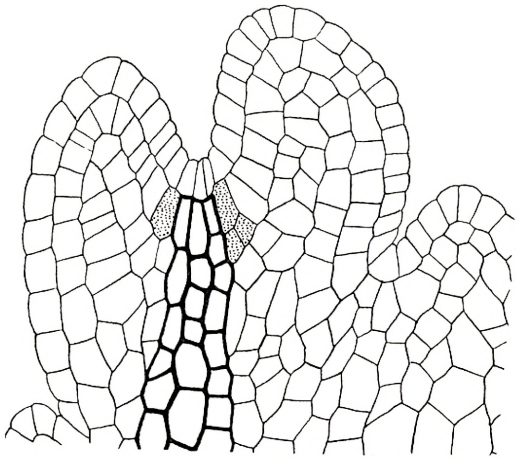
Fig. 8. Growing point of the tip of the floral axis just before the carpels are formed. × 700. — Fig. 9. First primordia for the carpels (a, b, c) and (p, q, r). × 700. Central rows of medullary cells (with heavily outlined walls) reaching upwards to the epidermis. Dotted cells are mother cells of the placentae. Cp. further the text.

vegetative leaves are formed (Fig. 6). Thus a carpel is initiated by the continuous periclinal division of such cells as are lettered a, b and c in Fig. 9. These cells become the mother-cells of long rows which compose the internal tissue of the carpels (Figs. 10—13), and which, as it were, lift the epidermis locally from its original position on the stem surface.

The gynaecium comes to be furnished with a central cavity due to the simple fact that such cells as i, j and k, Fig. 9.— and which are in central positions towards the summit of the growing apex — cease almost entirely to grow and form rows, while the surrounding tissue continues in upward growth.

Between the two carpel primordia indicated by a—b—c and p—q—r, Fig. 9, there lies a complete row of cells which are lettered d—o. These cells belong to the stem and are not involved in the carpel primordia. Thus the initial cone has not been "used up" in carpel initiation, nor has it ceased to grow except in its central portion which marked its prior summit (i—k, Fig. 9). But the stem cells, such as d—h and l—o — and which lie between the carpel primordia and the central meristematic cells i—k — divide once more at later stages, their progeny coming to be raised somewhat from the original and almost horizontal position. These further products of growth and division thus come to form downward continuations of the adaxial sub-epidermal layers of the young carpels (Figs. 10 and 11). In this way the floor of the ovary is partially composed of the almost crateriform apex of the floral axis.

If we now seek to probe into the nature of the placentae,



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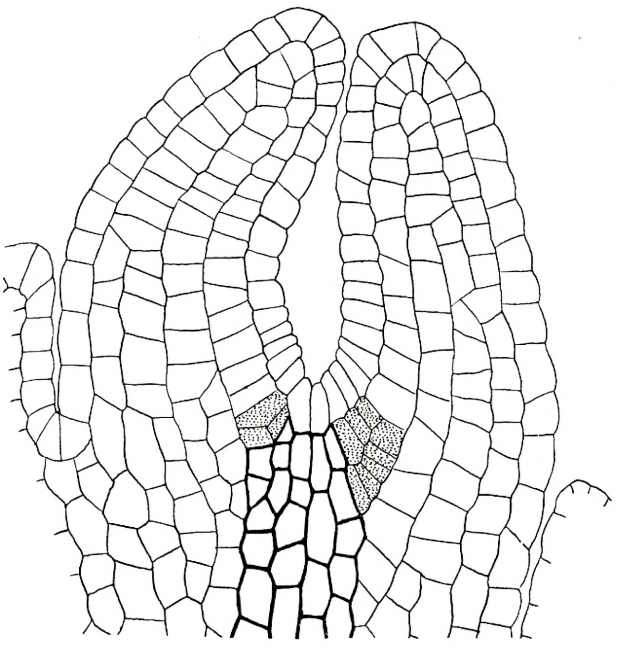

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Figs. 10—11. The first divisions of the mother cells of the placentae (dotted). The ovarial cavity is formed over the central medullary cells the growth of which ceases. × 750. Cp. also the text.

we may begin by examining the two sub-epidermal cells h and l, Fig. 9, and which are immediately adjacent to the core of medullary cells. These two sub-epidermal cells (which are shaded in Figs. 8 and 9) gradually acquire dense contents. They are especially capable of growth and division and soon become the mother-cells of large tissue masses. As is shown in subsequent figures, these tissue masses gradually become the placentae.

It will be further evident that these mother-cells (h and l, Fig. 9) belong to the floral stem, and not to the carpels, since in the early ontogenetic stages shown in Figs. 8 and 9, these mother-cells are in being before the carpels are formed. And indeed, as is shown in Fig. 9, there are several stem cells lying between such a mother-cell as h and the carpel primordium lettered a, b, c.

Fig. 10 shows a median longitudinal section through a somewhat older gynaecium. It will be seen from it that all newly formed cells composing the carpels are in regular rows, and that the sub-epidermal layers are particularly distinct. Despite the fact that the carpels are now fairly large, no placentae may yet be observed as prominences. The carpels grow especially at their bases, their first formed or older parts becoming the upper portions of the ovary (cf. Fig. 2). On these upper portions neither placentae nor ovules are formed.

It is not until the rather advanced stage in ontogeny shown in Fig. 10 is reached that the mother-cells of the placentae undergo their first divisions. The main directions of the successive walls whereby these mother-cells are divided are generally at right angles to each other. During the earliest of these divisions the strictly localised mother-cells are resolved by dominant anticlinal division into two

layers of cells (Fig. 11). It is in the outer of these two cell rows that growth and division is dominantly continued

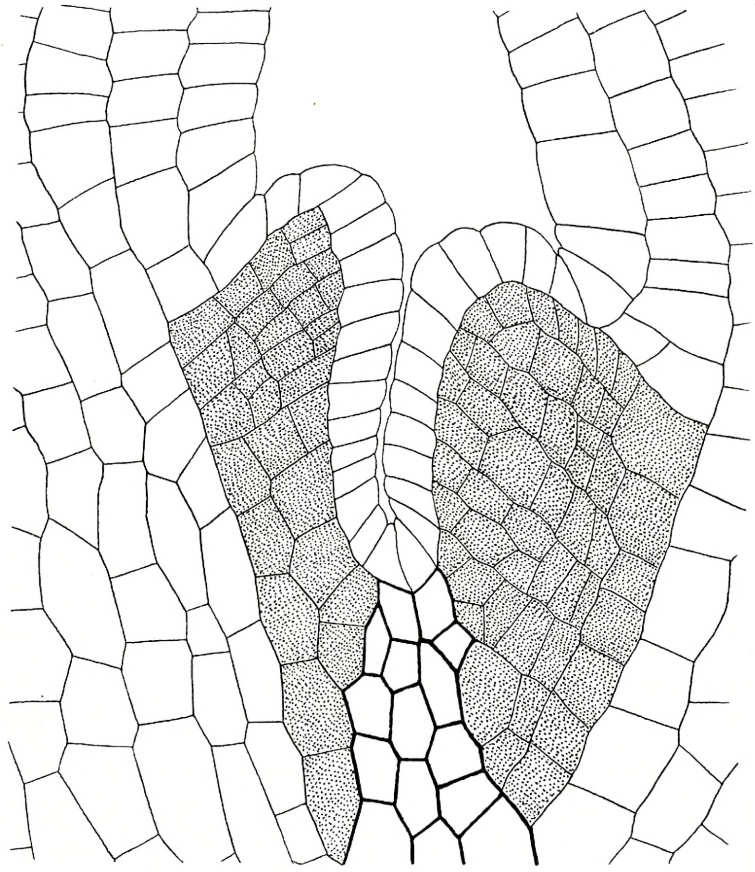

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Fig. 12. From the subepidermal layer of the tip of the axis are developed obliquely inward-directed cell rows (dotted) which form the placentae. × 1000. (Cp. the text).

(Fig. 12), the individual cells being divided at first by walls which are parallel to each other and to the inner surface of the ovary. In this way moderately long and curved rows of cells are formed directed obliquely upwards towards

the ovarial cavity (Fig. 13). The number of these rows is also increased by longitudinal walls which are placed anticlinally (Fig. 12).

In the older stages (e.g. Fig. 13), the original arrangement of the tissues of the axis and its appendages is so obscured that it is difficult of recognition. In the placentae, however, the bow-shaped and incurved rows of cells may still be observed, with clear indications of their origin from the stem apex at levels beneath the ovarial cavity. Taken by itself and judged by first appearances such an older stage as is shown in Fig. 13 seems to lend support to the suggestion that the classical theory is correct in maintaining that the placentae are essentially portions of the carpels. But the course of ontogeny shows that this is not a correct interpretation as has been demonstrated by the study of the changes in both the form and positions of the cells and their progeny shaded in Figs. 8—13. For the original blunt stem apex with its almost horizontal upper surface ceases to grow at the mid-point of its summit and continues in growth only along its margins which are raised almost in the form of a crater (J. M. Thompson), and along the inner faces of the carpels so as to constitute the placentae.

The form of the stem apex is not, however, that of a simple crater, for the crater rim is divided into two parts, which are the placentae, the division being evident almost to the crater floor, such as, for instance, the cupule of *Fagus* is subdivided into four portions. This subdivision is effected by inequalities in the rate of growth of the crater margin, growth being rapid along the mid-lines of the two placentae and being non-existent in the positions in which the clefts are formed.

The ovules are later initiated upon the placentae as local

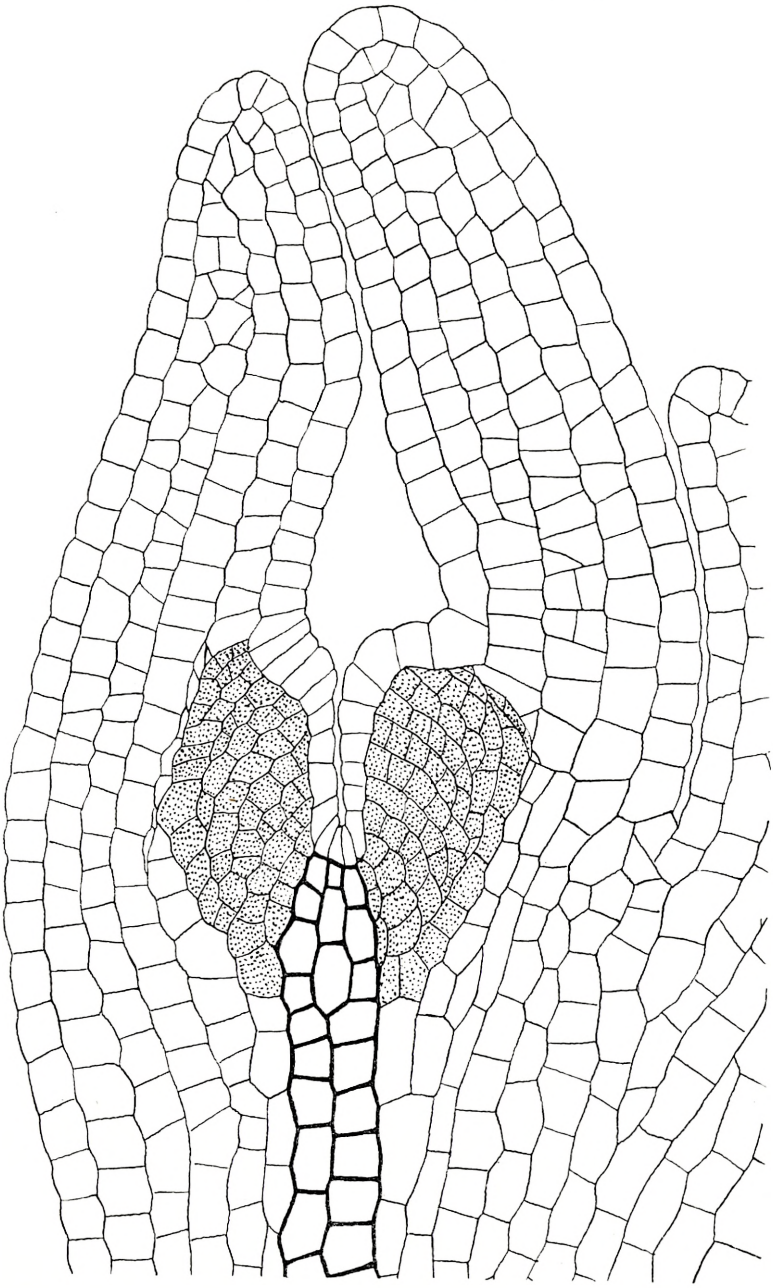

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Fig. 13. Older gynaecium before the forming of the ovules. The placentae form the crateriform tip of the floral axis. × 700. Cp. the text and Figs. 8—12.

growing points in the sub-epidermal and the underlying cells which are soon differentiated. They arise on the placentae in a manner similar to that in which foliage leaves and carpels are formed. This point is, however, so well known as to call for no emphasis here. It may suffice to state that on the evidence the ovules are inserted on those portions of the stem which are developed as placentae, and that they are completely dorsiventral and lateral organs like most other forms of leaves.

From the point of view of morphology there can then be no reasonable objection to regarding the ovules as individual, complete, and independent organs of foliar form. And it is to the abundant support of this interpretation that the consideration of the monstrosities illustrated below is directed.

But before these matters are considered, the results of our ontogenetic study may be presented in simple and clear diagrams such as Figs. 1 and 45.

It should, however, be kept clearly in mind that an histological analysis such as has been given above carries the drawback that it is not always possible to fix definitely the boundary between stem and leaf; and this in relation to the fact that these organs are phylogenetically of common origin and are interchangeable. The illustrations considered above are therefore open to other interpretations than those which I have proposed. And hence, so that there shall be no undue doubt on my reading of the facts of morphology, we shall now approach the matter from a different angle, namely that which is presented by the facts of teratology. In this way an opportunity may be given to check the conclusions of our histological study and to discover whether the results of teratological enquiry run

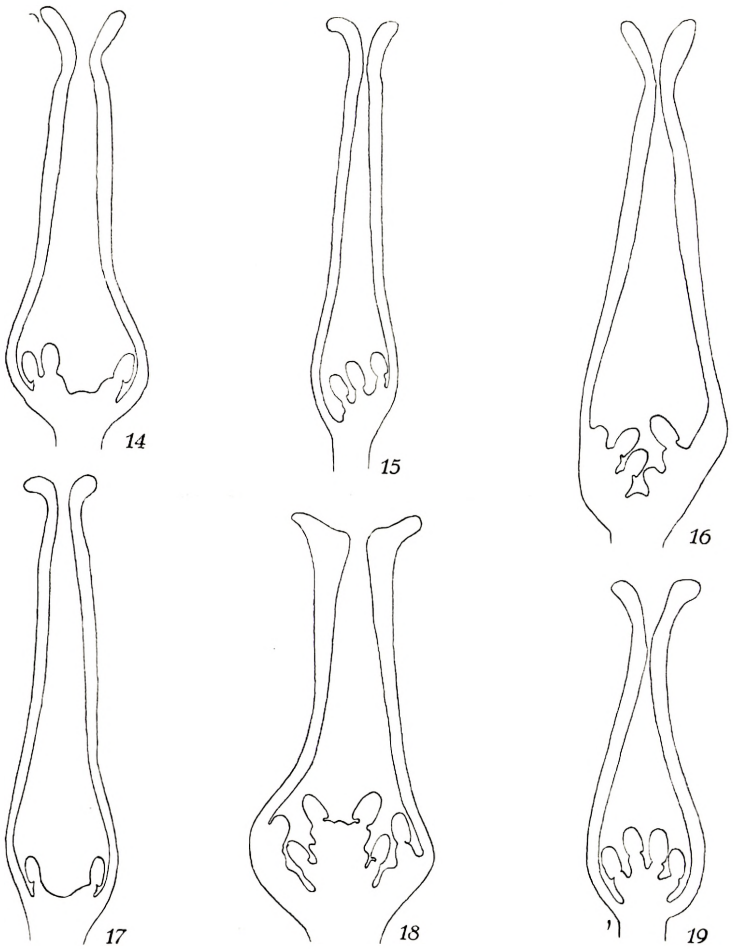
counter to our analysis, or the two methods of approach give compatible results.

III. Teratology.

During the past century many and varied malformations have been described among the flowers of numerous species of *Salix*. A good idea of the extensive literature on this subject has already been given in the long list of references provided by RAINIO and in the summaries of PENZIG. Many illustrations of anomalous flowers are available, and among those worthy of special mention are CHAMBERLAIN'S figures of "ovules" replaced by stamens. I think, therefore, that it may suffice to add to these illustrations Figs. 14—44. For they serve to confirm what has already been made known in the literature and warrant a generalisation which is founded on observation. And in particular, these additional figures provide a means of checking the histological features described and discussed above in relation to organogenesis.

Figs. 14—19 show longitudinal sections of a number of gynaecia taken from one and the same tree of *Salix cinerea*. It will be observed from these figures that the form, stature and fertility of the placentae may vary considerably. For comparison of the conditions shown, the reader is referred to Fig. 2 in which the normal placentae and their fertility are depicted. Fig. 15 shows, however, a flower with only one parietal placenta. Or again, the single placenta may be central, as in Fig. 19, in which case it forms a distinct prolongation of the floral axis as is habitual in the *Centrospermae*. And further, Fig. 18 shows that in one and the same flower the placenta may be central, with, at the same time, parietal portions as in the normal flower. In the light

of the ontogeny of the latter it seems reasonable to conclude that both the parietal and the central portions of the placenta



Figs. 14—19. Longitudinal sections of gynaecia showing that the placentae may be both parietal and central. × 20.

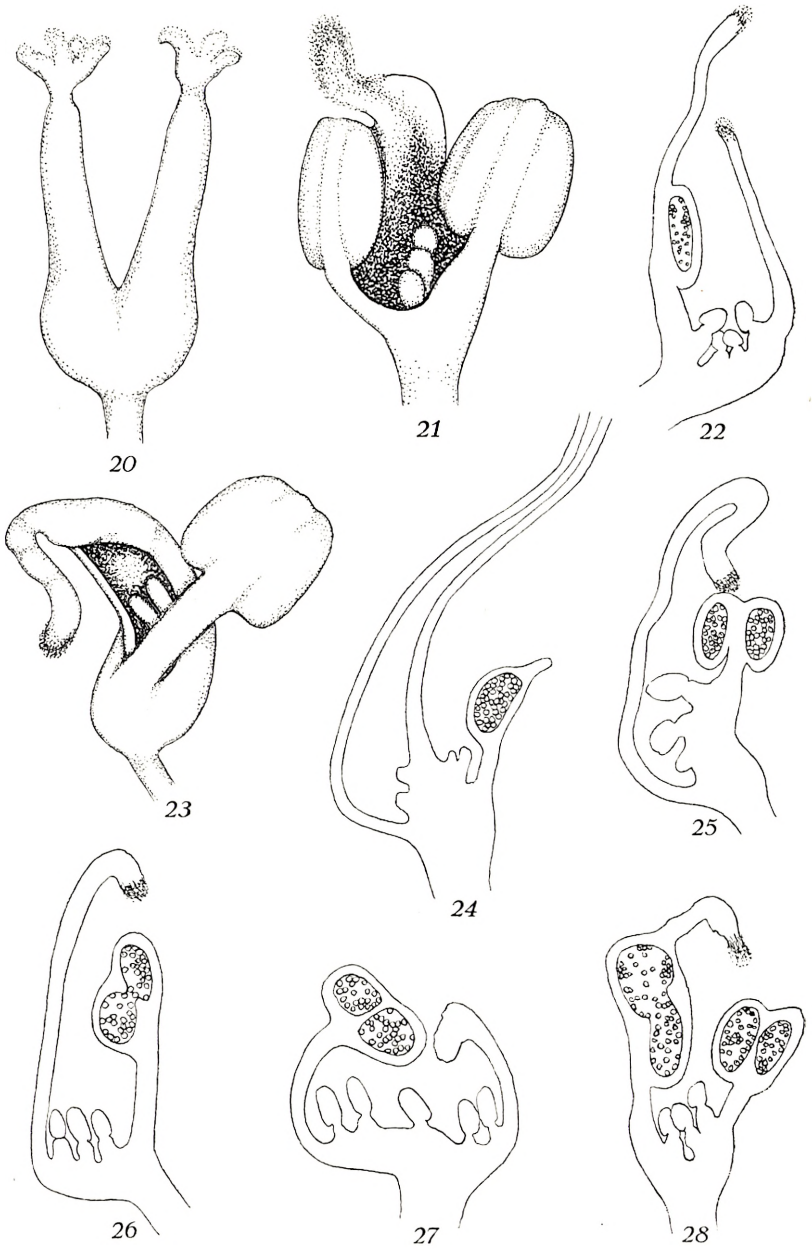
are here products of the stem apex. Finally, Fig. 17 shows a crateriform depression of the apex of a central placenta.

The peculiar apex of a floral axis may thus assume diverse forms and varied fertility. But the variations in

form, position, stature and fertility of the placentae do not entail differences of principle since one and the same organ is involved in their production. Conditions similar to these here discussed have already been recorded for *Mesembryanthemum* (HAGERUP, 1936) in which the placenta of some species is central, while in others it is parietal.

It suffices to make an analysis of the steps in organogenesis in order to establish the point, shown in Figs. 46—48, that the top of the gynaecium is composed of two lateral carpels. In the maturing flower such as is shown in Fig. 20, these two leaves are normally free from each other from a level at or above the upper limits of the placentae. The ovary here shown is reminiscent of the gynaecium in families in which the carpels are more or less independent, as, for example, the *Rosales* and the *Leguminosae*. It is in such families that one might expect to find support for the Cycadean homologies; but in point of fact it has been from these very affinities that the testimony on gynaecial organisation has caused the classical theory to be doubted (J. M. THOMPSON).

It will be seen, further, from Figs. 21—28; 36—44; and from Fig. 49 that either one or both of the normal carpels may be replaced by a stamen or by an anther-bearing organ. If then stamens are to be viewed as microsporophylls, the carpels of the normal flower must be considered homologous with such leaves. But in several of the flowers depicted it will be noted further that the stamen connective is prolonged as a stigmatic style, as is shown, for example, in Figs. 22 and 28, and, in lesser degree in Figs. 33 and 34: hence the stigmatic style and the connective may be considered homologous. In addition, two stylar branches may be formed as upward extensions of a single anther (Fig. 32).



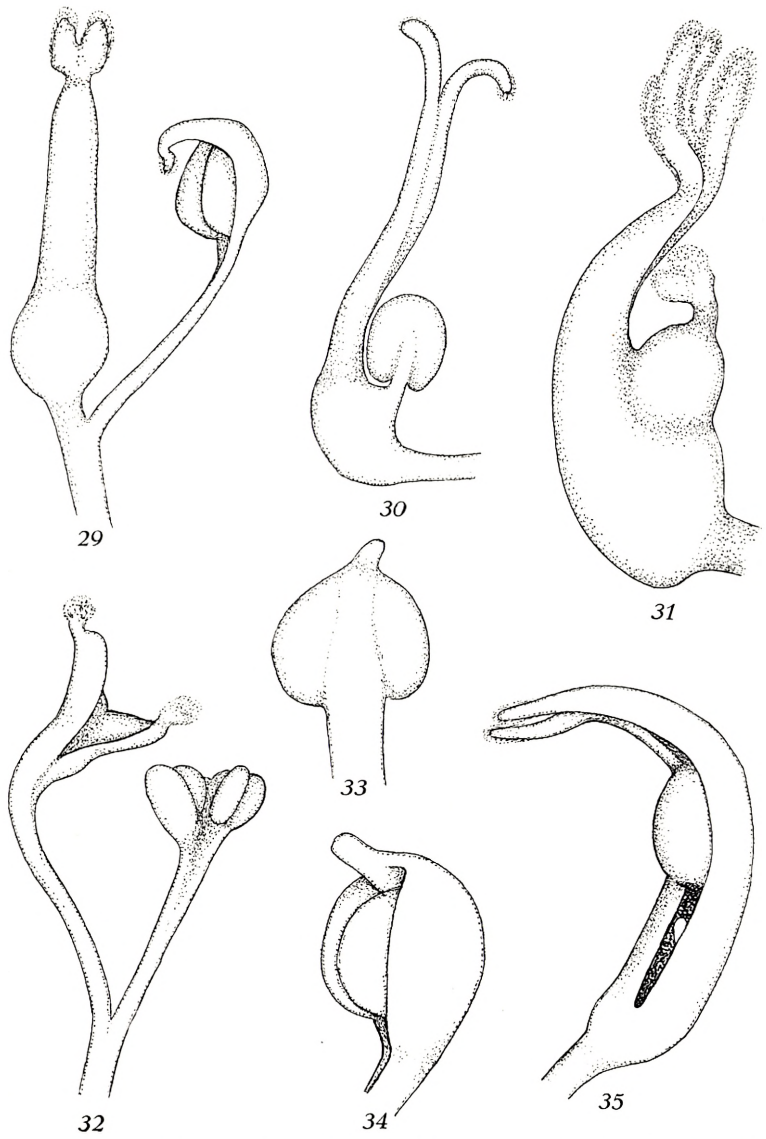
Figs. 20—28. Abnormal female flowers showing the homology of a carpel with a stamen. × 12. Cp. also the text.

The abnormalities here considered are very common, and their equivalents have frequently been figured.

To the left of Fig. 21 is an organ one half of which is matured as a half anther while the remaining portion is carpellate with an ovule-bearing placenta at its base.

It is, however, of special interest to note that throughout such variations the placenta may persist as if possessed of a definite morphological independence, irrespective of whether or not either or both of the carpels may be replaced by an organ which can no longer function in the normal way. Thus in the flowers shown in Figs. 28 and 49 the organs surmounting the gynaecium must be described as stamens rather than otherwise, despite the fact that in one instance the connective is replaced by a stigmatic style. And throughout all these variations it will be noted particularly that the ovarial base in each of these peculiar "male flowers" is female and is the bearer of several ovules.

The morphological explanation of this interesting condition may reasonably be taken to be that the placenta is the crateriform apex of the floral axis, and that along the rim of this "crater" (J. M. THOMPSON) are placed the "carpels" which are interchangeable with stamens and which may be replaced in part or in whole thereby. Reverting in this connection to Fig. 21 it will be noted that the ovules are inserted at the base of the crater, and it may be added that the condition here shown is one of the commonest in the abnormal flowers. Still further, it is most common to find flowers one of whose "carpels" is male while the other is normal in both form and function. And in such cases — as is shown in Fig. 25 — the ovules may be inserted immediately beneath the stamen upon their



Figs. 29—35. Malformations of female flowers. × 15. Cp. also the text.

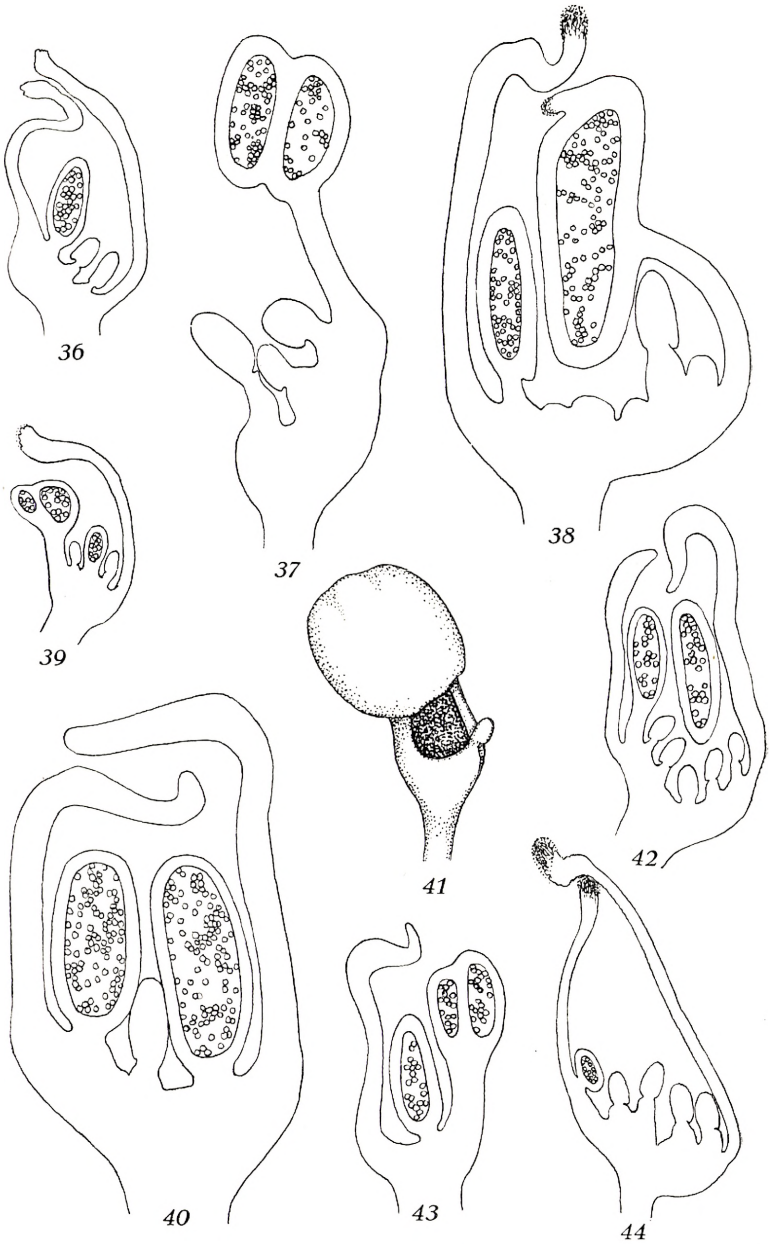
evident placenta which may now justifiably be considered a portion of the axis.

Among the many curious malformations which have

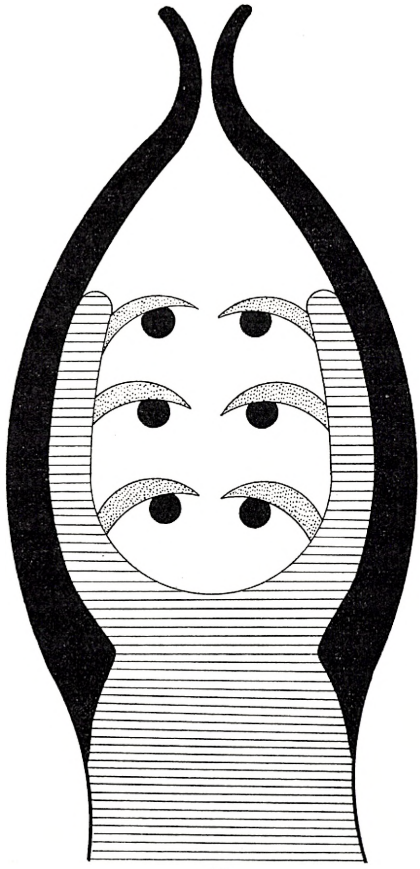
been observed there are some of outstanding interest in that the ovules may be replaced by organs of distinct and well defined morphological category. Such malformations have already been brought to our notice by HENRY, MARQUART and CHAMBERLAIN, the observations of the latter on American Salices being in marked agreement with those now recorded in Figs. 36—44 from our Danish *Salix cinerea*. One may therefore venture to believe that the facts here presented do not merely represent chance and localised occurrences in the genus but may help to express valid rules of variation and of interchange between the floral organs.

Fig. 41 shows a flower with the usual ovuliferous crater as its base and with two lateral "carpels" forming the margin of the crater. By far the larger of these marginal organs is a short stamen with expanded filament: the smaller is an ovule. In Fig. 37 is shown a longitudinal section of a somewhat similar flower in which there is an ovule in place of a normal carpel. It may be suggested that this solitary ovule is borne on the rim of the crateriform receptacle because the carpel to which it should be related has been greatly reduced, though still persisting as the rim upon which the ovule is raised. But against this possible view stands the fact that neither in this instance nor in any other which has been examined histologically has any trace been found of a residual sporophyll. It seems more reasonable to assume that the "solitary ovule" here considered is equivalent to a "carpel" and has replaced the latter in a manner comparable to that in which a stamen may be substituted.

The condition here considered is in direct opposition to the suggestions of the classical theory according to which



Figs. 36—44. Abnormal gynaecia showing that an ovule may be replaced by (and may be enquivalent to) a stamen. Figs. 37 and 41 show a „carpel" replaced by an ovule. × 15. Cp. also the text.



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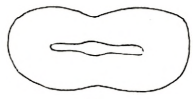

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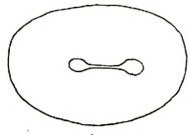

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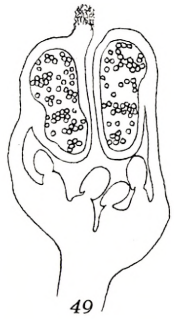

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Fig. 45. Diagram of the morphology of the gynaecium (cp. Fig. 1). Carpels black; the placentae are prolongations of the floral axis (horizontally shaded) whose leaves (macrosporophylls) are developed as ovules (dotted and in black). Cp. also the text. — Figs. 46—48. Transverse sections of young gynaecia. The carpellary edges do not curve inward (Fig. 46) and do not swell (Fig. 47). × 160. — Fig. 49. Longitudinal section of a "gynaecium" whose "carpels" are replaced by stamens. × 12.

ovule formation necessitates the presence of a megasporophyll beneath the solitary ovule to the left of Fig. 37. And further — so that the homologies proposed by this theory may be maintained — it would be necessary to hold that the stamen to the right of Fig. 37 is an equivalent micro-

sporophyll without which anther formation should not be possible. But if an homology is to be sought on the evidence as it stands it would seem more natural to propose that the ovule and the stamen are equivalent organs.

From such abnormalities as are shown in Figs. 37 and 41 the conclusion may be drawn that an ovule is a complete and independent organ. And by reason of its axial origin I have preferred to designate it a leaf. But, however, it may be named, the conclusion offered on this matter by the facts of ontogeny of the normal flower supports this interpretation which is in harmony with the abnormalities figured above. For in the flowers shown in Figs. 36—44 one or several ovules have been replaced by larger or smaller stamens with filaments of varied form and stature. And in this interplay — as has been held by J. M. Thompson — the anther is homologous with the nucellus and the filament with the funicle.

Further to this matter, I have examined in detail the flowering of a species of *Populus*, namely, *P. canescens*, and have found that the ontogeny of its gynaecium resembles so closely that of *Salix cinerea* that its description here would involve merely unnecessary repetition. It may then be held that *Salix cinerea* offers a general and representative picture of the events of gynaecial organisation in the *Salicaceae* as a whole.

IV. Summary.

1. The observations here detailed have been made and discussed so that, for the purposes of phylogeny, the classical carpellary theory may be put to the test for a genus possessing a gynaecium with parietal placentation. To this end, the ovary of *Salix cinerea* has been analysed in both its

normal ontogeny and in its teratological states at and towards maturity.

2. The ontogeny has shown (1) that the margins of the carpels do not curve inwards (Fig. 46); (2) that they do not swell (Fig. 47); and (3) that they do not form the placentae. This is in accordance with the facts disclosed by J. M. THOMPSON for a long series of Scitaminean plants.

3. The carpels form merely an involucre of coalescing and barren leaves. They do not bear the ovules but merely enclose them, thus producing the state of angiospermy.

4. The facts of ontogeny shown in Figs. 8—13 harmonise with the view that the placentae are prolongations of the floral axis, displaced, as growth proceeds, so as to be extended along the carpel bases.

5. The ovules are held to be complete and independent organs, which, in the absence of a more appropriate term by which they may be designated, I have called leaves. And this for the following reasons:—

a. They are inserted on the stem which comprises the placentae.
b. They are dorsiventral and they are lateral to the placentae.
c. When malformation occurs they may be replaced by independent organs of other categories, such as stamens (Figs. 36—44); or an ovule may completely replace a carpel (Figs. 37, 41).

6. The funicle is held to correspond to the stamen filament, and the anther to be homologous with the nucellus and its integuments.

7. Thus the classical Cycadean homologies are con-

sidered invalid in the case of *Salix* as has already been shown for many other genera, for example, by J. M. THOMPSON and O. HAGERUP.

8. The main points of interpretation of the floral structure of *Salix* offered in the text are expressed in Figs. 1 and 45.

9. According to this interpretation an ovule is a monosporangiate macrosporophyll. On this view it may even be assumed that a phylogenetic connection is possible between some Angiosperms and the *Lycopodiales*, the *Gnetales* and *Coniferae* lying on the lines of descent. The reader is referred to earlier phylogenetic work for the evidence on which this thesis has been made to stand (cf. HAGERUP).

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List of Symbols.

p = total amount of plasma-phosphate;
c = total amount of acid-soluble phosphorus in corpuscles
s_p = specific activity of plasma phosphate (activity per mgm. phosphorus);
s_i = specific activity of inorganic phosphorus in corpuscles;
s_c = average specific activity of total acid-soluble phosphorus in corpuscles;
a_p = total activity in plasma phosphate;
a_c = total activity of acid-soluble phosphorus in corpuscles;
S_p = value of s_p at the end of an experiment;
S_i = value of s_i at the end of an experiment;
S_e = specific activity of acid-soluble organic phosphorus in corpuscles at the end of the experiment;
S_c = value of s_c at the end of an experiment, etc.;
A_t = total activity of plasma ester at the time;
A_o = total activity of plasma ester at the start of the experiment;
α = coefficient of penetration;
k = rate-constant of the monomolecular decomposition of hexose-phosphate in blood.

The distribution of inorganic phosphate and of organic acid-soluble phosphorus compounds between plasma and corpuscles deviates from equipartition. This difference in the distribution of the phosphate ion could be due to the fact that during the life time of the corpuscles equilibrium between its contents and hose of the surrounding plasma is not yet reached, but it is more probable that we are faced with a case in which the partition coefficient of the ion in question between corpuscles and plasma actually differs from unity.

The distribution coefficient of inorganic phosphate and also that of the acid-soluble organic phosphorus compounds between plasma and corpuscles fluctuates within wide limits. HALPERN[1] investigated the inorganic P content of the plasma and corpuscles of rabbits blood in 33 cases. In 29 cases the inorganic P content of the corpuscles was found to be less than that of the plasma of equal volume, the ratio between the inorganic P content of the corpuscles and that of the same volume of plasma varying between 0.86 and 0.38. In our experiments we find an average content of the plasma inorganic P amounting to 7 mgm. % and of the corpuscles

[1] L. HALPERN, J. Biol. Chem. **114**, 747 (1936). In this paper earlier litterature on this subject is to be found. R. T. BRAIN, H. O. KAY and P. G. MARSHALL (Biochem. J. **22**, 628, 1927) found the inorganic P content of the human blood plasma to be 4.1 mgm. %, that of the corpuscles 2.4 mgm. %.

inorganic P to 4.5 mgm. % making the above mentioned ratio equal to 0.64. When taking into account that the water content of the corpuscles amounts to only 70 % of that of the plasma we obtain for the distribution coefficient of the inorganic P between the corpuscle water and plasma water a value differing not much from unity[1]. While the determination of the inorganic P of the plasma is not difficult, as the acid-soluble plasma P is mostly composed of phosphate ions, the analysis of the corpuscles often gives less reliable results. Some of the organic phosphorus compounds present in the corpuscles may decompose[2] in the course of the separation of the corpuscles, the decomposition giving rise to the formation of additional inorganic phosphate. On the other hand, when the corpuscles have to be obtained quite free of plasma as in our experiments (page 19) it is necessary to wash them with a suitable solution free of phosphate, for example with an isotonic sodium chloride solution; in the course of this operation some phosphate can be lost from the corpuscles by a diffusion process. In view of the great importance the plasma phosphoric esters play in Robison's theory of bone calcification he and his collaborators[3] made a careful study of the amount of phos-

[1] R. T. Brain, H. O. Kay and P. G. Marshall (Biochem. J. **22**, 629, 1928) find for human blood the same distribution coefficient as found by us for canine blood, namely 0.91

[2] Maitland, Hansman, and Robison, Biochem. J. **18**, 1152 (1924), have shown that, if the blood is made acid to $p_H = 7.3$, there is hydrolysis, if made alkaline to $p_H = 7.35$ there is for a short time synthesis of the esters; this, however, soon gives place to hydrolysis and to a corresponding increase of the inorganic phosphate; comp. also H. Lawaczeck, Biochem. Zeitsch. **145**, 351, 1924.

[3] Robison, R., The Significance of Phosphoric Esters in Metabolism, New York 1932, p. 68. Comp. also R. T. Brain, H. O. Kay and P. G. Marshall l. c.

phoric ester present in the plasma from human and rabbit bloods and ascertained an average value of about 0.5 mgm.%.

The problem in which we were interested was the determination of the rate at which phosphate ions and also the phosphoric ester molecules of the plasma penetrate into the corpuscles and vice versa. The usual procedure employed to obtain information on the permeability of the corpuscle membrane to phosphate ions is to introduce sodium phosphate into the plasma and to investigate if and to what extent the phosphate and phosphoric ester content of the corpuscles is increased. By using this line of attack HALPERN found that at 3° inorganic P does not enter or leave the blood cell to an yappreciable extent in the course of 9 hours. Above 23° a very slow, at 37° an appreciable penetration of the additional phosphate into the corpuscles was observed.

A very convenient way of the study of exchange between phosphorus components present in the plasma and the corpuscles is opened by the application of labelled (radioactive) phosphate. By introducing active sodium phosphate of negligible weight into the plasma all the phosphate ions present in the latter get labelled and, if after the lapse of some time radioactive phosphorus compounds are found to be present in the corpuscles, we can conclude that these penetrated during the time in question from the plasma into the corpuscles. We carried out experiments both in vivo and in vitro introducing active sodium phosphate into the plasma and investigating, after the lapse of few hours, the activity and the concentration of the inorganic phosphate and also of the phosphoric esters present in plasma and corpuscles. In other cases active hexosemonophosphate was introduced into the plasma and the activity and concentration of the above mentioned P compounds were measured. In view

of the very slow rate of the formation of labelled "non-acid soluble" phosphorus-compounds present in the blood (phosphatides and phosphorus containing proteins) ascertained in our former work[1] we left those substances out of consideration in this investigation. Some of the acid soluble phosphorus compounds, of which a great variety occurs in the corpuscles, were found to be labelled to a large extent after the lapse of a short time. The problem which first occurs is whether the labelled organic phosphorus compounds (phosphorus esters and others which we will in what follows denote as "phosphorus ester") are formed within the corpuscles from active inorganic phosphate or whether active esters diffuse from the plasma into the corpuscles. As we will see later the labelled esters found in the corpuscles are, at least to a large extent synthesised within the corpuscles.

As to the nature of phosphoric esters present in the corpuscles the presence of various compounds has been recorded, such as adenyltriphosphate, hexosephosphate, triosephosphate, mono- and diphosphoglycerate, glycerophosphate, and phosphopyruvate. The composition of the corpuscles of different animals was found to be markedly different: while the corpuscles of the blood of sheep contain[2], for example, 80% of esters which are hydrolyzed by boiling 1 n. HCl within 3 hours, the corresponding figure amounts, in the case of rats' blood, to only 30. The average ester P content of human blood corpusles is stated to be 20 mgm. per 100 cc. blood, of which about 68% is present as phosphoglycerate, 21% as hexosephosphate, and 11% as adenyltriphosphate[3]. Few data are available as to the

[1] L. Hahn and G. Hevesy, Memoir. Carlsberg Lab. **22**, 188, 1938.
[2] H. v. Euler and K. M. Brandt, Z. f. physiol. Chem. **240**, 215 (1936).
[3] E. Warneg and G. Stearns, J. Biol. Chem. **115**, 567 (1936).
S. E. Kerr and A. Antaki, J. Biol. Chem. **121**, 531 (1927).

phosphorus ester content of the plasma and its composition, the presence of small amounts of hexosemonophosphate being recorded[4]. The phosphoric ester content of the plasma varies within wide limits, the average value being about 0.5 mgm. %. In the normal human plasma values varying between 0.0 and 0.9 mgm. % and an average valwe of 0.33 mgm. % were recorded[5].

Diffusion of Phosphate Ions into the Corpuscles.

Radioactive sodium phosphate containing a negligible amount of phosphorus is added to 10 cc. of heparinised rabbit blood. The sample is shaken in a thermostat at 37° under a mixture of oxygen and carbon dioxid, after the lapse of few hours plasma and corpuscles are separated by centrifuging, the corpuscles washed 2—3 times with a physiological sodium chloride solution. The acid-soluble components of the plasma and also those of the corpuscles are isolated in the usual way (extraction with ice-cold trichloro-acetic acid). While the acid-soluble fraction of the plasma is practically (90% or more) composed of inorganic P, the corpuscles contain mostly organic phosphorus compounds and, in addition, an appreciable amount of inorganic P. The latter can be isolated by precipitation as ammonium magnesium phosphate. The organic phosphorus compounds present in the filtrate are then converted into inorganic salts and precipitated as such.

When carrying out such experiments we find that in the course of few hours an appreciable part of the labelled plasma inorganic phosphate diffused into the corpuscles. At the same time we find a formation of labelled organic

[4] Comp. R. Robison, The signficance of phosphoric esters in metabolism, New York 1932, p. 69.

[5] R. T. Brain, H. D. Kay and P. G. Marshal, Biochem. I. **22**, 635, 1928.

Table 1.

Activation in vitro of acid-soluble phosphorus present in the corpuscles.

Relative specific activities of corpuscles' phosphate and of organic acid-soluble phosphorus present in the corpuscles, taking the specific activity of the plasma-phosphate at the end of the experiment to be = 1.

	Time	Rel. spec. activity of corp. phosphate $\left(\frac{S_i}{S_p}\right)$	Rel. spec. activity of corp. org. acid-soluble P. $\left(\frac{S_e}{S_p}\right)$
Rabbit G	90 min.	0.25	0.14
	90 min.	0.38	0.21
Rabbit H	30 min.	0.27	0.11
	90 min.	0.46	0.23
	175 min.	0.69	0.36

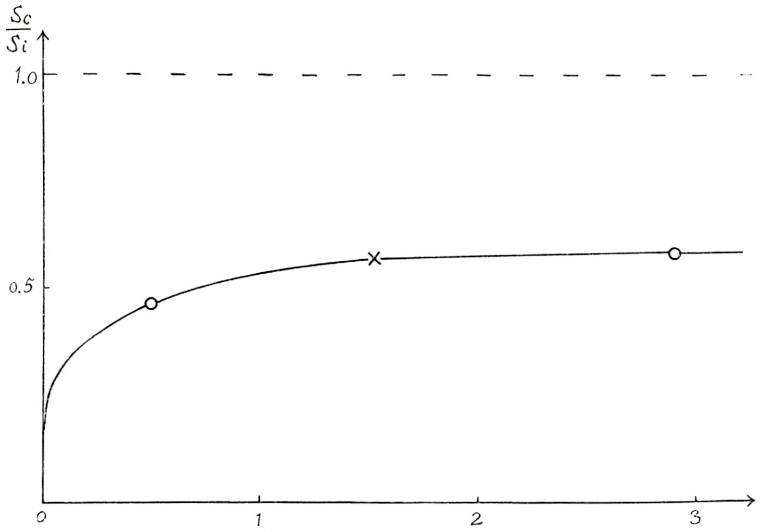
phosphorus compounds in the corpuscles. What actually happens is that the individual iorganic phosphate ions of the plasma diffuse into the corpuscles and are converted in the latter into phosphorus esters. The question, which now occurs, is which is the faster process, the diffusion of HPO_4'' into the corpuscles or the ester formation. This can be decided by comparing the specific activities of the different phosphorus fractions isolated from the corpuscles. Such a comparison is seen in table 1. After the lapse of only half an hour about half the ester was in exchange equilibrium with the inorganic P present in the corpuscles. The resynthesis of the acid soluble organic P compounds must thus be a fast process and, from the fact that with increasing time the ratio between the specific ativity of the ester P and inorganic P of the corpuscles only slightly increases and yet strongly differs from 1, we must conclude

that only one part of the diverse organic phosphorus compounds present in the corpuscles is renewed and thus activated in the course of the experiment while the other part, composed of diphosphoglycerate, hexosephosphate and other compounds remains, at least practically, inactive. This conclusion is supported by the results of the following experiments. Instead of destroying the total esters we hydrolysed[1] them with 1 n. HCl or H_2SO_4 for 100 min. at 100° and determined the specific activity of the hydrolysed P. While the average ester P secured from the corpuscles of a rabbit (G, comp fig. 1) was found to have a specific activity amounting to 55 % of the corpuscle inorganic P the corresponding figure for the hydrolysable ester was 80 %. In the case of another rabbit (H), the figures were 53 % and 100 % respectively[2]. From the facts mentioned above it follows that the exchange reaction between inorganic phosphate ions and the hydrolysable esters is a very fast process. That the inorganic P present in the corpuscles does not reach exchange equilibrium with the plasma phosphate in the course of a few hours is due to the fact that a large part of the active phosphate ions are incorporated into the organic compounds of the corpuscles, while simultaneously non-active phosphate ions are freed to take the place of the active ones and "dilute" the active inorganic phosphate which penetrated into the corpuscles, diminishing thus the specific activity of the corpuscle inorganic P.

[1] Under these conditions, diphospho-l-glycerate and also hexosediphosphate are only hydrolysed to a negligible resp. small extent. (G. Warweg and E. Stearns, J. Biol. Chem. **115**, 567 (1936)).

[2] The difference between the rate of activation of the "hydrolysable" and "nonhydrolysable" fractions is still better brought out when comparing the specific activity of the pyrophosphate, with of from adenosintriphosphate after 7 min. hydrolysis, to that the residnal P, as found in a recent investigation the result of which will be published shortly.

If the whole ester-phosphorus could exchange with the inorganic P, the activity-ratio should finally reach a value 1.0 (indicated by the dotted line). It is clear that the limiting value of $\frac{S_c}{S_i}$ is much lower, which is due to the presence of an organic phosphorus fraction in the corpuscles exchanging at a slow rate.



Ratio in the corpuscles of the specific activities of the total acid-soluble phosphate and the inorganic phosphorus. x = Rabbit G. o = Rabbit H.

Diffusion of Plasma HPO_4'' into Corpuscles.

We may obtain information as to the rate of penetration of the HPO_4'' from the plasma into the corpuscles by comparing, after a lapse of time, the specific activities of the plasma inorganic P and that of the total acid-soluble P present in the corpuscles. An alternative method would be to compare the specific activities of the plasma inorganic P and the corpuscle inorganic P, but in this way the rate of penetration would be underestimated for the following

reason: While active esters are forming in the corpuscle a corresponding amount of non-active P ester decomposes producing non-active inorganic P which dilutes the active inorganic P present. (We denote as specific activity the activity per mgm. P). Therefore to arrive to a proper figure for the rate of penetration into the corpuscles we have to consider the total acid-soluble P present in the corpuscles. As already mentioned, one part of the organic P of the corpuscles reaches exchange equilibrium very rapidly while the other becomes activated at a slow rate; and therefore the ratio of the specific activities of the corpuscle inorganic P and total acid-soluble P does not become = 1, but 1.8. This figure is only valid for the blood of the rabbit, while a different figure will presumably be obtained for blood containing appreciably more or less hydrolysable phosphorus. In the following discussion the calculation of the penetra-penetratition of HPO_4'', and of a magnitude which we will call a penetration coefficient and denote as α will be demonstrated. (Comp. the list of symbols).

Calculation of the Coefficient of Penetration.

The total activity of the plasma (a_p) is equal to the product of the specific activity (s_p) and the amount of phosphate (p) present in the plasma. (Definition of the specific activity.)

$$a_p = p \cdot s_p$$

and similarly for the corpuscles

$$a_c = c \cdot s_c$$

c denoting the total acid-soluble P of the corpuscles. During the exchange process $a_p + a_c$ remains constant, and therefore

$$-\Delta s_p = \frac{c}{p} \Delta s_c .$$

At the beginning of the experiment the specific activity of the corpuscles P was = O and, accordingly, when denoting the specific activities at the end of the experiment as S_p and S_c, we arrive at a value of the specific activity of the plasma phosphate at the beginning of the experiment

$$s_p = S_p + \frac{c}{p} S_c .$$

The rate of activation of the corpuscle P being proportional to $s_p - s_i$ we can write

$$\frac{ds_c}{dt} = \alpha (s_p - s_i)$$

were, as already mentioned, α is the coefficient of penetration. In an early stage of the experiment the change of s_p and s_i with time is linear and therefore, as far as we choose such experimental conditions that most of the activity is still to be found in the plasma, we arrive at the average value of s_p during the experiment

$$S_p + \tfrac{1}{2} \frac{c}{p} S_c$$

and in analogous way on the average

$$s_i = \tfrac{1}{2} S_i .$$

From which follows, considering that s_c increases linearly with time,

$$S_c = \alpha (S_p + \tfrac{1}{2} \frac{c}{p} S_c - \tfrac{1}{2} S_i) t .$$

We found in our best experiment $\frac{c}{p} = 4$ and, as we saw that $S_i = 1.8\ S_c$, we conclude that the end value of the specificativity of the corpuscles total acid-soluble P when the experiment, as was in our case, is of restricted duration,

$$S_c = \alpha (S_p + 1.1\ S_c) t .$$

The value of the penetration coefficient of the phosphate ions into the corpuscles, a magnitude we will make use of in our later calculations, is given in the table 2.

Table 2.

Calculation of the Penetration-Coefficient of Phosphate-Ions (α) into Corpuscles.

S_c denotes the specific activity of the total acid-soluble P in the corpuscles; S_p that of the plasma at the end of the experiment.

	Time in min.	$\frac{S_c}{S_p}$	α
Rabbit C	170	0.19	0.0009
	170	0.20	0.0010
Rabbit G	90	0.14	0.0014
	90	0.21	0.0019
Average value			0.0013
Average value (Rabbit C alone)			0.0010

In what follows we desire to compare the rate of interpenetration of labelled phosphate ions into the corpuscles with that of labelled hexose-monophosphate molecules. As the latter are easily decomposed in the plasma, we have first to discuss the behaviour of the hexose-monophosphate in this medium.

Rate of Decomposition of Hexose-monophosphate.

The usual method applied to the study of the decomposition of hexose-monophosphate under the action of enzymes is the determination of the amount of phosphate ions split off in the course of the experiment. This is a highly satisfactory method if, at the start of the experiment, no appreciable amount of inorganic phosphate is present. It is not very satisfactory, however, if the decomposition of a slight amount of phosphorus ester is to be determined in the

presence of large amounts of inorganic phosphate. In such a case the use of labelled hexose-monophosphate and the determination of the amount of radioactive phosphate split off is much to be prefered to the first mentioned method. Even large amounts of inorganic phosphate present at the start of the experiment will in no way influence the results as these are, in contrary to the phosphate split off from the labelled phosphorus ester, not radioactive, and thus not recorded by activity measurements.

In our experiments we have shaken 10 cc of rabbits' blood for a few hours in a thermostat, after the addition of labelled hexosemonophosphate containing about $^1/_{15}$ mg. P. The hexosemonophosphate (Embden ester) was prepared by Dr. Ostern and kindly presented us by Professor Parnas. At the end of the experiment the activity of the total acid-soluble P of the corpuscles, that of the organic acid-soluble P of the plasma, and also that of the inorganic phosphate present in the plasma was determined. The results obtained are seen in table 3.

Table 3.

Hydrolysis of Labelled Hexosemonophosphate Added to Blood in vitro at 37°.

	Time in min.	Fraction decomposed	Velocity constant of decomposition in min^{-1}.
Rabbit A	150	0.26	0.0021
	150	0.37	0.0031
Rabbit C	80	0.15	0.0021
	80	0.15	0.0020
	170	0.29	0.0020
	170	0.32	0.0023

The first two samples were shaken in air after the addition of oxalate, the four latter samples in a mixture

of CO_2 and O_2 after addition of heparin. The velocity constant was calculated by making use of the equation valid for mono-molecular reactions

$$\ln \frac{A_o}{A_t} = kt,$$

A_t being the total activity of the ester present in the blood at the time t, A_o at the beginning of the experiment. It follows from the fair constancy shown by the velocity constant recorded in table 2 that in the hydrolysis of the hexosemonophosphate equilibrium is far from being reached in three hours. It is of interest to compare the velocity constant of the hydrolysis of the labelled hexosemonophosphate which we obtained under the action of enzymes present in the blood with the value ROBISON[1] found when hydrolysing the ester by 0.1 n H_2SO_4. The acid was found to be much less effective in hydrolysing hexosemonophosphate than the enzymes, the value of $k = 2.2 \times 10^{-6}$ min^{-1} being found by him.

In one case labelled hexosemonophosphate was added to plasma and we found, after the lapse of 175 min., 23 % of the ester to be decomposed, thus approximately the same amount which decomposes during the same time in the presence of blood corpuscles (31 %).

When labelled ester disappears we have to distinguish between two possibilities, in one case the number of ester molecules actually diminishes, in the other case the active phosphorus atom present in the ester molecules is replaced through an enzymatic exchange process by a non radioactive one and no change in the number of ester molecules occurs. That in the above discussed cases we have to deal with

[1] R. ROBISON, Biochem. J. **27**, 2191 (1932).

the first mentioned possibility (decomposition of the ester) follows from the experiment to be described. To 10 cc. plasma non active hexosemonophosphate containing 0.15 mgm. P and radioactive sodium phosphate of negligible weight was added and after the lapse of 170 min. the activity of the plasma esters determined. If the loss of activity by the active ester in the experiments recorded in table 3 should be due to an exchange process we would have to expect about 5% of the activity of the sodium posphate to be incorporated in the originally non active hexosemonophosphate added to the plasma. The result of our experiment, however, has shown that the activity of the added hexosemonophosphate isolated together with the other minute amounts of esters present in the plasma amounted to less than $^1/_{10}$ of the calculated activity. We have therefore to conclude that at least 90% of the activity loss of the labelled hexosemonophosphate recorded in table 3 is actually due to decomposition and not to an exchange process.

General Remarks on the Resynthesis of the Molecules of the Acid Soluble Organic Phosphorus Compounds Present in the Corpuscles.

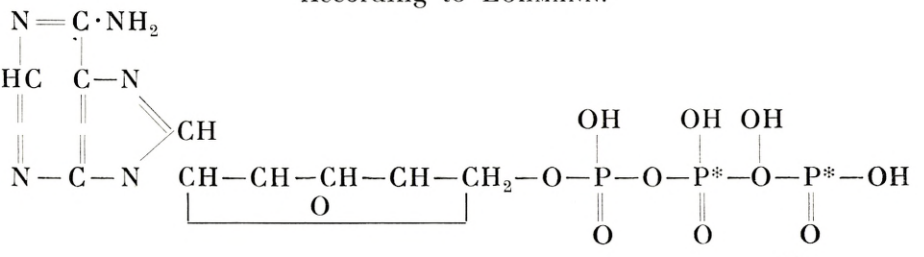
The process of the incessant new formation and thus activation of, for example, the adenosine triphosphate molecule present in the corpuscles shows a formal analogy to the incessant new formation of acetic acid radicals in aqueons solution. The acetic acid molecule is only dissociated to a small degree in turn; however, within the shortest time all molecules will pass alternatively through the dissociete and undissociated state. This can be best demonstrated by dissolving acetic acid in heavy water, in which case within

the shortest time an equipartition of the heavy hydrogen atoms between the labile H atom of the acetic acid molecule and the water will be noted. This is made possible by the incessant dissociation and rebuilding of the acetic acid molecule. In this process, however, only the acid H atom denoted by an asteric in the formula below

$$CH_3COOH^*$$

is involved, the CH_3 radical being held together by strong forces of attraction does not take part in such a new-formation process and is therefore not converted to CD_3 when acetic acid is placed in heavy water. Out of three P atoms of of the adenyltriphosphoric molecule only two, those denoted by an asteric, are moveable and involved in the alternative decomposition and new formation process going on in the corpuscles and made possible by the presence of a suitable phosphatase. The loosening of the third P atom of the adenosintriphosphoric molecule requires deeper going processes just as does the loosening of the H atoms of the CH_3 radical. The relative rate of replacement of the labile and stabile P atoms present in adenosintriphosphate extracted from the muscles of rabbits was recently found by PARNAS to be about 10 : 1.

Formula of Adenosintriphosphate.
According to LOHMANN.



Uptake of Hexosemonophosphate by the Blood Corpuscles.

We interpreted the formation of active phosphorus esters found in the corpuscles as due to an enzymatic exchange process inside the corpuscles, (the number of ester molecules decomposed being presumably replaced by an equal number of newly formed molecules) into which some of the active inorganic phosphate added to the plasma penetrated. An alternative explanation would be that active phosphorus ester molecules are formed in the plasma, diffuse into the corpuscles and are replaced, in experiments in vitro, by an equal number of non active molecules leaving the corpuscles. We can test the correctness of this explanation for cach compound by adding to the plasma the active phosphorus ester and determining, after the lapse of few hours, the activity of the phosphorus ester molecules and the inorganic phosphate of the corpuscles. So far we only carried out such experiments with active hexosemonophosphate, prepared by Dr. Ostern and presented to us most kindly by Professor Parnas. Several of the other labelled phosphorus ester compounds were also synthesized in the laboratory of the latter. The result obtained by us is that, if hexosemonophosphate molecules diffuse at all into the corpuscles, the rate of their penetration must be much slower than that of the phosphate ions. We arrived at this result by supposing: The amount of active P to be expected in corpuscles after the lapse of a certain time is wholly due to the penetration of active phosphate ions from the plasma into the corpuscles and independent of the presence of active hexosemonophosphate in the plasma. The next step is to compare the calculated values for the activity of the corpuscles P with those found by the experiment

and to ascertain if any difference is shown by the two values. Should such not be the case, then we must conclude that the rate of penetration of the hexosemonophosphate molecules into the plasma is negligible compared with that of the phosphate ions. The amount of labelled P in the corpuscles is zero at the start, i. e. after addition of active hexosemonophosphate to the plasma, and increases with time as discussed on p. 9. As in the course of the experiment only a small part of the active hexosemonophosphate is hydrolysed, we are entitled to make the simplifying assumption that the increase of the specific activity of plasma inorganic P takes place in a linear fashion. We also assume that the decrease of the difference in the specific activities of plasma phosphate P and corpuscle phosphate P with increasing time will also take place according to a linear function.[1] The average value (comp. p. 13) of the last mentioned difference will be

$$\tfrac{1}{2}\,(S_p - S_i) = 0.5\,S_p - 0.9\,S_c$$

and the specific activity of the corpuscles P at the end of the experiment

$$S_c = \alpha\,(0.5\,S_p - 0.9\,S_c)\;t.$$

The value of α being known ($\alpha = 0.0010$), the quantity $\frac{S_c}{S_p}$ can be evaluated. The figures thus obtained and also those supplied by the experiment are recorded in table 4.

Table 4.

	Time of experiment in min.	Values of $\frac{S_c}{S_p}$ calculated	found
Rabbit C	80	0.04	0.03
	80	0.04	0.04
	170	0.07	0.07
	170	0.07	0.09

[1] This assumption though incorrect does not cause an appreciable error.

Uptake of Phosphate Ions by the Blood Corpuscles in Experiments in vivo.

The interpretation of the results of experiments in vivo is much complicated by the fact that active phosphate introduced into the blood stream is rapidly exchanged with phosphate of bone tissue. Such an interaction leads to a very rapid decrease in the activity of the blood after intravenous administration of active phosphate as the specific activity of the plasma inorganic P at the end of the experiment differs very strongly from the value prevailing on the average during the experiment. To facilitate the interpretation of the result the labelled sodium phosphate was injected into the ear vein of a rabbit drop by drop in the course of the experiment which took 3 hours. We have to thank Professor Lundsgaard for injecting the rabbit in this and many other cases. During the experiment small blood samples (0.1 — 0.3 mg.) were taken from the vein of the other ear of the rabbit and their activity determined. The results of the experiment are seen in tables 5 and 6.

Table 5.

Percentage of the total labelled P injected during the course of the experiment present in the circulation.

(Labelled sodium phosphate administered drop by drop in the course of three hours).

Time in minutes	Percentage of the total P administered in the course of 3 hours present in circulation
18	1.16
61	2.64
117	4.00
168	5.36

Table 6.

Specific Activities Obtained.

Labelled P injected drop by drop during the course of the experiment (3 hours).

Fraction	Relative specific activity
Plasma inorganic P	100.0
Corpuscle inorganic P	11.0
Corpuscle acid-soluble organic P	7.7
Marrow inorganic P	7.5
Muscle inorganic P	6.9
Muscle creatine P	0.95
Muscle acid-soluble after removal of inorganic creatine P	0.78
Tibia diaphysis P average	0.27

As seen from the figures of table 5 the specific activity of the plasma inorganic P increases during the experiment somewhat slower than proportional to time. If the increase of the specific activity would be proportional to the time of the experiment the average specific activity of the plasma inorganic P, which we wish to know, should be $= \frac{1}{2} S_p$. This can be considered to be a lower limit of the average specific activity. To arrive at an upper limit we could assume the average specific activity to be equal to the maximum value observed, which is S_p. Making the assumption $s_p = \text{prop.} \sqrt{t}$, we arrive at the average value of the specific activity of the plasma inorganic P $= \frac{2}{3} S_p$. The value for the specific activity of the total acid-soluble corpuscle P (comp. p. 13) is approximately given by the formula

$$S_c = \tfrac{2}{3}\, \alpha t\, (S_p - 1.8\, S_c).$$

For the ratio $\frac{S_c}{S_p}$, after the lapse of 175 min., we arrive at the figure 0.12 (using the value of α found in our

experiments in vitro), while the experimental value of the specific activity of the acid-soluble P of the corpuscles (comp. table 6), after the lapse of 175 min., was found to be 8 % of that of the plasma inorganic P at the same time, or $\frac{S_c}{S_p} = 0.08$. The calculated and experimentally found values of the rate of labelling of the corpuscles acid-soluble P are thus in good agreement. We arrived at the calculated figure by making use of the α value obtained with rabbits' blood in experiments in vitro (taking $\alpha = 0.0013$ for rabbit I).[1]

Administration of Labelled Hexosemonophosphate.

Labelled hexosemonophosphate containing 6 mgm P was administered to a rabbit weighing 2.7 kg. by intravanous injection. After the lapse of $1\frac{1}{2}$ hour the animal was killed and the specific activity of the fractions recorded in table 5 determined.

When interpreting the above figures we must bear in mind that the greatest part of labelled inorganic P formed through the decomposition of active hexosemonophosphate enters the tissues. That the specific activity of the plasma inorganic P is, in spite of this fact, higher than that of the plasma ester P clearly indicates that a very large part of the active hexosemonophosphate injected into the blood must have been decomposed in the course of the experiment. We must also take in consideration that besides the 4 mgm. % hexosemonophosphate P added the plasma contained also its normal ester P content of about 0.5 mgm. % which became partly labelled, this being made possibly

[1] When isolating the inorganic P and the different fractions of the ester P present in the corpuscles and comparing their specific activity in experiments in vitro and in vivo some differences were found. The results of these experiments will be shortly published.

through the presence of labelled inorganic P formed through the decomposition of active hexosemonophosphate. But even disregarding the presence of esters other than hexosemonophosphate, by comparing the activity of the hexosemonophosphate introduced with that of the total plasma ester present, after the lapse of $1^1/_2$ hours, we arrived at the result that more than 99.9 % of the labelled hexosemonophosphate administered left the circulation within $1\frac{1}{2}$ hours. In Making the above calculation we assumed the blood content of the rabbit to amount to 160 cc. In our in vitro experiments $^1/_{15}$ mgm. of hexosemonophosphate was added to 10 cc. blood, thus the concentration of the latter was about $^1/_6$ of that in the experiment in vivo[1]. While in the in vitro experiment in the course of $2^1/_2$ hours $\frac{1}{5}$ of the labelled hexosemonophosphate was decomposed, in the in vivo experiment in the course of $1^1/_2$ hours more than 99.9 % was removed.[2] A powerful agency producing hydrolysis of hexosemonophosphate is ROBISON's bone enzyme. The presence of small amounts of this enzyme in the plasma was found by MARTLAND and ROBISON[3] which according to them is possibly derived from bone by slow diffusion. These small amounts of bone enzymes were presumably responsible for the hydrolysis of hexosemonophosphate in our experiments in

[1] When comparing the results of the in vitro and in vivo experiments we have to consider that the rate of decomposition depends on the concentration of the substrate as well, through a change of the concentration in a ratio 1:6 should not strongly influence the rate of decomposition.

[2] A large part of the hexosemonophosphate removed may have been taken up by the tissues. R. T. BRAIN, H. O. KAY and P. G. MARSHALL (l. c.) found namely that in the course of 5 min. more than three-quarters of the injected glycerophosphate left the human circulation and as in this time it had not been excreted in the urine, nor hydrolysed into inorganic P in the blood, it must have been taken up by the tissue.

[3] MARTLAND and ROBISON, Biochem. J. **20**. 847, 1926.

vitro, while the much more rapid disappearance of the labelled hexosemonophosphate from the blood in vivo is probably due to the much larger amounts of bone enzyme present in the bones and other organs, especially the kidneys[1]. As seen in table 7 the specific activity of the corpuscle inorganic P is appreciably lower than that of the plasma inorganic P, while that of the corpuscle ester P is only slightly lower than that of the corpuscle inorganic P.

Table 7.

Specific Activity of P Fractions $1^1/_2$ Hours After Administration of Labelled Hexosemonophosphate.

Fraction	Specific activity
Plasma inorganic P	1.
Plasma ester P	0.9
Corpuscles inorganic P	0.3
Corpuscles ester P	0.23
Liver inorganic P[2]	0.62
Liver ester P	0.22

On the Origin of Phosphatides of Plasma, and Corpuscles.

We have already mentioned that the amount of labelled phosphatides formed in the blood within a few hours is

[1] Bodansky (I. Biol. Chem. **118**, 391, 1937) concludes that the phosphatase in question comes from the bone, kidney ov some other tissue, but not from the intestinal mucose.

[2] We were prevented in extracting at once the inorganic and acid soluble organic P of the liver and therefore some of the latter may have been decomposed (comp. E. Jacobsen, Biochem. Z. **242**, 292, 1931); supplying inorganic P of low activity. The specific activity of the inorganic P present as such in the intact liver may therefore have been higher than stated above. In this connection it is of interest to know that in the case of a rat, which had been injected with labelled hexosephosphate and killed 2 hours later, no such difference was found. Relative specific activities: Plasma inorganic phosphate 1.0; liver inorganic phosphate 0.97; liver ester 0.58).

entirely negligible compared to that of labelled acid-soluble organic compounds formed. The phosphatides present in the plasma are released by the organs in which phosphatides are synthesised, primarily by the liver, but also by the intestinal mucosa, and possibly by other organs. The synthesis of phosphatides in the different organs was investigated in recent years using fatty acids, which could be traced by chemical analysis[1], and also by the use of radioactive P as an indicator[2]. In several cases the change in the degree of saturation of the fatty acid component of the phosphatides extracted from the intestinal mucosa, liver, etc., was studied after feeding cod liver oil which contains a large amount of unsaturated fats. Within a short time an increase in the iodine number of the fatty acids was found. For example, within 2 days after the change of diet the iodine number of phosphatides of the intestinal mucose increased from 93 to 160. From this result it follows that within 2 days an appreciable amount of the phosphatide molecules present in the intestinal mucose were renewed. Instead of feeding a mixture of fatty acids showing a different degree of saturation and having a high average iodine number, SINCLAIR fed, in his later experiments, fats containing pure (85 %) elaidic acid, a geometric isomer of oleic acid, an easily traceable substance, since it forms a lead salt which is insoluble in ether, differing in this regard

[1] Comp. R. G. SINCLAIR's report in Physiological Reviews **14**, 351 (1934). The papers of the same author and collaborators, J. of Biochem. **115**, 211 (1936); **118**, 122 (1937); **121**, 361 (1937). C. ARTOM, Archiv intern. Physol. **36**, 101 (1933).

[2] C. ARTOM, C. PERRIER, M. SANTANGELO, G. SARZANA, and E. SEGRE, Archiv Internat. de Physiol. **45**, 32 (1937); **47**, 245, 1938. L. HAHN and G. HEVESY, Skandinav. Archiv f. Physiol. **77**, 148 (1937). G. HEVESY and E. LUNDSGAARD, Nature **140**, 275 (1937). B. A. FRIES, S. RUBEN, I. PERLMAN and I. C. CHAIKOFF, J. Biol. Chem. **122**, 169 (1937); **123**, 587 (1938).

from all other unsaturated acids. 8 hours after feeding elaidic acid to cats, 15 % of the fatty acids extracted from the plasma phosphatides were found to contain elaidic acid, while hardly any was found in the phosphatides obtained from the corpuscles. This result may be interpreted as a proof of lack of a phosphatide turnover in the corpuscles. Another chemical indicator used to follow up the turnover of phosphatides is iodised fat which was fed by Artom to animals. Also in this case the presence of iodised fatty acid in the phosphatide molecule is a proof of their formation after the administration of iodised fat. By using this method Artom found the presence of iodised fatty acids in the phosphatides extracted not only from the plasma but also from the corpuscles. In the latter the concentration of the iodised fat was even higher (3.3 % of the total fatty acids) than in the former (2.0 %), a still lower content being found in the phosphatide fatty acids secured from the liver. The result obtained as to the turnover of phosphatides in the corpuscles, when using the elaidic acid method, is thus just the opposite of that arrived at when applying iodised fat as an indicator. While these indicators proved to be very useful to show that a rapid turnover of phosphatides actually takes place in some of the organs they are less adapted to permit conclusions of a quantitative nature to be drawn.

By introducing elaidic acid or iodised fat into the phosphatide molecule the properties of the latter are appreciably changed and not only will the different organs utilise the above mentioned substances in the formation of phosphatides only to a restricted extent, but the rate of uptake of these compounds may differ for different organs. In rats provided with large amounts of elaidic acid through-

out the entire period of prenatal and postnatal development the elaidic acid content of the fatty acids in the phosphatides of the brain was found to be only $^1/_4$ of that of the liver and muscles. If one finds a slower turnover of elaidic acid in the brain phosphatide than in other organs, this result can be partly due to a slower phosphatide turnover in the brain, and partly to a greater degree of selection in the building up of phosphatides in the brain than in the liver. The rate of the incorporation of elaidic acid into the phosphatide molecules will therefore fail to be a quantitative measure of the rate of phosphatide rejuvenation in the brain tissue, though this method revealed much important information as, for example, that the phosphatide turnover in the muscles is much slower than that of the liver and the intestinal mucose. In rats the incorporation of elaidic acid into the liver phosphatides was found to be essentially completed within one day but in the muscle transformation had occured only after a period of many days. One of the great advantages of the application of isotopic indicators is that the replacement of ^{31}P by ^{32}P for example in the phosphatide molecule does not change the chemical character of the substance to any noticeable extent and therefore any possible preference of an organ for the ^{32}P phosphatide can be disregarded. A quantitative comparison of the phosphatide turnover in different organs by using ^{32}P as an indicator was carried out by different experimentors, a slow turnover being found in the brain and muscles, a fast one in the milk gland, the liver, the kidneys and the intestinal mucose, while we find a fairly fast rate in tumor tissue. Taking the specific activity of the phosphatide P extracted from the liver of a mouse to be 100, we find for the specific activity of that extracted from the muscles

and the graft of the brest tumor 18 and 9 % respectively. These figures indicate the relative rate of resynthesis of the phosphatide molecules. They were obtained by extracting and analysing the phosphatides from the organs, 4 hours after the administration of labelled sodium phosphate. These figures, however, fail to inform us as to the percentage of the phosphatide molecules which were renewed within the last 4 hours. Most of the active phosphate ions will exchange with bone and other tissue phosphate and will thus be prevented from taking part in the synthesis of phosphatide molecules. Knowledge relative to the percentage of, for example the liver phosphatides, renewed within 4 hours can be obtained, as already discussed on p. 21, by administrering the sodium phosphate solution drop by drop and thus keeping the active phosphate concentration of the blood at an approximately constant level, or in perfusion experiments carried out on the isolated liver. In the latter case active sodium phosphate is added to the blood circulating through an isolated liver and, after the lapse of a few hours, the specific activity of the plasma inorganic P and that of the phophatide P extracted from the liver are compared. Through the kindness of Prof. Lundsgaard we were able to carry out such a determination from which it was concluded that, in the course of $2^1/_2$ hours, about 2 % of the phosphatides in the cat liver are renewed[1]. In the same time, when blood is shaken with active sodium phosphate, only a very slight amount of active phosphatide was formed. From these experiments it may be concluded that less than 0.1 % of the phosphatide molecules present in the isolated blood was renewed in the course of $2^1/_2$ hours. In experiments on living goats, 4 hours after injecting labelled sodium phosphate, less and probably

[1] L. A. Hahn and G. C. Hevesy, Biochem. J. **32**, 342, 1938.

much less than 1 % of the blood phosphatide molecules were labelled. We have therefore to conclude that the phosphatide molecules present in the blood can only get rejuvenated by an influx of molecules from the organs like the liver in which they were synthesised. As a possible source of formation of the blood phosphatides the liver is first to be considered in view of the fast phosphatide turnover found in the liver and the large amounts of phosphatides stored in it. In this connection the results obtained by NEDSWEDSKY[1] should be recalled according to which blood leaving the liver contains 23 % more phosphatide than that entering the liver from the portal vein. Laying hens are especially well suited to the study of phosphatide metabolism. A hen laying daily incorporates into the yolk 1—2 gms. of phosphatides corresponding to about 60 mgms. of P. We found that these phosphatides were not produced in the ovary but were carried by the plasma in the main from the liver. To what extent phosphatides are carried into the circulation through the lymph from the intestinal mucosa is not yet settled. In the above mentioned experiment the specific activity of the phosphatide P extracted from the hens' intestinal mucosa was found to be only 1/4 of that of the plasma phosphatide P. The greatest part of the active phosphatide molecules present in the plasma of the hen could therefore not originate from the intestinal mucosa but must have been formed in the liver and, possibly to minor extent, in other organs. As the plasma of the hen contains only about 20 mgm. % phosphatide P, thus only 1/3 of that incorporated in the yolks daily, over 9/10 of the plasma phosphatides of the blood is removed from

[1] S. W. NEDSWEDSKY and K. ALEXANDRY, Z. physiol. Chem. **219**, 619 (1928).

the latter, in the course of a day, and replaced by newly formed molecules. If the plasma phosphatides originate from the liver, after the lapse of a day, the specific activity of the plasma phosphatide P should no longer differ materially from that of the liver phosphatide P. In an experiment in which the hen was killed 28 hours after injecting the labelled phosphate, the plasma phosphatie P showed a specific activity amounting to 82 % of that of the liver phosphatide P. When interpreting the low figure found for the phosphatide turnover in the intestinal wall of the hen compared with that found in the liver we have however to bear into mind that the labelled inorganic phosphate reaches the digestive tract at a later and thus more "diluted" (with inactive phosphate) state than the liver. The active phosphate injected will be promptly carried to the liver while it enters the intestine only in the form of saliva, gastric juice, bile and pancreatic juice and, possibly to some extent, through the intestinal wall[1] in the digestive tract. Therefore, when comparing the specific activities of the liver phosphatide P with that of the intestinal mucosa phosphatide P after injection of active phosphate we are apt to overestimate the phosphatide turnover of the liver while, when feeding the active sodium phosphate, we must expect the opposite to be the case. A comparison of the specific activity of the liver inorganic P with that of the liver phosphatide P leads to the result that, after the lapse of 28 hours, the former was about two times greater than the latter. Thus only less than half of the phosphatide molecules present in the hen's liver was newly formed within that time, the rate of regeneration of the tissue phosphatides being thus a comparatively slow process, since the removal of the tissue phos-

[1] G. F. Youngburg, Proc. Exp. Physiol. Mediz. **36**, 230, 1932.

phatides into the plasma also takes time the low rate of rejuvenation of the plasma phosphatides is just what we would expect. In experiments we carried out on human subjects, in the course of a day less than 30 % of the plasma phosphatide was regenerated and a rough estimate, taking into account the change of the spec. activity of the phosphate, indicates that after the lapse of a week this fraction is still less than one-half. (comp. table 8).

We have already mentioned that in vitro experiments have shown that in the blood only a very minimal new formation of phosphatide molecules takes place; the phosphatides present in the corpuscles must therefore have been incorporated in the latter during their formation, or alternatively diffused from the plasma into the corpuscles.[1] As already mentioned, within a day, the plasma phosphatides of a daily laying hen were replaced up to 82 %, by phosphatide molecules carried into the circulation from the liver and other organs. In spite of this thorough-going replacement of the plasma phosphatides, the phosphatide molecules present in the corpuscles are renewed only to an extent equal to $^1/_3$ of that of the plasma phosphatides in the course of 28 hours. In human subjects, after the lapse of one day, the corpuscle phosphatide shows a specific activity amounting to only $^1/_5$ or less of that of the plasma phosphatide P and even after the lapse of 8 days the ratio is still $^1/_2$. When the corpuscles are formed from plasma containing labelled phosphatides they are bound to contain such. As seen above, even after the lapse of a week the

[1] An investigation carried out recently incollaboration with L. HAHN lead to the result that a fairly slow exchange between a part of the phosphatide molecules present in the corpuscles and those present in the plasma takes places. In the course of 4 hours about 5 % of the phosphatides present in the corpuscles were exchanged.

labelled fraction of the plasma phosphatides is still twice as high as that of the phosphatides present in the corpuscles. From this result we can conclude that less than half of the corpuscles are produced in a week. One could object to the above conclusions on the ground that the phosphatides are composed of different constituents, lecithin, cephalin and so on, each of which may contain very different fatty acids and, if those components which are mainly represented in the corpuscles are renewed at a slower rate than those chiefly found in the plasma, this difference may also tend towards a low value for the specific activity of the corpuscle phosphatide P. In human blood, the composition of the corpuscle phosphatides was found to be not very different from that of the plasma phosphatides[1] and as we have found that, after a lapse of a day, the specific activity of the corpuscle phosphatides is only 8—19 % of that of the plasma phosphatides (comp. table 8) we are justified in concluding that at least the most active component of the phosphatide mixture of the corpuscles must show a lower specific activity than its conterpart in the plasma.

This fact definitely excludes the possibility that the active phosphatides are formed in the corpuscles and diffuse into the plasma. The result discussed on page 27, according to which the corpuscle phosphatides contain more iodised fat than the plasma phosphatides, is presumably to be explained by a greater preference of the corpuscles for phosphatides containing an iodised fatty acid component.

[1] E. Kirk, J. Biol. Chem. **123**, 637 (1938). According to this experimentor plasma phosphatide contains as an average 13 % lecithin, 47 % cephalin, and 40 % sphingomeyelin, while the corresponding figures for the corpuscles are 16, 60, and 24.

Table 8.

Parts in Million of the Total Activity Injected Subcutaniously Present in 1 mgm. P extracted from the Plasma and Corpuscles of Human Subjects.

a) Boy	
Sample taken after 120 min.	
Plasma phosphate	201
Corpuscle total acid soluble P	57
Sample taken after 7 days	
Plasma phosphate	10
Plasma phosphatides[1]	14
Corpuscle phosphatides	6
b) Young female	
Sample taken after 130 min.	
Plasma phosphate	480
Corpuscles inorganic phosphate + hydrolysable ester P (hydrolysed for 7 min. at 100° in 1 n HCl)	87
Corpuscles non hydrolysable ester P	62
Sample taken after 1 day	
Plasma phosphate	105
Corpuscles total acid soluble	173
Plasma phosphatide	31
Corpuscles phosphatide	2.3
c) Old female	
Sample taken after 140 min.	
Plasma phosphate	224
Corpuscles inorganic phosphate + hydrolysable ester P (hydrolysed for 7 min. at 100° in 1 n HCl)	61
Corpuscles non hydrolysable ester	34
Sample taken after 1 day	
Plasma phosphatide	7.5
Corpuscles phosphatide	1.3
Sample taken after 8 days	
Plasma phosphate	10
Plasma phosphatide[1]	16
Corpuscles phosphatide	7

[1] We witness here an example of the case discussed in detail in former papers (for example HAHN and HEVESY, Skandin. Archiv f. Phys. **77**, 148 (1937)) in which, due to the rapid exchange between bone phosphate and plasma phosphate the active inorganic P is renewed from the plasma at a rapid rate while the removal of the labelled phosphatide P is a much slower one. Some of the phosphatide molecules were formed

Summary.

The rate at which labelled phosphate ions added to the plasma of rabbits' blood penetrate into the corpuscles was determined. A comparison of the specific activity (activity per mgm. P) of the inorganic P and of the ester P extracted from the corpuscles suggests that the penetration of the labelled inorganic phosphate into the corpuscles is a comparatively slow process while the process in which the labelled inorganic phosphate is incorporated into the easily hydrolysable organic compounds inside the corpuscles is a fast one.

The rate of increase of the specific activity of the total acid soluble phosphorus (s_c) present in the corpuscles with time (t) is proportional to the difference in the specific activity of the inorganic P of the plasma (s_p) and that of the corpuscles (s_i).

$$\frac{ds_c}{dt} = \alpha (s_p - s_i).$$

The proportionality factor of the above equation (α), denoted as coefficient of penetration, was calculated; the latter was found to be about the same in experiments in vitro and in vivo.

By making use of labelled sodium hexosemonophosphate it was found that, when shaking the latter with rabbits' blood, $^1/_5$ hydrolyses within $2^1/_2$ hours. From sodium hexosemonophosphate administered, by intravenous injection, to a rabbit after the lapse of $1^1/_2$ hours less than $^1/_{10}$ % was left in the circulation.

from a highly active plasma and were still present after a lapse of 7 days, while the greater part of the inorganic P dissappeared at this time from the plasma. Similar cases can also be found when investigating the acid soluble P.

By comparing the rate of formation of labelled ester P in the corpuscles after introducing in one case labelled hexosemonophosphate in the other labelled inorganic phosphate we arrive at the result that the labelled phosphate ions which penetrate into the corpuscles are utilised for the formation of labelled ester molecules. If any labelled hexosemonophosphate penetrates into the corpuscle at all, the rate of its penetration is much lower than that of the phosphate ions.

The analogy between the formation of labelled adenyltriphosphate and other acid-soluble P compounds present in the corpuscles and the formation of "heavy" acetic acid when dissolving acetic acid in "heavy" water is emphasized. The role played by the electrolytic dissociation in the latter case is taken over by the enzymatic action of the phosphatases present in the corpuscles.

The rate of regeneration of the phosphatide molecules present in the blood was found to be very much slower than that of the acid-soluble compounds. The regeneration of the former was found to take place in the organs from which they are carried into the circulation. After the lapse of a week, about one half of the phosphatide molecules present in human plasma was replaced by within that time newly formed molecules. The replacement of the corpuscle phosphatides is a much slower process than that of the phosphatides present in the plasma, the ratio in human blood after one day being 6 : 1, after a week 2 : 1.

We want to extend our thanks to Prof. Niels Bohr and Prof. J. N. Brønsted for putting many facilities at our disposal, to Prof. E. Lundsgaard for many valuble sugge-

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Despite the continuous efforts made during the past 30 years in muscle physiology, the extent of our knowledge in some parts of this field still remains comparatively slight. This is first and foremost due to the fact that as a rule the muscle has been considered experimentally as a structural unit, no attention having been paid to the many different elements of which it is composed, which may vary widely in their physical, chemical and physiological properties. Furthermore it has not been generally realized that the shape of the contractile elements themselves and their arrangment in the muscle will influence profoundly the mechanical behaviour of the tissue. Moreover, on the basis of experiments using whole muscles attempts have been made to draw conclusions concerning the phenomena taking place in the microscopic and ultramicroscopic parts of the muscular fibril. Thermal investigations also have met with difficulties arising from such causes; it has for instance been found impossible to secure a uniform temperature throughout the whole of the preparation, resulting in considerable uncertainty in assessing experimental evidence; and the importance of the shape of the fibres has not been appreciated. Investigations of the electrical properties of muscles have not been performed with regard to such considerations either. For instance the result of measurements of resistance in a whole muscle is of limited value, because we do not know the paths which the current will follow in the tissue. If it

is desired to study the electrical properties of the fundamental contractile mechanism, then the experimental object must be the single muscle fibre; because in the whole muscle such factors as connective tissue, blood vessels of varying calibre etc., will interfere considerably with the measurements.

There are, of course, problems which *can* be solved through experiments on whole muscles and some again which can *only* be solved by the use of such preparations. But in most cases this method of approach is ruled out, because the very numerous sources of error arising from the highly complicated structure of the muscle can neither be eliminated nor properly controlled. Therefore, more and more use has gradually been made of the functional unit — the single muscle fibre. The definition thus implied may be criticised on the grounds: firstly that even in muscles allowing the most finely graded movements, more fibres are present than axis cylinders in the corresponding motor nerve; so that a muscle fibre cannot come into action singly; secondly, recent electrostatic measurements show that the muscle is composed of a vast number of relatively independent subdivisions (sarcomeres); yet nevertheless, it must for the time being, be considered permissible to regard the fibre as the *functional* unit under physiological-conditions, as in such circumstances a partial contraction of a fibre is unknown. Since the muscle fibre is thus neither structurally nor functionally considered to be the *ultimate* unit, the value may be questioned of employing these small objects, in many respects so difficult to handle; the more so since the isolated fibre, despite all possible care, has a very limited life as an experimental object.

It is, of course, for such reasons that where experimental

conditions have permitted, bundles containing a small number of fibres have been used. But greater difficulties are met with in the whole muscle. Even pairs of symmetrically situated muscles in the same animal differ in structure, so that the variations in the results of experiments with whole muscles will in many cases become so great that it becomes possible only to discuss the functions of the muscle in approximate terms. Therefore, when the essential features of muscular function had to be investigated attempts have always been made to infer from such results the behaviour of the single fibre. This has, however, often proved to be impossible, not only because the experimental errors may become of a higher order of magnitude than the reaction which it is desired to study, but also because variations are so great as to require an impracticable number of experiments before reliable results can be obtained by the use of statistical methods. By working with the single muscle fibre we eliminate a number of the major sources of error, the conditions of the experiments become far simpler, and unavoidable errors become of more reasonable size in proportion to the physiological reactions. This method of approach, during the few years in which it has been used, has proved fruitful and has thrown new light on previous investigations, which may be said to justify its introduction.

Anatomy of the Striated Muscle Fibre.

On the basis of the general concept of the organic kingdom that structure and function are intimately related, it would be appropriate to begin every account of the function of an organ with a discussion of its structure.

The anatomical investigations to be dealt with here are limited to the skeletal muscles of the vertebrates, especially of the frog, chiefly because the great majority of our own physiological experiments were performed with frog's muscles. We have, however, also experimented with lizard's muscles, especially so where the motor endplates were involved in the experiments.

Shape and Size of Muscle Fibres.

In order to determine the shape and size of the muscle fibre, it must be completely isolated. Within the bundles the fibres are often interwoven to such a degree that it becomes impossible to trace any one fibre for a considerable distance, especially towards its terminal portion, which is often extremely slender (Fig. 1.). As the fibres moreover are often overlapped in part by others, or deformed through pressure by adjacent fibres it will almost always be impossible without complete isolation to measure their thickness with requisite accuracy. When isolating the fibres, the bi-refringence method gives valuable information as to whether one or more of these is being dealt with, and has the particular advantage that the decision can be made without touching the fibre with any instrument.

If the isolated fibre has to be employed in physiological experiments there is only one method of isolation possible, viz. dissection. If, on the other hand, we only want to measure the shape and dimensions of the fibre, other methods may also be employed; but as not all those suggested seem to be reliable, only a few will be referred to here.

In Schwalbe's laboratory Mayeda (1890) employed the following procedure: He immersed the live animal in 20

per cent. nitric acid and then placed it in an incubator at 40° (C) for 24 hours. The preparation was then removed and carefully rinsed with water. Examination had to be made immediately after this preparation was completed. The advantage of this procedure appears to be that the animal whilst alive would have to swallow some of the fluid in which it was immersed, so that the action of the latter would presumably be more effective and more rapid than by penetration through the skin alone. This method, though unattractive, may be expected to give reliable results.

Fig. 1. Muscle bundle with the conical and spindle shaped fibres of which it is composed. Entrance of nerve twigs marked. (BARDEEN).

Another method, given by F. C. C. HANSEN (see LINDHARD (1926)), consists in boiling the muscle for 2 hours in a quantity of water that will just cover it, after which the boiled muscle is clarified in glycerine. The following modification of this method has been employed by LINDHARD. The whole of a frog's leg was placed in a beaker filled with ordinary tap-water, and covered by a glass-plate. When the water is heated to boiling, whether slowly or rapidly, a sudden extension of all the joints of the limb will occur at a certain temperature, and during the subsequent boiling the limb will remain in the same position. When boiling is finished and the skin cut off, the muscles can be removed. Muscles prepared in this manner have the same shape as fresh muscles but are slightly smaller in all dimensions. As a rule there are no signs of contracture, but if a single muscle is treated in the same manner it will be found after boiling in a state of maximal contracture.

The individual fibres may now be isolated with comparative ease under the low power binocular microscope. After it has been ascertained, using the high power, that the sarcolemma is uninjured, the fibre is then measured with the aid of an ocular micrometer.

It appears from such measurements and from similar observations on uninjured living fibres (see later), that the muscle fibres vary greatly both as regards dimensions and shape, and not only do such variations occur between different species but also between different muscles in the same animal. Great variations may even be found between fibres of the same muscle.

In the majority of the cases examined the muscle fibre is shorter than the bundle, sometimes much shorter, but there are also muscles in which the fibres run from one terminal tendon to the other. Most text-books appear to give rather superficial statements. However, Heidenhain's statement (1911) that only in muscles more than 12 cm. long are fibres found terminating within the bundle, whilst in all shorter muscles the fibres run from tendon to tendon, is probably incorrect. Moreover, his observation that the thickness is from 9—60 μ does not cover the whole range of variations.

Schäfer's (1893) statements are more accurate, saying that the length (with the exception of the sartorious) rarely exceeds $1^1/_2$ inch., whilst the thickness is 0.1—0.01 mm. — Mayeda (l. c.) examined the thickness of the fibres in the muscles of different animals throughout the whole range of vertebrates; but unfortunately he gives one measurement only, which makes it impossible to determine the shape of the fibres. In only a few cases was the length measured. These measurements show that the minimal thickness of

the fibres is approximately the same in all vertebrates, whilst the maximal thickness varies, decreasing in the following order:— Fishes, toads, reptiles, mammals, birds. The two latter classes are, however, only represented by one species each. The thickest fibres in a single individual muscle were found in the gastrocnemius, where this muscle occurred, and the thinnest in the ocular muscles.

In one case MAYEDA measured the length as well as the thickness of 48 fibres from a 26 mm. long sartorius of the R. esculenta, (giving, however, only one measurement of thickness). His results appear in Table I.

Table I.

	2	12	11	24	18	17	20
	2	14	24	11	24	24	23
	8	12	16	21	10	13	16
	9	4	16	19	22	16	25
	11	18	19	6	11	20	20
	7	11	7	11	22	22	..
	..	..	24	10	24	25	..
	..	..	15	..	13	25	..
Length, mm.	6.5	12	16.5	14.5	18	20	21
Thickness, μ	34—42	49—53	57—61	68—89	91—106	114—118	122—141

It will appear from the table that there is an approximate relation between length and thickness, the thickness increasing with increasing length. The average length of the 48 fibres is 15.7 mm., which shows that two fibres originating from opposite ends of the muscle overlap for a length of 5.4 mm.— .

BARDEEN (1903) has given an excellent description of the structure of the obliquus abdominis externus in a number of mammals. Figure (1) represents a bundle consisting of three "fibre lengths", the centre one of which is composed of elongated spindle-shaped fibres, the tapering ends of which both end within the bundle.

Lindhard (1926) has measured the dimensions of the fibres of the gastrocnemius and the sartorius in the two experimental animals usually employed, namely Rana esculenta and R. temporaria. The gastrocnemius is composed of fibres of unequal length, the longest being those in the centre of the muscle. The average length of 10 fibres from a muscle 30 mm. long, was 4.6 mm.; the distal fibres are the shortest, hardly more than 1—1.5 mm. in length. In R. esculenta the fibres are bluntly conical. The ratio between the thick and the thin end of the fibre was 5.2:3, this being the mean of 18 measurements. In R. temporaria most of the fibres were irregularly cylindrical and arranged in pairs, a thick and a thin fibre alongside each other, the following measurements show:—

	I	II	III
Length in mm.	3.1—2.8	3.1—2.8	3.1—2.9
Thickness in μ	122—52	113—52	116—34

In the gastrocnemius the fibre thus runs from one terminal tendon to the other; but as a rule this is not so in the M. sartorius, especially not that of R. esculenta. The following figures are illustrative of the M. sartorius (R. esculenta):—

Muscle (longest fascicle)	27.9 mm.
Average length of fascicles	25.5 -
Average length of 117 fibres	17.2 -

The fibres will thus overlap each other for 9 mm., on the average. The ratio between length and thickness is best illustrated when the thickness of the fibres is measured in two places near the ends of the fibres:—

Length in mm.	13.5	14.8	25.5	15.1
Thickness in μ	9—104	46—122	9—101	49—107
Length in mm.	18.2	13.1	14.4	21.5
Thickness in μ	9—61	21—80	46—168	24—128

In a sartorius, 26 mm. long, the following fibre lengths were found:

5.2 7.6 8.6 11.5 12.4 13.9 16.2 19.6 22.0 24.0 mm.

In the sartorius of temporaria the average fibre length is greater in proportion to the total length of the muscle; the fibres frequently pass through the entire length of the muscle, but are rarely cylindrical.

From what has been stated above it seems highly improbable that any part of the sartorius is devoid of nerve fibres. This is shown by KÜHNE's early investigations (1860) and is borne out by SCHIMERT's more recent publication (1934). PÉTERFI and BUCHTHAL in unpublished experiments, have recently demonstrated with the aid of vital staining, fine nerve twigs in the pelvic end of sartorius.

Despite the considerable variation in shape of the muscle fibres, and the absence of any systematic investigations on this point, it is, however, possible to establish certain general types.

The muscle fibre is sometimes cylindrical; such fibres occur in various muscles of different species of animals, and are doubtless always comparatively short. The same applies to the bluntly conical shape described above in the gastrocnemius of R. esculenta. Long muscle fibres, on the other hand, are flagelliform (F. C. C. HANSEN) or lanceolate and are then connected with the terminal tendons by their thick, rounded end whilst the thin "tail" is lost in the endomysium. Moreover MAYEDA's work as well as that of BARDEEN's and LINDHARD's suggests that in certain muscles the fibres are shorter than the bundle, often considerably so, but on the average their length is more than half the length of the bundle, so that the thin fibre-ends overlap

for some distance. As fibres in the same muscle vary considerably in length this arrangement does not give rise to the occurrence of mechanically weak parts in the belly of the muscle. An additional mechanical support is provided by the occurrence in the muscle of varying numbers of elongated spindle-shaped fibres, i. e. fibres which are thickest in the centre and have long pointed ends, both of which are lost in the endomysium. The M- and N-fibres mentioned by Schiefferdecker (1902) are probably to be considered as artefacts, as the illustrations in his paper seem to indicate. It is often stated that in the tongue or the basihyoid membrane of the frog there may be found muscular cells showing tree-like branching. This statement, too, must at present be regarded with reserve. (Cf. Nogamitu (1931) cit. Rona's Berichte 65, p. 215). The isolation of muscle fibres is often so difficult, especially when they are very thin, that only with the utmost care and proper technique will it be possible to avoid errors such as those indicated here.

The fact that the muscle fibres are rarely cylindrical structures running parallel to each other from one terminal tendon to the other, ultimately "passing into tendinous fibres", makes it difficult to realise the exact nature of muscular contraction, or more precisely the process of relaxation. A muscle fibre can contract actively, but it cannot relax actively; so that if a fibre terminating in the endomysium contracts to, say, half its resting length, it will not be able to revert to its original length without the assistance of some external force. Häggquist (1920) has tried to overcome this difficulty. On the basis of his observations, he maintains, in agreement with Péterfi, that muscle fibres never pass directly into tendinous fibres. Neither does

he consider it possible that muscle and tendon fibres could be connected together by any cementing substance. In contradiction to W. J. SCHMIDT (1937) and LUBOSCH (1937) HÄGGQUIST, therefore, considers the terminal tendons to be continuous with the connective tissue stroma of the muscle, in the meshes of which the contractile substance is embedded and fixed by means of the Z-membranes (see later). In this manner the muscle fibres become closely connected with the surrounding tissues. One consequence of this conception is, that in any stable condition there exists in the muscle an equilibrium between the connective tissue stroma of the muscle and the active and inactive contractile elements, and that any transition from one stable state to another must be characterized by a disturbance of this equilibrium. Therefore comparisons of length and tension can only be made between muscles which have attained such stable conditions.

The functional significance of the shape of the muscular fibres is not quite clear, but it is not difficult to realise that the more or less pronounced conical shape, in particular, may give rise to considerable experimental difficulties, especially in connection with myothermal investigations.

Microscopic Structure of the Fibre.

Evaluation of the very comprehensive histological literature that exists concerning the muscle fibre is beset with great difficulties, because the great majority of the observations have been made on fixed and stained, i. e. dead material, and only in very few cases have attempts been made to verify the results so obtained by examination of living fibres. It is notorious that fixatives frequently change the tissues to a great extent, so that it frequently becomes im-

possible to decide whether the structure seen in the stained preparation is a picture of the structure preexisting in the living cell, or whether it is an artefact. In such work it is difficult, if not impossible, to safeguard against systematic errors.

It may be unwise to draw conclusions as to function only from observations on cadaver material; and it is an especial task of the physiologist to estimate the value of such work, in its applications to his own problems.

Microscopic examination of the muscle fibre shows that it is enclosed in a membrane, the sarcolemma, consisting of fine fibrils arranged in a network, the meshes of which are filled by a homogeneous substance (Péterfi).

Immediately beneath the sarcolemma are found the nuclei of the muscle cells, the number of which varies, but at any rate is very large. The nuclei are oval and are surrounded by a small amount of granular cytoplasm located especially towards the ends of the nucleus. During development the nuclei are at first situated throughout the fibre substance, but as the contractile elements proper are developed, the nuclei move towards the periphery of the cell. In the cells of frog's muscle, however, a few nuclei are to be found in the fully developed muscle fibres scattered irregularly throughout the fibre substance. The fibre is composed of an apparently undifferentiated substance, the sarcoplasm, in which a number of highly differentiated fibrillar structures, the myofibrils, are deposited. The deviation of the interference stripe, which indicates the degree of bi-refringence (see Fig. 9) shows also the presence of numerous spikes, pointing to the existence of fibrillar structure. The myofibrils occur either more or less uniformly scattered throughout the sarcoplasm or united in

bundles of various sizes (sarcostyles), which appear in transverse section of the fibre as irregularly polygonal figures (COHNHEIM's areas). Two types of muscle fibre are distinguished, according to the proportion existing in them between sarcoplasm and fibrils. In one of them, the "red" fibres, there is a relatively large amount of sarcoplasm, whose content of myoglobin determines the red colour, whilst in the other type, the "pale" fibres, there is only quite a small amount of coloured sarcoplasm.

In the majority of muscles of vertebrates a mixture of the two types is found; but in certain cases the whole of a muscle is formed by one or other type of fibres, forming respectively "red" and "pale" muscles. Various workers claim to have shown that the two types of fibres are responsible for differences in the behaviour of the muscle, pale muscles being said to contract more quickly but also to fatigue sooner than the red. It seems, however, as if the differences pointed out may all have their explanation in the varying content of myoglobin in the two types of fibre. As far as is known, no differences have so far been shown between the contractile elements proper, the myofibrils, in red and pale fibres and, therefore, these questions will not be treated further here.

Under the microscope the myofibrils prove to consist of two substances forming regularly alternating discs of different height. One of these substances is clearly doubly-refractive, whilst the other as a rule has been considered to be singly refractive; however, according to W. J. SCHMIDT this substance also shows a slight degree of double refraction. As the isotropic and anisotropic layers of the fibril are lying at the same level in all the fibrils, the fibre as a whole thus becomes "striated". The border between the isotropic and

the anisotropic layers is not sharp but still distinctly recognizable. When the muscle fibre is treated with KOH of suitable strength it falls apart into discs (Bowman's discs). If, in fibres isolated according to the method of boiling referred to previously, the sarcolemma is torn, it will be possible to isolate the fibrils, at any rate for short distances. Various workers have observed and described numerous details in the microscopic picture of the muscle fibre. For example Krause has demonstrated that corresponding to the middle of the isotropic layers there is a fine dark line (the "Z-Membrane"), which was considered by him to be a membrane. This finding has been accepted by most subsequent workers, and in the histological literature references are generally made to "Krause's membrane", or the basal membrane dividing the fibre into "compartments" corresponding to Bowman's discs. The same arrangement in "compartments" is also seen in the so-called "Festonbildung", where the fibre assumes the appearance of a string of pearls and the retracted parts correspond to the Z-membranes. Häggquist states that, like the sarcolemma, the basal membrane gives a connective-tissue-staining reaction and, as mentioned above, believes that these membranes fasten the fibre to the surrounding endomysium. Against this view, however, v. Möllendorff (1925) has objected that the staining method employed by Häggquist is non-specific; and other workers (Hürthle (1909)) have maintained on the basis of observations on living fibres that a basal membrane does not exist. We shall deal with this question again later, in connection with our own experiments. Otherwise it is not intended here to deal with the numerous histological data referred to in the literature, since the great majority of them were performed on fixed

and stained preparations. The method of fixation with glycerine-chloral hydrate employed and recommended by v. STUDNITZ has given no better results in our hands. A comparison of measurements using this method (Table II) with those stated in the later tables, and with similar comparative measurements made by LANGER (1937) show that the method is unsatisfactory.

Table II.

Experiments with Glycerine-Chloralhydrate Fixation according to v. STUDNITZ.

Preparation	A	I	A + I	
	μ	μ	μ	
I	1.28	0.73	2.01	Without Fixing.
Other parts I	1.02	0.98	2.00	
II	1.69	0.53	2.22	
Same Bundle, Other Fibres II	1.11	0.67	1.78	Isometric Suspension.
Other Bundle II	1.19	0.51	1.70	
Same Bundle, Other Fibres II	0.72	0.34	1.06	
III	1.75	1.26	3.01	Small bundles isometric.

No further proof need be given that at present only those structures found in fibres which are living will be of interest when the functions of the muscle fibre are under consideration. As early as 1873 this was pointed out by ENGELMANN, and has been strongly emphasized since by HÜRTHLE (1909); but a perusal of the literature shows that only a very few workers have been guided by this obvious criterion. HOLTZ (1932) has indeed paid attention to this; but his results are only of limited interest, because his experiments were not performed under mechanical conditions that can be defined and reproduced.

When it is desired to investigate the microscopic structure

of the fibre under different conditions there are two further points to which attention must be paid. If it is necessary to record photographically changes taking place during contraction, the exposures must be less than $^1/_{10}$ sec. Such an exposure requires a very intense source of light of sudden action, especially where high magnifications are necessary as in the case of fibres with a small "height of compartment". These difficulties have been overcome by means of the "flash-light" lamp (Fig. 2) (Buchthal and Knappeis, 1935). For details the reader is referred to the original paper; it may be mentioned here that a considerable quantity of flash-light powder is ignited in a closed metal case provided with a silencer and outlet, so that the smoke formed during the combustion can be removed by means of a vacuum cleaner in a few seconds. The light is focussed on the mirror of the microscope by passing through two lenses and sometimes also a colour filter. The flash-light powder is ignited by a spark and the time of ignition is practically constant. By means of this lamp the time of exposure can be reduced to less than $^1/_{30}$ sec. and the amount of light emitted has proved to be adequate even where very high magnifications are employed.

A further requirement, and one which, in contrast to the above, does not depend on special technical aids, is that the mechanical state of the fibre in the moment of exposure shall be defined with certainty, and accurately reproducible. Disregard of this point is doubtless the cause of discrepancies so common in the literature, and of endless controversies. As with all other elastic bodies there will be some length at which the tension exerted by the muscle fibre is zero. In practice the procedure we adopted was as follows: The isolated fibre was subjected to the minimal

tension necessary to straighten it, i. e. to remove all kinks. The experiments shown below on passive extension of different degrees, followed by release of the fibre, show that even with varied material there is a close correlation between compartment height and resting length ("equilibrium length") so that the starting point is reproducible within

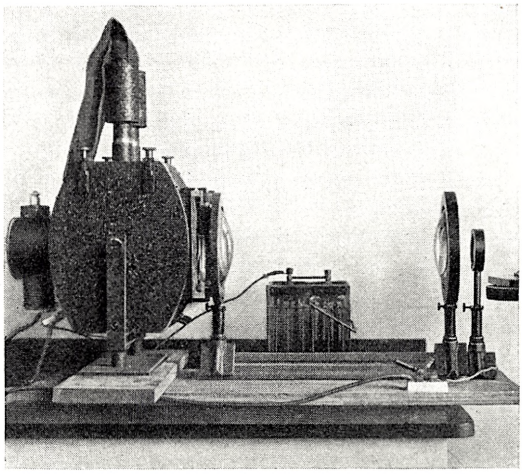


Fig. 2. Flash-light lamp.
(Buchthal-Knappeis).

narrow limits. If we consider the above position as the resting length, which in this case is the same as the equilibrium length, and determine all other starting lengths in relation to it, and if, moreover we make use of isometric contraction, the measurements will be properly controlled and experiments therefore reproducible. As will appear from Table III, isotonic contraction is, on the other hand, useless for this purpose.

The results will always show whether or not the contraction has been completely isometric; for, if so, the aggregate height of 10 or 20 muscular compartments, which

Table III.

Preparation	Rest A	Rest I	Rest A+I	Preparation	Contraction A	Contraction I	Contraction A+I	Isotonic Shortening in %
	μ	μ	μ		μ	μ	μ	
IX. B 351	1.32	0.85	2.17	IX. B 350	1.08	0.96	2.04	6.0
VIII. B 326	1.73	1.02	2.75	VIII. B 325	1.37	1.06	2.43	11.5
VIII. B 324	1.73	1.03	2.76	VIII. B 323	1.35	1.02	2.37	14.1
128	1.73	0.78	2.51	127	1.01	0.90	1.91	23.9

is of sufficient size to be measured from the photographs with considerable accuracy, should be identical in both the resting and the contracting fibre. At the conclusion of all experiments a control determination was made again with the fibre at its resting length (Table IV) to make certain that we had been working with living uninjured fibres.

Table IV.

Preparation	A μ	I μ	A+I μ	Extension in %	State of Fibre
VI. A 298	1.36	0.89	2.25	..	Length of Equilibrium.
VI. A 299	1.58	0.89	2.47	9.8	1. Extension.
VI. A 300	1.99	0.89	2.88	28.0	2. Extension.
VI. A 301	1.34	0.83	2.17	..	Length of Equilibrium.

Where it is desired to compare different conditions in the same fibre (passively extended, contracted etc.), care must be taken that in all circumstances it is the same part of the fibre that is photographed and measured. It is true that within the individual fibre, variations in the compartment height are very slight, but since the measurement of very small quantities is involved, possible sources of error, however small, must be excluded. Moreover, in this connection it should be borne in mind that the different fibres of a bundle often have different equilibrium lengths, and that in passive extension of the bundle we cannot be certain that all fibres will undergo the same degree of stretch.

In view of the above results, Buchthal, Knappeis and Lindhard (1936) have examined more closely the behaviour of the cross-striation in the frog's muscle fibre under varying experimental conditions.

An isolated fibre or a bundle of the frog's semitendinosus muscle was placed on a cover-glass in a drop of Ringer's solution and held in place by two fine glass or metal clips. The cover-glass was then inverted over a moist chamber. The fibre was also held in place by micro-electrodes, which were led into the chamber from below and the preparation could be observed through the cover-glass under the high power. The micro-electrodes, which consisted of Hg-calomel-potassium chloride electrodes connected with micro-pipettes filled with Ringer-agar, were adjusted by means of the micro-manipulator devised by Buchthal and Persson (1936). The micro-electrodes were used for extension of the preparation and for electrical stimulation.

The fibres were usually stretched so that a bundle was split in the shape of a V, one branch being fixed at equilibrium length, the other being stretched from 10 to 50 per cent of this.

An induction coil was used for stimulation, which was either limited to a single fibre (direct stimulation) or included the whole of the bundle (indirect stimulation). Where single contractions had to be recorded microphotographically the rapid sequence of stimulation and ignition of the flash lamp was secured by means of a Helmholtz pendulum. It soon appeared, however, that there was no demonstrable difference in the structure of the fibre during a single contraction and during a tetanus of short duration, and therefore in most cases it was preferable, for technical reasons, to employ tetanic stimuli of a few seconds duration. A

muscle fibre can stand repeated tetanic stimuli of short duration without alteration of its structure, but powerful stimuli of longer duration will produce a disturbance of the striation of the fibre. It appears to us unphysiological to apply to single fibres, as v. STUDNITZ (1936) has done, tetanic stimuli of some minutes duration. Our microphotographs have been taken either during a single contraction, or at the beginning of a tetanus of a very few seconds duration only. Intense stimuli will also produce disturbances for another reason than the above; namely that where larger bundles or whole muscles are employed there is a serious risk of displacement of those fibres situated in the field of vision, rendering a reliable estimation of the result of the experiment impossible. The effect of focussing on the measurements was carefully ascertained. It proved to be possible to vary the focus only within very narrow limits before the picture became blurred; and moreover that when sharply defined pictures were measured focussing was without demonstrable influence on the results.

For microphotography various cameras were used, especially Phoku-Kolibri (ocular 10 ×) with objective Zeiss Apochrom. 40 (N. A. 0.95) or 90 hom. Im. (N. A. 1.3). The photographic arrangement was calibrated photographically by means of a Zeiss object micrometer. A considerable number of measurements were made with different photographic materials in order to find plates with a sufficiently fine grain and to devise a suitable developing technique (BUCHTHAL & KNAPPEIS 1936). Negatives intended for comparable pictures were always treated in the same manner.

The measuring of the microphotographs was performed on positives from unenlarged negatives by means of a

Leitz ocular screw micrometer with movable line using 12 × magnification. One division of the micrometer then corresponded to 3.79 μ when parallax errors were eliminated. The measurements were made by two independent examiners on photographs, which, in order to minimise personal errors, were provided with random serial numbers. As a further check, photoelectric measuring was performed in several cases by means of a micro-densitometer.

The height of the isotropic (I) and the anisotropic layers (A), was measured, as well as the height of a single compartment and the total height of 10 compartments. The photographs were also examined for the presence of a basalmembrane (Z), the possible occurrence of Hensen's line in A, and any longitudinal fibrils or nod-shaped formations. Finally the thickness of single fibres, where visible, was measured.

The results of this series are briefly stated in the following tables.

All values given represent the mean of 10 determinations.

Table V shows comparative measurements of fibres at rest at their equilibrium lengths and during contraction. As will be seen, the variation in the single determinations in each column is very slight although it includes experimental errors as well as physiological variation in the material. The errors of the mean figures appear to be insignificant.

In the resting fibre the anisotropic disc is higher than the isotropic, namely 1.37 as compared with 0.81 μ; on isometric contraction A is shortened whilst I is lengthened. The aggregate height of a compartment will, of course, remain unaltered. A is thus 63 per cent. of the height of the

Table V.

Preparation	Rest		
	A	I	A + I
	μ	μ	μ
I. B 263	1.35	0.74	2.09
II. A 267a	1.33	0.83	2.16
II. A 267b	1.37	0.91	2.28
III. A 271	1.35	0.89	2.24
III. B 275	1.33	0.79	2.12
VIII. F 335	1.41	0.76	2.17
IX. B 349	1.36	0.80	2.16
IX. B 353	1.48	0.79	2.27
Mean of 80 determinations	1.37 ± 0.008	0.81 ± 0.01	2.18 ± 0.01
Average error of a single determination	0.07	0.09	0.12

Preparation	Contraction		
	A	I	A + I
	μ	μ	μ
I. B 262	1.11	0.96	2.07
II. A 266a	1.12	1.02	2.14
II. A 266b	1.14	1.09	2.23
III. A 270	1.07	1.17	2.24
III. B 274	1.06	1.02	2.08
VIII. F 334	1.23	1.00	2.23
IX. B 348	1.10	1.05	2.15
IX. B 352	1.21	1.06	2.27
Mean of 80 determinations	1.13 ± 0.009	1.05 ± 0.01	2.18 ± 0.01
Average error of a single determination	0.08	0.11	0.11

compartment in the resting fibre, I is 37 per cent. — In the case of the isolated contracted fibre the corresponding figures are 52 and 48, from which it would appear that during the contraction A is shortened by 18 per cent. whilst I is lengthened by 28 per cent.

Corresponding measurements on lizard's muscle at rest give as mean figures, $A = 1.34\ \mu$, $I = 0.90$ and thus the height of the compartment: $2.24\ \mu$.

Passive extension of the fibre gives the results seen in Table VI.

Table VI.

Preparation	Rest A	Rest I	Rest A + I	Extension %
	μ	μ	μ	
IX. D 361	1.65	0.94	2.59	about 17.
VIII. G 339	1.69	0.87	2.56	
VIII. H 343	1.70	0.93	2.63	
VIII. E 333	2.00	0.99	2.99	about 40.
VIII. G 341	2.15	0.96	3.11	
VIII. A 322 β	2.10	1.05	3.15	

	A	I	A + I
	μ	μ	μ
Unextended fibres	1.37	0.81	2.18
Extended fibres: Group I, Mean	1.68	0.91	2.59
Group II, Mean	2.08	1.00	3.08

	Proportional height A %	Proportional height I %	Extension A %	Extension I %	Extension A + I %
Unextended fibres	63.0	37.0	..	..	..
Extended fibres: Group I, Mean	65.0	35.0	22.6	12.4	18.8
Group II, Mean	67.5	32.5	51.8	23.5	41.3

Preparation	Contraction A	Contraction I	Contraction A + I	Extension %
	μ	μ	μ	
IX. D 360	1.38	1.15	2.53	about 17.
VIII. G 338	1.49	1.06	2.55	
VIII. H 342	1.39	1.17	2.56	
VIII. E 332	1.68	1.29	2.97	about 40.
VIII. G 340	1.70	1.37	3.07	
VIII. A 321 β	1.46	1.63	3.09	

	A	I	A + I
	μ	μ	μ
Unextended fibres	1.13	1.05	2.18
Extended fibres: Group I, Mean	1.41	1.13	2.55
Group II, Mean	1.61	1.43	3.04

Table VI (continued).

	Proportional height		Shortening of A in %	Lengthening of I in %
	A %	I %		
Unextended fibres	52.0	48.0	18.0	28.0
Extended fibres: Group I, Mean	55.3	44.7	16.1	24.2
Group II, Mean	53.3	46.7	22.1	42.0

As will be seen, A is more markedly influenced by the extension than I, 51.8 as against 23.5 per cent with 40 per cent. extension. If the passively extended fibre is made to contract isometrically, then A will be shortened and I lengthened; that is with 40 per cent. extension the changes are 22.1 and 42.0 per cent. respectively. The significance of these facts will be discussed later.

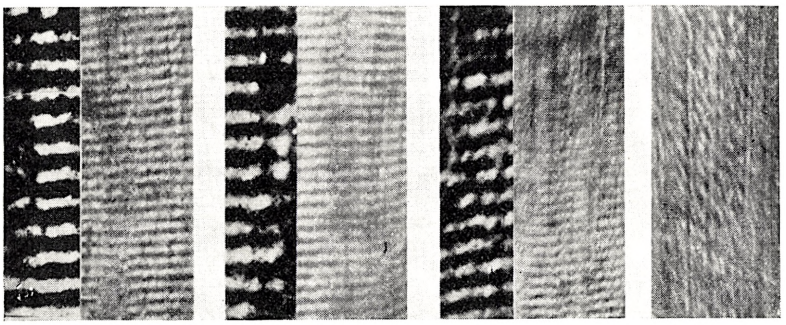
With regard to the degree of extension our experiments show that a passive extension of about 30, at most about 40 per cent. of the equilibrium length is reversible and without any permanent influence on the structure and function of the fibre. If the degree of extension is increased beyond 40 per cent. a disturbance of the structure occurs; first a displacement longitudinally of the contractile elements, relative to each other, their striations becoming indistinct, and then a complete disturbance of the picture, the fibre assuming the appearance of a sarcolemma filled with a granulated mass. Even before the latter stage has been reached, the fibre has lost its irritability (Fig. 3).

There has been general disagreement as to whether the A or the I-stripe is the broadest. However, the results of the above work on living fibres appear to show conclusively that under well-defined and reproduceable experimental conditions, the A-substance, both at rest and under passive extension within physiologic limits and

under isometric contraction, forms the greater part of the height of the compartment (Fig. 4).

The remaining problems over which there has been dispute are not so readily resolved. The question of the existence of the so-called basal membrane (the "Z-stripe") is of special interest, since the views expressed concerning this question are so divergent, some workers denying the existence of a basal membrane, others maintaining that it forms a continuous spiral throughout the whole length of the fibre and having the character of a nerve ramification (Tiegs (1922)). The latter views will not be discussed in detail here; but as to a possible helicoidal arrangement it may be mentioned that this idea is recently supported by Aurell & Wohlfart (1936) from Häggquist's laboratory. This work which was performed on fixed and stained material, appears open to criticism and is, moreover, so decidedly at variance with functional viewpoints, especially Buchthal & Péterfi's potential measurements (see later), that we cannot accept the authors' conclusions. On the basis of his experiments with hydrophilus muscles Hürthle regards the Z-membrane as a structure that is absent from the living fibre but only appears when the fibre is moribund. We have been unable to confirm this statement in observations on frog's muscle where stimulation before and after photographing proved that the tissue was in normal condition. It is true that the Z-stripe is not invariably seen in the living fibre, but it is frequently seen (Fig. 5) and under conditions which certainly do not suggest that the fibre is abnormal.

The fact that photographs of the stripe are only successful in a minority of cases is due to the fact that the Z-stripe



a. b. a. b. a. b.

1. 2. 3. 4.

Fig. 3. Structure of resting living fibre with the following degrees of extension:

1. stretch over 23 % of resting length.
2. — — 34 - - — —
3. — — 39 - - — —
4. — — 50 - - — —

a. = magnification × 1000; b. = magnification × 600.
(Buchthal, Knappeis & Lindhard).

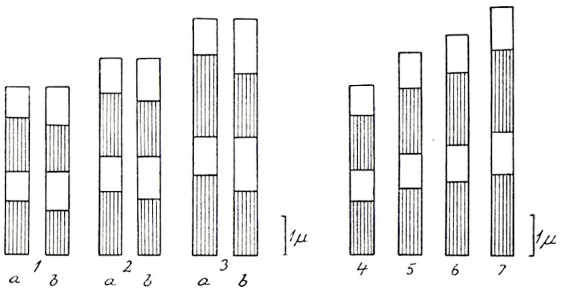


Fig. 4. Relation between A and I substance in the resting, stretched and contracted fibre (diagrammatic).

a. = at rest; b. = isometric contraction.
1. fibre at resting length.
2. — 25 % stretched.
3. — 40 - —
4—7. resting fibres stretched from 0—45 % of resting length.
(Buchthal, Knappeis & Lindhard).

only appears distinctly as the focus is changed, which cannot be done in the course of photographing. According to statements by v. MURALT after WOHLFART (1933), who examines the fibre by means of ultraviolet light and a quartz optics system, the Z-stripe is always found in the living fibre. CIACCIO (cit. PÉTERFI, 1937), has found that during metamorphosis in frog larvae, the basal membranes in the otherwise empty sarcolemma persisted after the myofibrils had been resorbed. It may be added that BUCHTHAL and LINDHARD in unfinished experiments on the micro-incineration of single fibres, have in several cases been able to demonstrate a distinct Z-stripe in the reproduction of the striation seen in the "ash-picture"

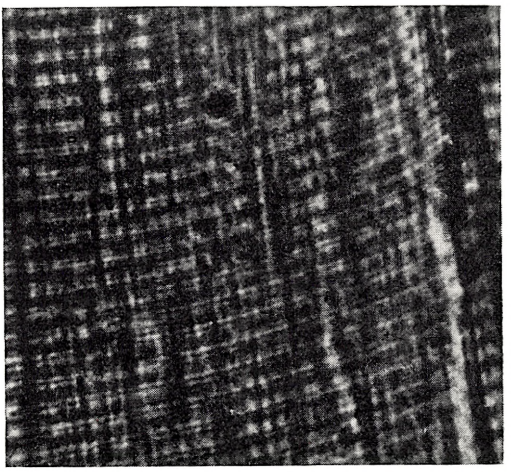


Fig. 5. Microphotograph of living single fibre with visible Z-membrane. Magnification 1000×.

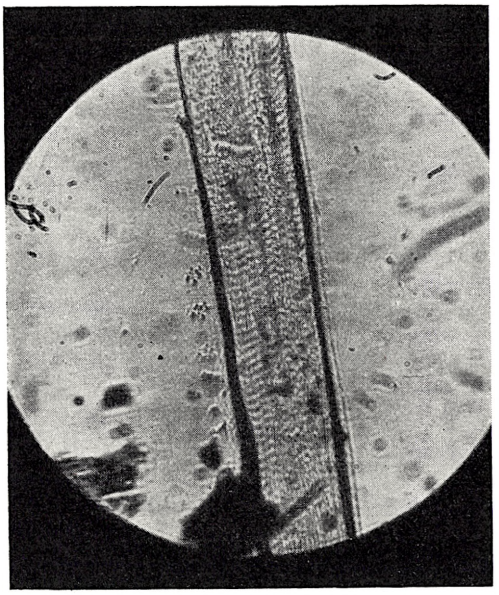


Fig. 6. Ash-picture of single muscle fibre, obtained by micro-incineration.

(Fig. 6). In the latter case we are not concerned with living fibres, nor even with fibres at all; yet this observation implies that in the part of the fibre where the Z-stripe is seen there is something of a substantial nature differing from the surroundings. The same fact appears from HÄGGQUIST's staining experiments, which show that the Z-stripe is stained like the sarcolemma; and even though the staining method employed was not a specific one this does not affect the point at issue. Lastly BOWMAN has shown that fibres treated with a KOH-solution of suitable strength fall apart into transverse discs limited by the basal membranes.

To these histological grounds for the existence of the Z-stripe as a pre-formed, normal constituent of the fibre there must be added the important evidence from physiological observations. Electrostatic measurements of single fibres by BUCHTHAL and PÉTERFI (1934) have shown that the potential difference between the two electrodes increases in proportion as the number of intervening muscular compartments; on extension of the fibre the potential difference decreases, and disappears completely when the Z-stripe is disturbed. This fact implies some kind of an isolation of the compartments from each other, which is difficult to imagine except with the aid of some structural elements. As already mentioned, it must be supposed on physiological grounds, that an intimate connection exists between the sarcolemma and the endomysium, and it seems logical in agreement with HÄGGQUIST to refer this connection to the basal membrane. Finally, the basal membrane probably serves as a point of fixation for the muscular compartments during contraction. In this connection it is not sufficient to suppose that by means of these membranes the fibre as a

whole is attached to the endomysium, leaving the fibrils free. When it is remembered that in a conical fibre during contraction, one end is extended and the other shortened, it becomes difficult to see how a fibril 20 mm. long, containing about 10^4 alternately isotropic and anisotropic discs, would be able after contraction to rearrange itself in relation to the adjacent fibrils, so that the striation remains distinct. After the contraction the striation is as distinct as before, unless the fibre has been injured by strong stimuli applied for a longer period. Therefore, the suggestion that the basal membrane may be fenestrated in order to allow the passage of the fibrils seems improbable. And such fenestration has actually never been observed. On the other hand both Péterfi (1937) and Nagel (1935) describe the sarcolemma, which we may consider to be of structure similar to the basal membrane, as consisting of a network of filaments, the meshes of which are filled with homogeneous substance. Such an observation could be interpreted as fenestration. However, the fibrils though no doubt continuous, are "linked" by the basal membranes.

We do not know the details of the character of the basal membranes, but Häggquist's supposition that they consist of collagenous substance has some foundation in fact, inasmuch as the tissues separating the single plates of the electric organs of fishes are known to be of a connective tissue character. Likewise it seems probable that the thickened sarcolemma covering the motor end-plates (the telolemma) serves as an electrical insulator between end-plates and adjacent fibres. But this does not imply that the inter- and intra-fibrillar basal membranes are of the same thickness or in the same physico-chemical state. When the fibre is treated by Bowman's method it falls apart in transverse

discs, the basal membrane being dissolved as a whole; but when the fibre is boiled, at any rate if the boiling is not carried too far, the fibre is disorganized into fibrils, the intra-fibrillar membrane remaining intact, when the sarcolemma is torn. Corresponding with APATHY's (1907) & PÉTERFI's view this fact shows that the inter- and intra-fibrillary parts of the Z-membrane must be of different natures. Again, in decomposing fibres it may sometimes be observed that some fibrils (sarcostyles) retain the power of contraction whilst others have already lost their irritability. In this way longitudinal displacement of the different fibrils will take place, the striation being so considerably displaced that a rupture of the basal membrane between the active and the inactive parts of the fibre must be inevitable.

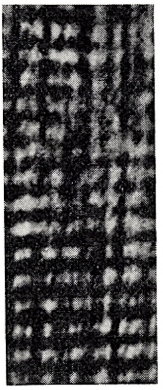


Fig. 7. Longitudinal striation in a single fibre. Magnification 1000 ×. (BUCHTHAL, KNAPPEIS & LINDHARD).

Although in such cases as these there can be no doubt as to the existence of longitudinal fibrils (sarcostyles), the longitudinal fibrillary striation in the living fibre is generally not nearly so distinct as the cross striation. It is, however, possible by means of ordinary illumination to ascertain the presence of longitudinal fibrils which are about 1.5 μ thick. We do not find a completely well-defined transition between the A- and the I-stripe in the single fibril; which agrees with W. J. SCHMIDT's (1937) and D'ANCONA's (1931) views on the distribution of the anisotropic substance; whilst the longitudinal striation can be followed from one basal membrane to the other (Fig. 7).

On the basis of our investigations in connection with the mechanical phenomena we must suppose that the

individual muscular compartments consist of rod-shaped formations (fibrillary sections) with a greater or smaller amount of intervening sarcoplasm. In the middle the rod-shaped formations consist of A-substance, at either end of I-substance. The rods forming the highly anisotropic A-substance are on an average 1.6—1.7 μ long and 1.3 μ thick, and are probably composed of a number of contractile elements. As previously mentioned (p. 25), A is shortened during isometric contraction, and I extended; as this takes place simultaneously in all compartments under normal conditions, they will act as a single unit, the tension exerted being transmitted to the tendons via the basal membrane and the endomysium.

A question of considerable interest is that relating to the volume of the fibre during contraction. Here, also, there exists considerable uncertainty. Most workers have either found the volume to be constant, or very slightly changed. Buchthal, Knappeis and Lindhard, too, found the volume of the muscle compartment to be constant during contraction, the thickness of the fibre being unchanged; but these experiments were not primarily concerned with the determination of possible changes of volume. Some of the most recent and best observations in this field, those by Meyerhof and Möhle (1933) and Meyerhof and Hartmann (1934) give as their results that during contraction, when the muscle is covered by liquid paraffin during the experiment, there is a slight constriction of volume, which in the authors' opinion might be explained by the chemical changes taking place during the period of restitution. In the case of isometric contraction the volume changes amount to 2.0×10^{-5}, in the case of isotonic contraction to 6.4×10^{-6} cm.^{3} per gram of muscle. In other words the change of volume

is 3 times as large in isometric as in isotonic contraction, a difference that is difficult to understand if the diminution in volume is considered to be a direct result of the chemical changes, unless the tension in the two cases presents corresponding differences. Volume changes of this order could, however, hardly be detected with the technique employed by BUCHTHAL, KNAPPEIS and LINDHARD. Other workers, however, (E. FISCHER (1935)) have claimed to be able to demonstrate a slight increase in volume; moreover, such an increase, even of a very considerable size, is shown indirectly in the measurements made by HÜRTHLE.

If calculation is made from HÜRTHLE's mean figures the increase in volume of the fibre during contraction amounts to no less than 38 per cent.; and if his single measurements be used even 50 per cent. increases may be indicated. An increase in volume of such an order has no real existence; such increases in size if they occurred would be easily detected in our experiments; but in all cases where we have been able to measure exactly the thickness of completely isolated fibres we have, in the case of isometric contraction, found the volume of the muscular compartment to be unaltered. In such cases the volume of A or I is thus proportional to the height of the respective sections. The discrepancy between our own and HÜRTHLE's results are, we feel, due to the unreliable nature of HÜRTHLE's measurements of thickness, either because the fibres had not been isolated, or because they were not normal. It is not permissible to measure, as he has done, "resting values" in one section of a partially contracted fibre, as the dimensions of the passive parts of the fibre may be influenced by the contracting parts. Moreover the possibility cannot be completely excluded that the cover-glass may have compressed the con-

tracting parts of the fibre, making the latter appear thicker than it really is. Finally, when in the muscle fibres of insects which normally react very rapidly, slow localised contractions are found to occur, such a reaction must be considered abnormal in character; in such circumstances it cannot be assumed that in other respects the fibre will react normally or display normal conditions of diffusion.

Minute Structure.

The study of minute structure is concerned with the constituents of the smallest tissue elements visible under the microscope. These are the structural elements intermediate in size between these and the molecule, the molecular aggregates once termed "micellae" by Nägeli. The methods that have come into use for observation in present fields of research are determinations of double refraction, roentgen-optical methods, and thermoelastic methods. It seems to us, however, that the latter methods have not been employed with the necessary reservations. As to the roentgen-optical methods it seems preferable, at the present stage of development of the technique, to confine its uses to the examination of myosin. The muscle "mummies" that are so often examined are of little use in the study of the living musculature, and this is especially so when muscles of quite different types are used indiscriminately. As to thermo-elastic experiments, it should be borne in mind that the results of heating living tissues may be entirely different from those obtained on dead material. The living tissues, which at present must be studied through their metabolism, react to changes of temperature with far more complex reactions than do the physical objects usually employed and, therefore,

erroneous conclusions may easily be drawn, especially so long as our knowledge of them is imperfect.

It has long been known that the A-substance of the muscle fibre is anisotropic, and hitherto the belief has been wide spread that the I-substance is isotropic; more recent investigations by W. J. SCHMIDT and D'ANCONA, however, seem to indicate, as already referred to, that the I-substance, also is anisotropic, though to a far less degree than the A-substance. Whilst a rod-like structure can be demonstrated with some certainty in the A-substance we have no certain knowledge of the structure of the I-substance. According to measurements performed by BUCHTHAL, KNAPPEIS & LINDHARD A is shortened during isometric contraction and I is lengthened, but this does not necessarily mean that A alone is contractile, as a faint contraction of I might be masked by contraction of the more bulky A-layer. Any contraction of I, however, must be of minor importance. Many years ago ENGELMANN (1873) maintained that contractility, not only of the skeletal muscle but also of other contractile substances, was always associated with the occurrence of anisotropic uniaxial bodies arranged parallel to the direction of contraction of the elastic substance. Such bodies can be demonstrated in A, but it must not be concluded that they are not therefore found in the I-layer at all.

H. H. WEBER (1934) and NOLL & WEBER (1934) have performed comprehensive investigations comparing the double refraction of very fine myosin filaments with the A-substance of the muscle fibre, and have found a very far-reaching correspondence between the minute structure of the two. This applies especially to the so-called "Rod-double refraction" ("Stäbchendoppelbrechung") (WIENER), which must be attributable to the difference in refractive

index between the rods and the surrounding medium. As shown in Fig. 8, this "Rod-Double-Refraction" diminishes as the refractive index of the medium approaches more and more to that of the Rods. However, the position of the lowest point on the curves shows that some Double Refraction always remains, which is due to an inherent property of the molecular structure of the rods. Values of the latter component obtained for myosin threads may not be comparable with those for intact muscles, since the conditions affecting the protein molecule may be quite different in each case.

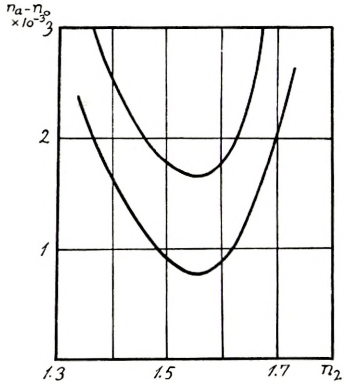


Fig. 8. Striated muscle (lower curve) and myosin thread (upper curve). Ordinate: total double refraction measured after immersion in fluids of different refractive indices. Abscissa: refractive indices of the fluids. (E. Fischer and H. H. Weber).

According to Weber a myosin thread, which contains 20 per cent. of protein, is composed of longitudinal micellae, containing 70—80 per cent. of protein and separated from each other by waterfilled interstices. The distance between the individual micellae, is only a few $\mu\mu$, so it cannot be supposed that there would be room for other protein molecules in the interstices. Weber estimates the distances between the filamentous molecule chains composing the micellae to be about 11 Å. Each micelle is supposed to contain 10—20 "main-valency-chains" ("Hauptvalenzketten"), the dimensions of each being: thickness 4 $\mu\mu$, length 50—100 $\mu\mu$, and volume about 1100 $\mu\mu^3$. The molecular weight of myosin is said to be of the order of 10^6.

Though Weber gives many good reasons for supposing

that the A-sections of the fibrils of the cross-striated skeletal muscles consist of myosin, this does not necessarily apply to the A-sections of the muscle-fibre, for the fibrils must be supposed to be embedded in a greater or smaller amount of sarcoplasm. WEBER's statement of the quantitative proportions in the muscle is inaccurate, since his contentions are based on incorrect statements of the proportion between the dimensions of the A- and the I-segments. As to the amounts of the different proteins he considers that about 40 per cent. of the protein is myosin; to this must be added 15 per cent. of myogen, 15 per cent. of globulin, and nearly 30 per cent. of stroma-protein. WEBER does not say how these substances are distributed between the I-substance of the fibrils, the sarcoplasm and the stroma; his supposition that the I-section of the fibrils consists of stroma-proteins is probably also derived from the erroneous statements concerning the relation between the A- and the I-substance during contraction, found in the literature.

v. MURALT (1932) has recently examined the double refraction of whole muscles in isometric contraction and has tried to establish the time relation between bi-refringence and the mechanical response by means of a method giving a reliable record of the changes in double refraction. Among other things the experiments showed that the variations of double refraction preceded the development of tension.

WEBER's work has usually been performed on fixed material, which it has been emphasized several times, has numerous drawbacks. Other experiments, chiefly owing to the use of whole muscles, have not given quantitative results. Therefore BUCHTHAL & KNAPPEIS (1937) have re-determined double refraction on living material under various conditions. The bi-refringence of resting, passively extended and con-

tracting fibres was examined by means of a Babinet-ocular compensator.

The double refraction can be calculated according to the formula:

$$n_a - n_o = \frac{\gamma\lambda}{d},$$

where γ is the phase difference, λ the wave length of the light employed, in this case 546 $\mu\mu$, d the thickness of the object determined by means of an ocular screw micrometer, and n_a and n_o the refractive indices of the ordinary and the extraordinary ray respectively. Fig. 9 shows the course of the stripe of interference when the light passes through a muscle fibre, and the displacement of this line is a measure of the bi-refringence. In the case of a single living fibre of the frog's semitendinosus it is:

$$(1.70 \pm 0.016) \cdot 10^{-3}$$

as a mean figure from 230 preparations examined. As the thickness of the fibre and the difference in phase change proportionally, the quotient $\frac{\gamma\lambda}{d}$ remains a constant. Injured fibres give far greater values of bi-refringence (up to $2.30 \cdot 10^{-3}$), which taken in conjunction with the structural changes makes such preparations easily recognizable. With advancing, decomposition of the structure the double refraction, however, also decreases considerably; it may then become uniform throughout the whole of the fibril, which

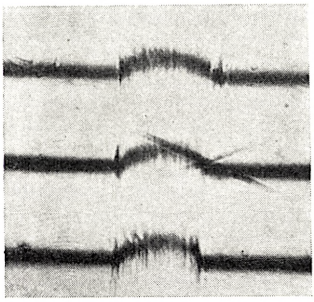


Fig. 9. Bi-refrigence of a single living muscle fibre measured with Babinet-Compensator. (Buchthal & Knappeis).

apparently has caused some workers (MEYER & HÜRTHLE) to believe that the whole of the fibril should consist throughout its length of more or less orientated myosin micellae. Similar phenomena may appear when whole muscles are used for the experiments instead of isolated fibres. Therefore, neither whole muscles nor injured fibres can be employed for determinations of double refraction. Experiments with vital staining (PISCHINGER) have shown that the A- and the I-segments stain differently and must thus consist of different substances. Moreover H. H. WEBER has put forward sound reasons why the myosin is found exclusively in the A-segments. The structure of the I-segment is unknown, but this substance too may on mechanical grounds be expected to be composed of molecules longitudinally orientated or arranged in reticular formation. BUCHTHAL & KNAPPEIS found no difference between the double refraction of the resting and the passively extended fibre, which is apparently due to the fact that the decrease in the double refraction which may have occurred during the extension, has been compensated by the decrease of the thickness of the fibre which results from stretching. During, and after the contraction, fluctuations in the double refraction occur, which in their time relations closely resemble the fluctuations occurring in fibre potential and electrical resistance. A corresponding analogy between the double refraction and the fibre potential is found in the case of the effects produced by different substances applied to the fibre. For example, lactic acid in a suitable buffer mixture gives rise to a simultaneous, reversible decrease of the fibre potential and of the double refraction. This observation suggests that there is a close relation between potential difference and bi-refringence which in turn is intimately concerned with the contractile substance (Fig.

10). Buchthal & Knappeis have also made use of the changes in double refraction which take place when the muscle fibre is influenced by different substances, to obtain direct information as to the permeability of the fibre itself to substances in the external fluid. Bi-refringence is thus employed as an actual indicator of the penetration of sub-

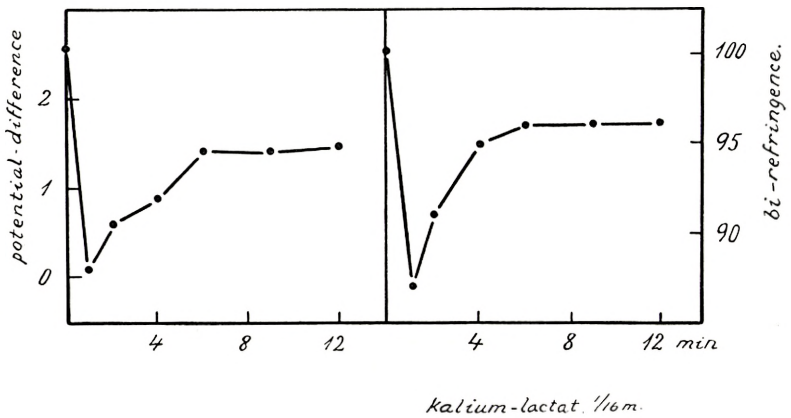


Fig. 10. Bi-refrigence and potential difference of a single fibre after application of potassium lactate.
(Buchthal).

stances into the fibres. It was found that acids cause a diminution of the bi-refringence, which progresses constantly with increasing concentration and time of exposure (Fig. 11). On the other hand it seems that the possibility of a specific anion effect on the double refraction can be excluded. Anions and cations invade the fibre in equivalent amounts. Changes of the double refraction under the influence of neutral salts must be supposed to take place by exchange of anions, e. g., the carbonate ion is exchanged with lactate ion in the external fluid, causing a change in the pH of the contents of the fibre. But it is improbable that an exchange of cations takes place, since phosphoric acid, even in high concen-

trations, does not influence the double refraction, whilst lactate—and acetate—RINGER's fluid with a pH of 7.4 causes a spontaneous, reversible decrease of the double refraction.

As to the roentgen-optical methods ASTBURY and his collaborators in a series of publications, have especially contributed towards the elucidation of the minute structure

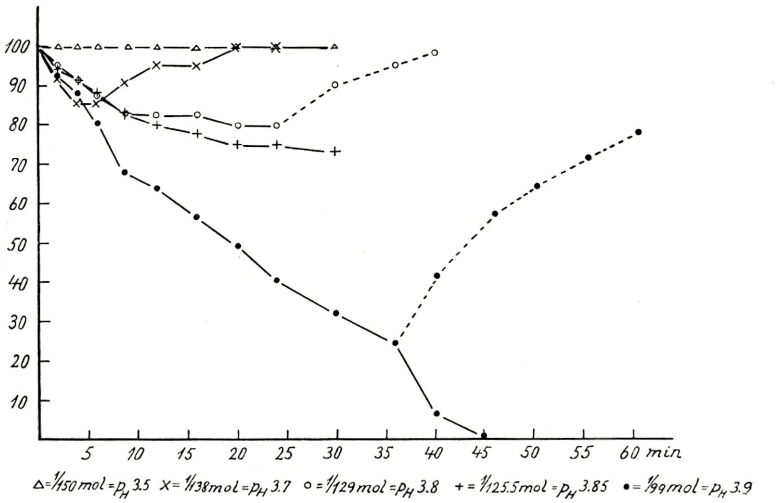


Fig. 11. The influence of lactid acid in different concentrations upon birefringence. Stippled lines: recovery after washing in Ringer. (BUCHTHAL & KNAPPEIS).

of the myosin. ASTBURY & DICKINSON (1935) have moreover demonstrated a certain analogy between the structure of myosin and keratin; but for the time being it must be left as an open question whether this demonstration is of any value to the understanding of the muscle function, for although much is known of structure of dead myosin threads, little can be said of the state of myosin in the living fibre.

KURT H. MEYER's investigations are in the main applicable to biological problems. MEYER & FERRI (1936) have examined the relation between the elastic properties of a

body and its molecular structure, especially with regard to elastic and collagenous connective tissue. Previously K. H. Meyer and his collaborators examined carefully the thermoelastic conditions in rubber and were able from their experiments to draw certain conclusions as to the force with which stretched rubber will contract. Wöhlisch (1932) and others from their work on different organic tissues, have found that the yellow cervical ligament of the ox will contract when heated within certain limits, whilst tendinous tissue lengthens. Meyer & Ferri have attempted theoretically to show how, on the basis of the thermoelastic conditions of a substance, we can analyse the force of contraction of the passively stretched material into its component forces. The authors draw attention to the fact that the amount of work any system is able to perform is determined by its internal energy, the attractive forces between the single elementary constituents, and the molecular movements, as is also the state of equilibrium of the system. What the system loses in internal energy it will gain in molecular movement, i. e., in heat; the movement of heat tends to produce complete irregularity in the arrangement of the molecules, and its proportion in any amount of work performed will, therefore, become greater the more organised the structure of the substance is. Any system in which the molecules display a certain orientation has a lower entropy than the corresponding system in which the molecules are arranged in less orderly fashion. With increasing temperature the internal energy will decrease whilst the heat movement will increase. The authors establish the equation

$$dA = dE - T\,dS,$$

in which A is the free energy of the system, E the internal energy and S the entropy, and determine dE and $T\,dS$ by

means of the temperature coefficient of the force of contraction. Further consideration of these formulae need not be made here; the question of special interest in this connection, is that of the conclusions which can be drawn from the experimental results regarding the molecular structure of the substance. Thus dE denotes change in the internal potential energy between the molecules or parts of the molecules. If this quantity is negative during the contraction, it indicates that internal forces are absorbed e. g. through crystallization, whilst a positive value for dE is indicative of a process in the opposite direction, i. e. the transition from the crystalline to the amorphous state. If, lastly, dE is equal to 0 it must be supposed that the contraction is due exclusively to the movement of heat.

If S increases when the substance is passively stretched, it means that through the stretching the atoms will obtain greater freedom of motion, especially in the direction of the extension; if, on the other hand, S decreases on extension but is increased during the contraction, as is the case when rubber is stretched under certain conditions, it must be supposed that the freedom of motion of the atoms decreases on extension owing to an orientation in the direction of this extension. It is well known that vulcanised rubber consists of a mass of long flexible "main-valency chains" ("Hauptvalenzketten") united into a network by means of sulphur bridges. When the substance is passively extended these molecular chains will arrange themselves parallel to the direction of extension, gliding towards each other as in a viscous fluid, and the movement of heat will try to re-establish the previous chaotic state. The requirement for contraction is, however, that the substance shall consist of long "main-valency chains" in which the molecules, or

parts of molecules, are combined through very firm valencies. If these unions do not exist, the molecules may be disarranged solely through turning, no change of the outer shape being necessary, as is the case with dipoles orientated in an electric field when the action of the field ceases.

When these considerations are applied to the results of experiments with elastic connective tissue and tendinous tissue it appears that elastic connective tissue consists of long, flexible "main-valency chains" which, on passive extension, become orientated in the direction of the traction, whilst the movement of heat re-establishes the disorderly condition during the contraction. Tendinous tissue also consists of long "main-valency chains" but they are already orientated longitudinally when the tendon is at its length of equilibrium and so cannot be stretched further by passive extension. In a more recent publication, Meyer & Picken (1937), have tried to extend to muscles the results obtained by them in observations on various organic substances. Though realizing that the muscle consists of different heterogeneous constituents the authors believe that the thermoelastic methods of examination may still be employed with advantage, since by their aid information can be procured about precisely those substances which are responsible for the contraction. We consider, however, that this view is improbable. The force of contraction measured by Meyer & Picken is the resultant of several components which, at any rate quantitatively, are completely unknown. Moreover the authors work with muscles of vertebrates and invertebrates indiscriminately and draw conclusions from one kind of muscle tissue to the other, a view that has resulted in a great number of misconceptions. Finally, they take for granted that as regards minute structure the resting muscle

can be compared with unstretched rubber; this, see later, is not supported by our results. Despite objections which may be raised to the above work, it must be granted that the authors observations show that the muscle, or perhaps more correctly certain parts of the muscle fibre, must be composed of long, flexible main-valency chains, which become orientated longitudinally on passive extension and revert, at any rate, to a less extended condition when the muscle is allowed to resume its resting length.

As to the sarcoplasm no conclusions whatever can be drawn from our experiments. It is known that the sarcoplasm contains myoglobin and it must also be supposed to contain the glycogen of the muscle, as there would scarcely be room for the large glycogen molecules in or among the myosin micellae. Therefore a nutritive role may be suggested for the sarcoplasm. The suggestion that the sarcoplasm possesses contractile properties is so far removed from facts of histology or physiology that it need not be discussed here.

The Motor End-Plate.

Anatomically the motor end-plate belongs to the muscle fibre, being situated beneath the sarcolemma. Functionally it is so closely connected with the muscle function that it becomes impossible to examine and describe the latter except in conjunction with the function of the end-plate. From a phylogenetic point of view the end-plate must, however, be considered an organ sui generis, as a structure that both ontogenetically and histologically is homologous with the electric organs of fishes. A single summarizing statement by BABUCHIN (1870) will suffice: ". . . . . dass die elektrischen Organe eigentlich Muskeln sind, aus denen nur die Muskelsubstanz entfernt ist; und umgekehrt, die Mu-

skeln sind elektrische Organe, in welchen unter allen Platten Muskelfasern eingeschoben sind . . . . . Die elektrischen Platten und die motorischen Endplatten sind in morphologischer Hinsicht identisch." This view has been well confirmed by later investigations of Ewart (1892) into the development of the electric organ in Raia, and also by Boeke's observations (1927) on the development of the motor endplate. In mammals, birds, reptiles and fishes the motor end-plate is an organ shaped like a biconvex lens with a diameter of 40—60 μ. Its size appears to have no relation to the size of the species of animal in question but seems proportional to the thickness of the muscle fibre. It is not bounded on the side towards the fibre substance by any membrane demonstrable by histological methods; it is covered by the sarcolemma, which is strengthened by the covering of Schwann's and Henle's sheaths of the nerve filament (the telolemma of Kühne). The end-plate consists of embryonic fibre substance in which there is a number of great, ellipsoid nuclei with 1 or 2 nucleoli (the "Sohlenplatte" of Kühne). The nuclei are formed by metamorphosis of the nuclei of the muscle fibre. To this "sole" comes a somatic motor nerve branch, the external sheaths of which, as already mentioned, pass into the sarcolemma, whilst the medullary sheath disappears and the axis cylinder alone perforates the sarcolemma, whereupon it breaks up into numerous fibrillary loops in the sole.

In several publications Boeke has maintained that the actual distribution of nerves does not take place in the end-plate but that beneath this, in the sarcoplasm, there is another network of extremely fine neuro-fibrils, which in turn are connected with the myofibrils. On this view then, the end-plate is not a terminal organ, and the whole of this

histologically well-known and well-characterized structure would therefore be without any biological meaning whatsoever. BOEKE's nervous network has no foundation, in fact, either. The specific substance of the neuron is in fact delimited from the effector cell as the classical neuron doctrine states (PÉTERFI, (1935)). PÉTERFI moreover points out that the potential difference between end-plate and muscle fibre demonstrated by BUCHTHAL & LINDHARD could hardly exist if there was a continuous transition from neuroplasm to cytoplasm. Furthermore, BOEKE's conception is incompatible with our knowledge of curare, and it is quite unphysiological to imagine a connection at random between ultramicroscopical nerve branches and any muscular compartments.

Mechanical and electrostatic findings make it necessary to assume that the muscle fibre consists of compartments, which are isolated from each other. As the height of these compartments is 2—2.5 μ there will thus be about 25,000 compartments in a muscle fibre about 5 cm. long, each compartment containing something like 12—1300 fibrils. It must either be supposed that the natural stimulus proceeding from the end-plate is propagated to the whole of the fibre without special conducting tracts, or it must be supposed that each of the units mentioned above is innervated separately, a view which to say the least appears improbable. The most serious objection to BOEKE's theory is, however, the fact that the secondary network of nerves in the fibre cannot be demonstrated with any degree of certainty (cf. J. V. WILKINSON (1934)). The only picture which may be relied upon is obtained by making transverse sections through end-plate and fibre; but the very few pictures of such a transverse section found in BOEKE's publications (as

far as we know only 2), do not display secondary nerve ramifications under the end-plate (Fig. 12). These networks are not seen, either, in exact longitudinal sections and the shadows appearing in obliquely projected longitudinal sections prove little. Such appearances may often be found when we are approaching the limits of resolving power of the microscope, and might very well be artefacts produced through fixing and staining. For the time being it may be supposed that the irregular shadows which at present form the basis of Boeke's so-called secondary network of nerves, belong to this category.

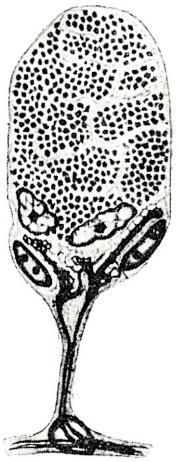


Fig. 12. Cross-section of end-plate and muscle fibre. (Petersen).

In amphibia the somatic motor nerve distribution is of a somewhat different form, the end-plate not appearing as a circumscribed lenticular body, but the axis cylinder being seen to end in a root-shaped ramification ("Stangengeweih" of Kühne), covering a relatively large area. The sole follows to some extent the nerve branches, to which also the nuclei connected with the end-plate attach themselves. The difference between this form of the terminal organ and the typical form described above is thus a purely morphological one.

As a rule there is one end-plate on each fibre, in most cases situated near the middle of the fibre or, in the case of conical fibres, a little nearer the thicker end. In certain forms of fishes the end-plate is, however, situated near the end. Some workers e. g. Agduhr (1916), describe 2, even 3 end-plates on each fibre, a phenomenon associated with plurisegmental innervation of the fibre. This question,

on the elucidation of which a considerable amount of work has been expended, does not, however, seem to be of corresponding functional importance and, therefore, the details will not be considered here.

Besides the somatic motor end-plates we find, usually in close proximity, a small end-plate of similar structure, to which a non-medullated nerve fibre leads; but the question of the autonomic innervation of the muscle fibre is not so far clarified histologically that we can deal with it in detail here.

Boeke makes the following statement about the development of the organ. Even before the individual muscle fibres are differentiated the motor nerve grows into the embryonal muscular plate. The nerve forms a network, the greater branches of which run across the muscle fibres whose cross striation has now become visible. Moreover the muscle nuclei have now begun to move from their original central position towards the surface of the fibres. The sarcolemma has not yet been formed. At this juncture a thickening develops on the transverse nerve branch, corresponding to the individual muscle fibre. The thickening develops into a fine fibrillary network, gradually moving away from the nerve branch and ultimately being connected with the latter only by a thin stalk. Simultaneous with this development the sole is formed from the embryonal protoplasm, and muscle nuclei invade it and are then transformed to nuclei characteristic of the motor end-plate.

Previously it was taken for granted that the essential part of the end-plate was the ramification of the axis cylinder, and the muscular part of the organ, the sole with its nuclei, was considered as something accessory, unimportant, or even as a resistance interposed between the nerve ramifica-

tion and the contractile fibrils (E. du Bois-Reymond). Ranvier compares the sole with the glass of a Leyden jar, a comparison perhaps more allied to functional viewpoints. However, a few workers (e. g. Babuchin) had noticed that the intensity of the discharge in the electric fishes did not appear to be in direct proportion to the extent of the nerve ramification in the electric plate, whilst on the other hand it increased with the mass of the sole. Thus Babuchin finds that the electric "shock" from a young malapterurus is much weaker than the shock from an older animal, though the young animal has the full number of electric plates and the nerve ramification is practically fully developed; the part of the organ that increases in mass with the growth of the animal is the sole. It appears highly probable that this view of the sole, as the structure characteristic of the motor end-plate, is the correct one. As mentioned previously, there is no demonstrable limiting membrane between the sole of the end-plate and the remaining fibre substance; but the electrostatic determinations to which we shall refer later, show that a limiting boundary exists there which permits the maintenance of a fairly considerable potential difference between the end-plate and the remaining part of the fibre.

The Elastic Properties of the Muscle Fibre.

The elasticity of muscle has been the subject of investigations that have gone on for more than a century, and still no general agreement has been reached in results. The determination of elastic moduli by physical methods on a complex living subject such as the muscle has been and is still the centre of much debate, and there is no general agreement as to the usefulness and suitability of the various methods usually employed.

It may be permissible to determine the modulus of elasticity of a muscle, a bundle or a fibre by the usual methods provided the object in question is considered as a homogeneous physical object. In a similar fashion the technologist determines the modulus of elasticity of oak etc. In either case it is impossible either in theory or in practice to take account of the different structural elements of which the object is composed. It has been maintained that the contractility of the muscle should interfere with determinations of elasticity; but this will not be so if care is taken that the experimental technique employed does not stimulate the muscle. Resting and active muscles can simply be considered as different physical objects whose elasticities are determined separately under precisely defined experimental conditions. The existing determinations of the elasticity modulus of muscle give widely fluctuating absolute values, which is not to be wondered at, because it must always be extremely difficult, if not impossible, to obtain a series of preparations which are in the same condition, if only for the reason that we possess no criterion of this and are therefore unable to control it. But we can obtain reliable relative elasticity moduli for one and the same muscle in the resting and active states. In all cases care must be taken that the changes in the muscle are reversible and that the experiments are reproducible. This as a rule presents no difficulties.

The criticisms directed against these methods have most often been evoked by cases of their wrongful use. In certain cases, however, objections may be raised on theoretical grounds, e. g. against the extension method, since the marked elastic after-effects of the muscle may easily be responsible for unreliable measurements. However, even by means of this method comparable results have been obtained. The

same applies to Bethe's elastometer. In its original form this apparatus was unsuitable, as tension was measured instead of elasticity; but Steinhausen has succeeded in modifying the apparatus so that it can be used for determination of elasticity. Objections can also be made against the apparatus employed by Gasser & Hill.

Apart from such errors as omitting to determine one or more of the factors concerned in the formulae used in calculations of the elasticity, the most common experimental error is overloading of the preparations, often to an absurd degree, and omitting to employ definable and reproducible experimental conditions. These errors are to some extent due to the fact that often preparations quite unsuitable for the purpose have been employed, as for instance whole gastrocnemii, the longest fibres of which are about 6 times longer than the shortest ones, and the shape of which will from the start render it impossible to analyse the results of the experiments. Whole sartorii are unsuitable in such experiments, at any rate when torsion experiments similar to those of Lindhard and Möller (1926—28) are performed, even though these muscles, owing to their shape and structure, are much to be preferred to gastrocnemii. On the other hand, it is extremely difficult to work with isolated fibres, because it is difficult to keep them alive for a sufficient length of time, especially when they are subjected to mechanical influences. Determinations of elasticity are therefore best made on bundles consisting of a fair number of fibres so as to minimise the inevitable effects of the mechanical influences on the individual fibres. Otherwise the preparation should, of course, be selected with due regard to the method which it is intended to employ. In all cases the preparation must be in a state of relative equilibrium when

measurements are made. In any stationary state there exists an equilibrium between the tensions exerted by the different elements of which the muscle is composed. When the equilibrium is disturbed through external interference such as change of intensity of a stimulus, or alteration of the length of the muscle, the different elements composing the muscle (stroma tissue, active and inactive fibres) will tend to attain another state of equilibrium. Such adaption requires, however, an appreciable time for its completion which, among other things, is shown by GASSER & HILL's thermoelastic experiments, and until this has occurred it is, of course, quite impossible to obtain information about the structural elements of the fibre. Changes in friction, viscosity etc., which may be demonstrated during transition from one stationary state to another, are mainly referable to the macroscopic parts of the muscle; they should not be brought into any direct relation to the microscopic constituents of the individual fibre. It must be considered highly probable that the greater part of the elastic afterchanges of the muscle are due to such disturbances of equilibrium. But the fibre, too, consists of a complicated system of microscopic elements, as already mentioned, and their relative positions of equilibrium are also altered when the equilibrium between the macroscopic elements is disturbed, so that if it is desired to obtain more detailed information of the microscopic changes, experiments must be performed with isolated fibres.

As the great majority of the experiments hitherto performed were made with whole muscles, the existing absolute values of the elasticity moduli are quite useless, but the ratio between the elasticity moduli of the resting and the active muscle can be determined with considerable success

on whole muscles, provided that their shape is suitable for the purpose.

The classical experiments in this field are due to E. Weber (1846), who made use of extension experiments and calculated the elasticity modulus from the formula

$$A = 2\,\frac{l_1 - l}{l_1 + l} \cdot \frac{1}{p_1 - p}$$

that is the lengthening of the unit of length on loading with 1 gm. — The experiments showed that the stimulated muscle was more easily stretched than the resting, and moreover that the elasticity modulus of the muscle increases with the degree of extension. These extension experiments were controlled by means of torsion experiments, when it was found that the time of oscillation was greatest in the case of the stimulated muscle, despite the fact that it was both shorter and thicker than the resting muscle.

Objections have been raised against both methods. Against the extension experiments it has been asserted that the elastic after-effects will invalidate the results. Of the older authors Blix especially has opposed this method, and most recently Wöhlisch has drawn attention to the difficulties attending the exact measurement of the muscle length. Thus a sartorius will gradually lengthen appreciably under the load of its own weight alone. Nevertheless, it is possible after a few minutes to obtain a sufficiently constant measurement of the length, especially if the muscles are not too heavily loaded; it is at any rate sufficiently constant if the requirements are limited to comparative measurements of elasticity on stimulated and resting muscles. A number of workers have also been able to confirm Weber's results as far as the extension experiments are concerned. The

torsion experiments employed by WEBER have not been described in detail; but objections have also been raised to this procedure, namely that torsion experiments should not be employed because, normally, the muscle is never twisted in this way. Such objections are, however, irrelevant if the muscle is being considered simply as a physical object. Generally the torsion elasticity gives no information concerning the elasticity in the longitudinal direction, unless, as is the case with muscles, the volume remains unaltered when there is a constant ratio between the two elasticity moduli. Torsion experiments may then be employed; and one of their advantages is that errors arising from measurements of length are abolished.

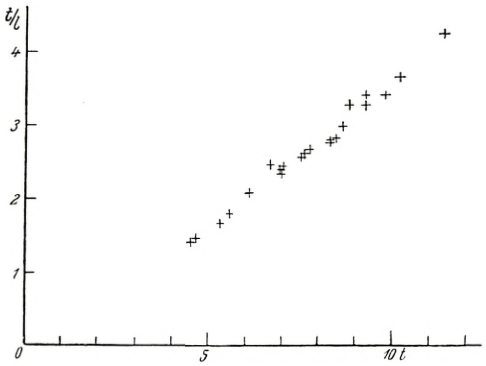


Fig. 13. $\frac{t}{l}$ in arbitrary units as a function of t. t = time in sec.; l = length of bundle. (LINDHARD & MØLLER).

A. V. HILL has objected to the torsion experiments on the grounds that when the muscle fibres are twisted spirally round each other in such experiments, components of force will arise which, irrespective of the elasticity, will cause oscillation of the muscle. According to HILL this would manifest itself by the ratio $\frac{t}{l}$ being constant in the oscillation experiments. LINDHARD & MÖLLER have, however, shown that $\frac{t}{l}$ is not constant, but is a function of t (Fig. 13). If, therefore, under properly controlled conditions there is agreement between the extension and torsion methods, the

results may be relied upon. In the literature a fair number of torsion experiments are reported and in all cases, as far as can be seen, the results confirm Weber's observations. The difficulty in assessing these published results is usually due to the fact that the duration of the torsion but not the length of the muscle is stated; in many cases a statement of whether the muscle was shortened or not will, however, afford sufficient information. Weber has observed that when overloaded to a certain extent a muscle will lengthen when stimulated. This observation, known as "Weber's Paradox" is also reported by other observers. Thus Gad (1879) states that he has seen the writing point fall below the base line during the latent period when a loaded muscle attached to a myograph is stimulated. It is, of course, not necessary merely to overload the muscle in order to elicit the phenomenon. The muscle may be overloaded to such an extent that it fails to appear. Where overloading is sufficiently great the time of oscillation is found to be unaltered on stimulation of the muscle, which is probably due to the fact that the oscillation time of the stroma is involved. Bethe & Happel (1923) by means of special techniques, have obtained corresponding results in certain cases. Steinhausen (1928) employing a modified Bethe's elastometer, has obtained results similar to Weber's. Steinhausen determines the elasticity modulus of the muscle according to the following equation

$$m \cdot \frac{d^2x}{dt^2} + r \cdot \frac{dx}{dt} + ax = 0,$$

in which x is the coordinate of a point in the track of the hammer, m is the mass of the hammer, t the time corresponding to x, r a friction constant, a the elastic antagonistic

force of the muscle corresponding to its starting length. This force can be determined from the following equation

$$a = \frac{Eq}{l},$$

in which q, when the muscle fibres are arranged in parallel formation, may be substituted by $\frac{v}{l}$, in which v is the volume of the muscle. If E remains unaltered during the contraction, t must decrease proportionally to l. This is, however, not so in STEINHAUSEN's experiments; they show that E decreases when the muscle is stimulated. STEINHAUSEN's explanation of this result is that the muscle is perhaps only partially contracting; but it should be noted that whilst a resting muscle is one whose fibres are all at rest, a contracted muscle is one in which many or few fibres are active. Corresponding to a given length a resting muscle has a certain modulus of elasticity, whilst a stimulated muscle of a given length has as many elasticity moduli as it has degrees of stimulation; but all these values are at a lower level than the elasticity modulus of the resting muscle as ascertained by STEINHAUSEN and others.

Apart from some experiments by ERNST and collaborators (1935), based on very doubtful suppositions, there is among experiments on whole muscles only one series reported by GASSER & HILL which is in definite disagreement with WEBER's results. Serious objections have, however, been raised against the technique they employed. GASSER & HILL used a heavy steel spring to which the muscle was fixed at a point "determined by experiment to be optimum". It should have been fixed at the free end of the spring. As pointed out by STEINHAUSEN (1928) the spring does not oscillate about the same point in each experiment so that

its oscillations become highly asymmetrical. The time of oscillation of the resting and the contracting muscle are not, therefore, directly comparable. Moreover, the objection can be raised to Gasser & Hill's technique that the spring may stimulate the muscle by pulling upon it; for both Dreser (1890) and Blix (1891) have observed that sudden loading as well as a sudden removal of loading may produce fibrillary contractions of the muscle. A similar objection may be made to Steinhausen's procedure and, therefore, it appears inadvisable to employ the so-called Bethe-elastometer in future experiments on the elasticity of muscle; but in the present instance possible errors of this nature do not, however, invalidate Steinhausen's results.

Lindhard & Möller have performed experiments with muscle strips of the central part of the frog's sartorius muscle. The preparation employed, as far as possible circular in transverse section, was loaded with 0.363 gm. Stimulation was effected by induction shocks via the myograph and a copper wire, 0.04 mm. thick, which hung from the lower end of the preparation into a beaker of water. The elasticity modulus of the muscle was determined by torsion experiments according to the formula,

$$t^2 = \frac{1+k}{E} \cdot \frac{16\pi lI}{r^4}, \qquad (1)$$

in which t is the time of oscillation, k a constant, l the length of the muscle bundle, r its radius, and I the moment of inertia. As the volume of the muscle during the contraction is practically constant we find

$$\frac{l_1}{l_2} = \frac{{r_2}^2}{{r_1}^2}, \qquad (2)$$

if we introduce these indices into (1) and at the same time instead of r insert the value determined by (2) we find by division

$$\frac{E_1}{E_2} = \frac{t_2^2}{t_1^2} \cdot \frac{l_1^3}{l_2^3} = \frac{\alpha_1}{\alpha_2}, \tag{3}$$

as $\alpha = \frac{l^3}{t^2}$. In order to determine $E_r = \frac{E_2}{E_1}$ we have only to measure l and t, which can be done with considerable accuracy. In the present experiments l was determined with an accuracy of $\pm$ 0.1 mm.; timing was performed by means of a stop-watch by two observers, which gave an accuracy of $\pm$ 0.1 sec. — As even the slight loading used in these experiments caused a measurable lengthening of the muscle, an attempt was made to correct the length back to l_0 by means of the formula

$$l - l_0 = \frac{k}{E},$$

in which k is a constant. When k is found by other means, the equation enables a determination of l_0 to be made. The

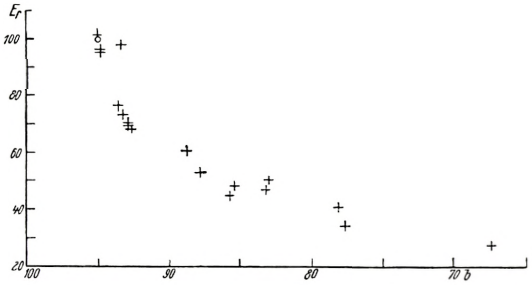


Fig. 14. Ordinate: relative elasticity modulus. Abscissa: length of the contracted bundle in % of the initial length.
(LINDHARD & MØLLER).

correction is not exact, but is obviously necessary and its incorporation results in an improvement of the figures

obtained. The damping was not considered, as its influence had to be regarded as being $^1/_2$ per cent. of the time of oscillation. The results of these experiments (Fig. 14) correspond with Weber's results and with the results of experiments previously mentioned, except those of Gasser & Hill.

Quite recently (1935) Sichel has attempted to determine the elasticity modulus on a single resting muscle fibre. This author used the formula $E = \frac{l}{\pi r^2} \cdot \frac{F}{l - l_0}$, measuring l, l_0 and $d = 2r$ under the microscope and determining F, the tension, by means of a glass lever previously calibrated. It must, however, be considered doubtful whether this method of measuring tension is satisfactory where the end in view is to obtain exact absolute measurements. Neither can the preparations used by Sichel be considered suitable for the purpose. The author uses fragments of fibres of $^1/_6$—$^1/_1$ mm. in length by stretching them by means of needles, which by his own admission damage the preparation. The maximum extension possible is 15 per cent. Sichel states E for the resting fibre to be 2.5×10^6 dyne/cm^2. In the two published tables the average is 1.56 (0.5—2.8) and 1.57×10^6 dyne/cm^2 respectively; the mean figure stated, therefore, does not appear of much value, less so because the error is not stated, or the number of experiments. These experiments are, however, worth continuing using more suitable methods for the measurement of tension and the degree of extension.

As already mentioned the muscle fibre consists of sarcoplasm and of fibrils, which in turn are composed of short cylinders consisting of alternating anisotropic (A) and isotropic (I) substance. From the measurements referred to previously (Buchthal, Knappeis & Lindhard) the relative elasticity moduli of these two substances can be calculated,

but absolute values can not be obtained because the tension was not measured in these experiments. We may consider that A is contractile, I non-contractile. If we make use of the experiments given in Table VI and compare the unstretched fibre with the extended one, we find, since the tension is the same in the whole of the muscle compartment, e. g.

$$\frac{2.08 - 1.37}{\frac{1.37}{A_r}} = \frac{1.00 - 0.81}{\frac{0.81}{I_r}}, \quad A_r = 0.45\, I_r.$$

On an average we find $A_r = 0.47\ I_r$ where A_r and I_r denote the elasticity moduli of A and I during rest. Correspondingly we find in the case of the contracting fibre $A_k = 0.93\ I_k$[1], where A_k and I_k designate the respective elasticity moduli of the contracting fibre. If we assume on the basis of the existing determinations of elasticity moduli on muscle bundles (LINDHARD & MÖLLER) that the elasticity modulus of the whole muscle compartment is less during contraction than at rest, it follows from the figures above that I_k is about $\frac{1}{2}\ I_r$, while A_k is about equal to A_r. The results of the above elasticity determinations cannot be used quantitatively here; but an examination of all existing measurements indicates that the decreased elasticity modulus of the whole muscle compartment during contraction will more readily be produced by a change in the elasticity modulus of the extended I-substance than by a change in the modulus of the greatly shortened A-substance. A further decrease in the A-substance would otherwise imply an improbably large decrease in the resting length. As the most probable result it must, therefore, be concluded that:— In the resting fibre

[1] This figure is less reliable than the resting value as the changes in length and elasticity modulus may not necessarily run parallel.

the elasticity modulus of the *I*-substance is about twice as great as that of the *A*-substance, whilst during contraction the two moduli are nearly equal, for on stimulation the elasticity modulus of the *I*-substance is reduced to about half the resting value, whilst the elasticity modulus of the *A*-substance remains unchanged. A possible explanation of this apparently uneconomical phenomenon appears to be that the decrease of the elasticity modulus will act as a "buffer" in the case of sudden, strong, mechanical stresses. At the same time it must be admitted that the assumption that *I* is non-contractile is open to question in so far as a slight contractility of *I* might be disguised by contraction of the stronger *A*-section.

The breaking strain of muscle tissue determined in extension experiments with whole muscles is only of slight physiological interest, because it must depend chiefly on the muscle stroma; at any rate it is impossible to draw conclusions from such experiments regarding the elasticity of the muscle. Carvallo & Weiss (1899) have examined muscles in situ with intact circulation in guinea-pigs and frogs. The authors find that frog's muscles are generally torn by a traction of 6000 gm. per cm.^{2}, a little more for stimulated muscles than for resting muscles, which is attributed to "the counter-traction of the contracting fibres against the loading". The limit of elasticity, on the other hand, is a quantity which, especially in experiments with isolated bundles and single fibres, is of some importance in the estimation of the results of such experiments. Hitherto a good deal of such work has been in vain because due regard was not paid to the existence and importance of physiological limits.

Much earlier work was performed in order to determine the shape of the curve of extension, whereas subsequent

experiments have aimed at determining the tension-length diagram and then have shown that the *stretch- and release*-curves of the innervated muscle did not coincide, so that the latter therefore could not be considered to be "a new elastic body". None of these experiments have given satisfactory results because the necessary conditions for such experiments have not been observed, and because different workers have taken widely differing views of their material. A number of experiments by H. H. WEBER, performed with a view to comparison between the curve of extension of a whole muscle and that of a myosin thread, are especially open to such criticisms. Such analogies are now unnecessary, since we are able to determine the stretch-curve of the single sections of the muscle fibril. Further discussion of tension-length experiments on whole muscles will not be considered here; reference will only be made to a series of experiments by ASMUSSEN (1936) performed on bundles and single isolated fibres. A close examination of these experiments will show their superiority over similar experiments on whole muscles.

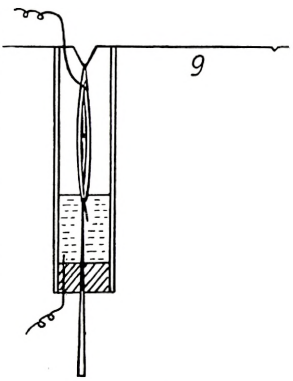


Fig. 15. Device for recording the tension of single fibres or small bundles. g = glass rod. (ASMUSSEN).

In this experiments ASMUSSEN employed the semitendinosus muscle of the frog, a muscle that is wellsuited for such experiments, because its fibres are comparatively short, very nearly cylindrical, and of the same length as the bundle, so that they can be fixed by means of the tendinous tissue to which they are attached, without injury to the fibre substance. The arrangement of the experiment will appear from Fig. 15.

The tension measurements were made with the aid of a glass lever, previously calibrated. This method is obviously of limited accuracy, but that the results obtained bear some relation to the conditions really existing, is shown from the fact that measurement of the area of the tension-length curve for contraction within physiological limits gives the expression $^1/_5\ Tl$ for the potential energy, where T is the isometric tension and l the resting length; a value corresponding very well to the one stated by A. V. Hill. Thus the accuracy is at any rate sufficient for the experiments discussed here. The difficulties in obtaining exact results in muscle physiology are as a rule associated not only with the apparatus but with the preparation also, so that in many cases the limits of accuracy of the experiment will be determined, less by the perfection of the apparatus, than by the condition of the preparation.

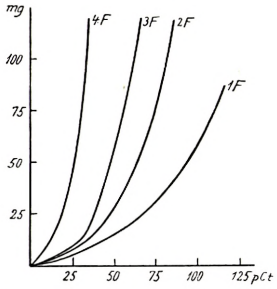


Fig. 16. Length-tension diagram of small bundles (1—4 fibres). Ordinate: tension in mg.; abscissa: length in % of resting length. (Asmussen).

Passive extension of one or a few muscle fibres will result in curves such as are shown in Fig. 16, where the abscissa represents extension in per cent. of the resting length, the ordinate representing the tension in mg. As will be seen the curves are not straight lines but are chiefly of the shape seen in extension experiments on whole muscles. This shows that the shape of the curve of extension of whole muscles within physiological limits is due primarily to the fibres, and that the connective tissue stroma becomes of importance only on more marked extension. This shape of the curve moreover explains why Sichel, who works with

the lowest and least reliable part of the curve, arrives at the result that the curve of extension is a straight line. When experiments are performed, as are those of SICHEL, with short, more or less damaged fragments of fibres, where the initial length is in doubt and the extension amounts only to 10 per cent. of the latter, the result must be unreliable; for the shape of a curve cannot be determined on the basis of so short a portion of the whole. ASMUSSEN moreover finds that the curve of extension of a resting fibre or bundle is completely reversible up to a degree of extension of a little more than 40 per cent. If this length is exceeded the curve is no longer reversible, but the curve of relaxation will lie at a lower level than the curve of extension. The author concludes from this that the limit of elasticity of the fibre must lie at this degree of extension. That this conclusion is correct will appear from the histological measurements of BUCHTHAL, KNAPPEIS & LINDHARD, which show that the histologic structure of the muscle fibre, especially the cross-striation, disintegrates at a limit lying at about 150 per cent. of the resting length. This limit is not sharp and cannot be so, because a fairly considerable individual variation will always exist, so long as we have no exact criterion of the physiological condition ("Vollwertigkeit") of the fibre. The wider the units with which we work, the more does this apply, because all the fibres of the bundle are not necessarily in the same condition or extended to the same degree in any given experiment.

If then we consider the stimulated muscle fibre it can be seen that it is a new elastic body with a shorter length of equilibrium and lower elasticity modulus than the resting fibre. If the stimulated fibre is prevented from attaining its new resting length a tension will be produced in it. Owing

to the rapid course of the contraction it is theoretically impossible to determine the extension curve corresponding to a single stimulus. Therefore it becomes necessary to work with tetanized preparations. There are then two possible procedures to choose; either the tetanized bundle can be stretched, or the bundle previously extended, can be tetanized. So long as we remain within physiological limits the results will in the main be identical, but if these limits are exceeded the result will become different in the two cases as may be expected.

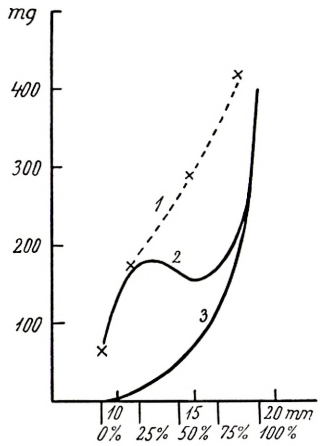


Fig. 17. Length-tension diagram. Small bundle of 12 fibres. 1. stretched during contraction. 2. stretched and then stimulated. 3. resting fibre. Abscissa and ordinate as in Fig. 16. (Asmussen).

If the muscle, extended beforehand, is stimulated we shall obtain a tension-length diagram of the shape shown in Fig. 17. As will be seen it corresponds fairly closely to the curves arrived at by experiments with whole muscles (Sulzer and others). In different experiments the point of the curve will occupy somewhat different positions depending on the shape of the fibres and their individual lengths as well as on the general condition of the preparation. This type of curve, whose shape has never been explained, has been used in attempts to prove that the stimulated muscle cannot be considered a new elastic body. However, when the change in excitability of the muscle produced by stretching is taken into account, such arguments cannot be sustained. Clinical investigations of Brinch-Eliassen (1925) suggest

that the threshold value of the stimulus is lower when the muscle is relaxed than when it is passively extended. If the threshold value of the direct stimulus is determined for a muscle bundle, the result shown in Fig. 18 will be obtained. The curve shows that when the extension reaches about 25 per cent. of the resting length the threshold value of the stimulus begins to rise, slightly at first, but gradually more and more until at an extension of 50—60 per cent. of the resting length, the excitability disappears. If the stimulus is not of too long duration nor forced to extreme limits, the change in excitability is reversible. The bundle will resume its original resting length, and regain its former excitability and corresponding tension. The changes of excitability referred to here do not appear when indirect stimulation is employed, because the stimulus proceeding from the end-plate is at a higher level than the threshold value; therefore the effect of an indirect stimulus will remain almost unaltered until, at an extension of about 60 per cent., it suddenly disappears. When this point is reached the bundle will behave as though unstimulated.

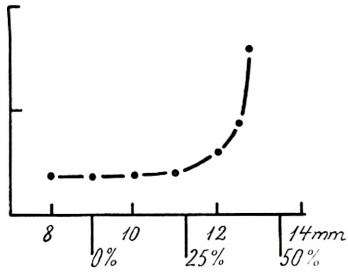


Fig. 18. Excitability of the directly stimulated fibre with increasing stretching. Ordinate: threshold in arbitrary units; abscissa: stretch in % of resting length. (ASMUSSEN).

If, on the other hand, a resting bundle is stimulated and then, under constant stimulation, passively extended, the conditions become so complicated as to be beyond our control. To begin with, the muscle will assume its shortest length of equilibrium; but gradually as the extension continues stimulation becomes less effective and simultaneously

there will occur, as shown by various experiments of ASMUSSEN (1936) and others, a slow regression of the change of elasticity. Since the tetanic curve is formed by the summation contractions of curves of single fibres this will cause the tension to be added to an existing "residual tension" (tension remainder) so that the resulting curve will be at too high a level. The curves arrived at by these two different procedures will, therefore, coincide only in their first part. (Fig. 17.) This does not imply, however, that the stimulated muscle is not a new elastic body, but it means, as has already been pointed out, that the active muscle when stimulated at different lengths is transformed into different elastic bodies. From this point of view it appears inadmissible to employ the term "force of contraction", or similar terms when referring to the difference between the tension of the resting and the contracted muscle corresponding to a given length.

It is assumed that stretch and release of the bundle must take place so smoothly that possible displacements between the individual fibres, relative to each other, will soon be evened out. If the contraction or the relaxation takes place suddenly it will be impossible to obtain smooth tension-length diagrams, for a sudden jerk may act as a stimulus, and possible displacements cannot pass off until some time after the movement has ceased. The same applies to an even greater degree when whole muscles are used. On the other hand it will be impossible to work exceedingly slowly, for then further complications will occur owing to the onset of fatigue. For these reasons, therefore, it is impossible to construct a tension-length diagram representing the theoretical working maximum of the muscle.

Stimulation of the Muscle Fibre.

In the preceding chapter it has been shown that the resting, unstimulated, muscle fibre is a body possessing certain elastic properties, and the stimulated fibre a body with certain other elastic properties. When it is undisturbed by stimuli, the resting state is relatively stable. On the other hand, the fully developed state of contraction is labile, so that even under the most favourable conditions it can only be maintained for a very short time, a few seconds at the utmost. The apparent difference in the case of whole muscles is due to the fact that in this case interference phenomena are mostly being observed. Despite the variations which may be observed experimentally in these two conditions, there will always exist a characteristic difference between them, for the stimulated fibre has a shorter length of equilibrium than the resting fibre. Within physiological limits both the resting and the stimulated fibre will behave in general like elastic bodies. Especially characteristic of muscle fibres is the fact that, under the influence of a stimulus, the resting fibre will pass into a different elastic state and then, after stimulation wears off, will return through a stage of restitution to the resting condition. During the transition from one elastic state to another the fibre will pass through an active state, the actual stage of contraction, in which it will be able to perform work under suitable external conditions. This, however, must not be considered to be some special property inherent in the organ, but is a direct consequence of the characteristic elastic property of the muscle. During the "process of contraction" the same things happen in the fibre as in a stimulated muscle which had been passively extended to the equilibrium length of

the resting muscle and then released. The contracted muscle taking up its equilibrium length is no more active than the resting muscle. The activity of the muscle may be considered to be of the same nature as the activity of the rubber cord, both phenomena being simply the consequence of an elastic body having been disturbed from its position of equilibrium.

With gradual increase in our knowledge of the events taking place in the muscle fibre as a result of stimulation, and with extended understanding of muscle function, we are faced more and more with difficulties arising from the inadequacy of the terminology at our command. Thus, to describe a whole series of, apparently, highly specialised phenomena we have only the single term: contraction. This term must, for example, serve to describe the shortening of the thick end, and the elongation of the thin end of the conical muscle fibre; its use results for instance in such linguistic monstrosities as "isometric contraction" and "excentric contraction", and the terms "static" and "isotonic" contraction. This incomplete terminology only serves to reflect the corresponding confusion which exists as regards definitions and ideas of muscle function. The phenomena taking place in whole muscles, which in part must be related to the conditions of stimulation and the architecture of the muscle, have been and still are confused with the phenomena of the individual fibre, whose shape and innervation must likewise influence the ultimate result of the process of stimulation. Finally these processes are in turn confused with those taking place in the single muscle compartment, the essential unit of muscular activity.

An attempt will be made here to give an account of the process of stimulation in the single fibre under different conditions, whilst the mechanism of muscular activity itself,

so far as it is known, will be discussed in a subsequent chapter. In most cases it is impossible to follow the process of stimulation in the muscle than by observation of the effects it produces, so that it may become necessary even here to refer to muscle tension etc.; but where the description of a highly complicated phenomenon is described under several headings, a certain amount of repetition is unavoidable.

The Stimulus.

The muscle fibre may be stimulated directly or indirectly. The physiological stimulus is, however, always indirect; that the somatic motor nerve branch also may be influenced by artificial stimuli is of no importance in the study of muscle function proper. There is general agreement on these elementary points. A great deal of confusion in this field has resulted from badly arranged experiments and ill-controlled conditions, in consequence of which indirect stimulation has taken place very frequently when it was believed that a direct stimulus had been applied.

The muscle fibre can be directly stimulated by mechanical, thermal, chemical, and electrical means. The latter has, however, mainly been employed hitherto in experimental work as it is difficult in the case of the first three forms to adjust the strength, to limit the time of action, or to employ them in such a manner that they do not damage the fibre. Provided the electrical stimulus is not made unnecessarily strong or continued beyond a certain time, depending empirically on the conditions of the particular experiment, it will cause no injury to the fibre, which may indicate that it is of similar nature to the natural stimulus. Therefore, it may be supposed that the results obtained by direct and indirect electrical stimulation are comparable.

According to the classical theory of the physiological process of excitation, a nerve impulse from some ganglion cell, arrived at the motor end-plate and was "transmitted" in some way to the "contractile substance" of the muscle fibre, causing this to become active, "the state of contraction". Since muscle physiology is concerned only with the subsequent processes, it is of no consequence whether the nerve impulse is produced by natural processes, or by an artificial stimulus applied to the peripheral nerve. At the point of entry of the nerve, the so-called "nervous equator of the muscle", the arrival of the nerve impulse produced a "wave of contraction" which was propagated at a fairly slow rate towards the ends of the muscle.

This conception was ultimately elaborated about 1870 as the doctrine of the so-called "action current". Even though this is now of historical interest only, at least so far as muscle is concerned, nevertheless it played so important a role in the investigations into muscular function as to merit its brief mention here, if only for the reason that discussion of this question directed attention also to the part played by the motor end-plate in the natural excitation of muscle.

When two non-polarizable electrodes were placed some distance apart on an undamaged muscle and connected with a galvanometer a deflection of this would be observed when the muscle was stimulated electrically. This deflection would appear as a diphasic curve and was termed the "action current".

The phenomenon was carefully studied by Hermann, who explained it as due to a "wave of contraction" in the muscle which first passed under one conducting electrode, whereby a deflection of the galvanometer occurred in one

direction, and then under the other electrode, causing a deflection in the opposite direction. HERMANN, however, soon modified his account to the effect that it was not the wave of contraction itself which gave rise to the electric phenomena, but that the latter were associated with the process of stimulation, as any part of the muscle when excited became negative to a resting part. In this manner a "wave of negativity" occurred, which advanced over the muscle at the same rate as the "wave of contraction".

Many years later the doctrine of the "action current" was revised by PIPER (1912), who worked with improved technique, including EINTHOVEN's string galvanometer. PIPER was fully aware of many of the difficulties involved in the support of HERMANN's theory but had no other explanation to offer. PIPER moreover extended the existing data by experiments on humans, some with voluntary muscle function, some with application of an artificial stimulus to the nerve trunk. In these experiments both stimulation and leading off of action currents took place percutaneously, and groups of muscles were employed. The curves so obtained were in complete correspondence with those obtained from isolated muscles, but the difficulty of the theoretical explanation of the action current was increased. As recently as 1925 FULTON has repeated the early theory quite uncritically, and no further facts of importance have since appeared.

The first real opposition to the theory of the action current which PIPER's work supported came from TSCHIRJEW (1913), who pointed out that by leading off transversely from a muscle, action currents of the usual form were obtained, which was unexplained by the theories then in vogue; he considered the action currents to be artefacts.

The validity of Hermann—Piper's hypothesis was again questioned by Henriques & Lindhard (1920), who criticised some of Piper's hypotheses and raised a number of anatomical as well as functional objections to the theory. We shall not revive the discussion here but only emphasize certain important points. Henriques & Lindhard maintained that the innervation of muscle is of such a nature as to preclude the possibility, in the whole muscle of the occurrence of a progressive wave-motion of the kind required by the theory, particularly in the case of groups of muscles (cf. for instance Fig. 19 reproduced from Bardeleben's Handbuch). Thus, even in a small muscle bundle we find not one, but many points of entry of the nerve branches (see Fig. 19), which excludes the possibility of a "wave of negativity" proceeding in the same direction in all fibres. Henriques & Lindhard found that in certain cases action currents could be obtained in the absence of muscle contraction, and that under other conditions powerful contractions could be obtained without action currents; e. g. the curarized muscle apparently produced no action current on direct stimulation. They showed, moreover, that the two phases of the diphasic action current were not invariably symmetrical, which was considered to be in contradiction to the theory. From their experiments Henriques & Lindhard concluded that Hermann—Piper's theory was untenable; moreover that the action current must be considered to be connected, not with the substance of the muscle fibre, but

Fig. 19. Distribution of nerves in the flexor group of the fore arm. (Frohse & Fränkel).

with the motor end-plate. This view aroused much criticism, which was especially centred on the behaviour of curarized muscles, as it was claimed that curarized muscles gave as powerful currents of action as did non-curarized muscles. The experiments were later resumed by LINDHARD (1932) with similar results to those previously obtained; and experiments by ASMUSSEN (1935) and BUCHTHAL & LINDHARD (1934—35—36—37) and HÖFER (1934) have now suggested that "action currents" originating from curarized muscles may be due either to defective curarization or are artefacts due to spread of stimulus. ("Reizeinbruch", c. f. SCHÄFER (1936)). In such work experimental errors are easily made, since the stimulus used to obtain the mechanical record may easily interfere with the arrangements for recording action currents. If care is taken, it is, however, possible to prevent this, not only on fresh tissues but also on curarized or paralysed muscles. The chief difficulties are those referred to above. As to curarization, it is a wellknown fact that curare is an inconstant and difficult drug with which to work. In view of this difficulty ASMUSSEN (1935) has systematically examined different methods of curarization and has shown that the effect varies greatly; but by far the most serious difficulties are those caused by the stimulation. Working with indirect stimuli we can easily prevent stimulus "escape", and ascertain whether this requirement has been fulfilled or not; but working with direct stimulation, which in the case of curarization is unavoidable, we have no effective control with regard to spread of stimulus. The only criterion lies in the result itself. If the galvanometer gives no deflection at all, there has been no spread of stimulus and then there is no "action current". Care is necessary in order to achieve this result. First, large frog gastrocnemii may be

employed, so as to ensure that the leading-off electrodes, which are placed on the lower third of the muscle, shall be as far away from the stimulation electrodes as possible. This is essential, because the threshold for direct stimulation is far higher than for indirect. Again, the fibres may be stimulated in the transverse direction of the muscle, and one of the stimulating electrodes may be earthed. Lastly, portions of the muscle where a large nerve branch approaches the surface, should be avoided; but in this respect individual differences may complicate the conditions of the experiment. The experiments are easily performed on large gastrocnemii, but are far more difficult with sartorii. The reason may be that the paths taken by the stimulating current are more or less confined to the comparatively large muscle mass in the upper part of the gastrocnemius, whose fibres, and blood capillaries also, follow an oblique course and are not directed towards the leading-off electrodes. In the strap-like sartorius which may be preferable from another point of view also, the reverse is the case, for all the conducting elements pass in the same direction from the point of stimulation to the conducting electrodes.

In a subsequent publication Henriques & Lindhard (1923) emphasized that it is only possible to determine the frequency of the action current when the curve is regular and shows no signs of interference. To attempt, as Piper has done, to distinguish between "major and minor waves" ("Haupt- u. Nebenwellen"), is valueless. In the case of very vigorous and thus comparatively short voluntary muscular contractions it is possible from a group of muscles such as the flexors of the forearm to obtain smooth curves, the frequency of which lies between 40 and 65 per sec., which was supposed by Piper (1912) to be the frequency

with which the motor centres sent out their impulses. This smooth rhythm can only be demonstrated in the case of maximal contractions. It cannot be decided with certainty whether the rhythm of the action currents corresponds to the rhythm of the ganglion cells or not, even if the rhythm of the action current follows the rhythm of an artificial stimulus.

The whole of the question of the essential nature of the action current and its possible importance in the organism was reconsidered when LINDHARD (1924) revived the old "Discharge Theory" (Entladungshypothese). HENRIQUES & LINDHARD's paper (1920) made it evident that in some way the electrical phenomena must be associated with the motor end-plate and, therefore, a comparison was suggested between this organ and the electric organs of certain fishes. It soon became apparent that we possessed so complete a literature on the subject that it could be approached both ontogenetically and histologically. In the case of one of the strong electric forms, however, the question is still unsettled. This is the Malapterurus, whose electric plate is associated with the skin and does not seem to be developed from musculature. The young of this fish have, however, never been found, despite very careful search. In the Malapterurus the whole of the electric organ, which is capable of producing an E. M. F. of about 250 volts is innervated from two large (macroscopic) nerve cells in the central nervous system, whilst in all forms where it is developed from skeletal muscles it is innervated from the somatic motor nerves attached to the latter. The correspondence demonstrated between the electric plates and the motor end-plate is so great that they must be considered morphologically identical organs and, therefore, the electric "shock" of the fishes in question was considered to be a phenomenon analogous with the "action

current'' of the muscle. This concept was further supported by experiments on Astrape japonica of Fuji, who employed an oscillograph and photographic recording, and examined the electric discharges from a portion of the electric organ of this fish. He obtained a curve which bore a striking resemblance to the so-called single-contraction curve of a skeletal muscle. After a latent period (7.6 σ after the commencement of the stimulus) it rose comparatively abruptly to a maximum, then fell again more slowly to the base-line. Finally there was a slight, secondary rise in the curve (Fig. 20). The curve conforms to the equation

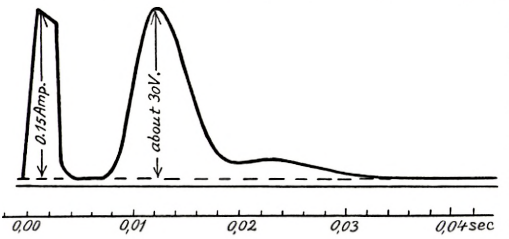


Fig. 20. Stimulation and response of electric organ of Astrape japonica. (Fuji).

$$y = Ae^{-b^2 \log^2 \frac{x}{x_0}},$$

which has been derived from the equation for the exponential curve of errors. On the basis of his analysis of the curve Fuji believes that the shape of the curve is due to the fact that not all the plates react simultaneously but in succession, the beginning of the curve corresponding to the plate first reacting, whilst its maximum represents the greatest number of plates reacting simultaneously. According to Fuji the secondary rise is due to the discharge of the last reacting plates acting as a stimulus on the first plates, the reaction of which by that time has already passed off.

If this is correct it would be expected that the first phase of the curve of the action current should correspond to the above equation. That there might possibly be some basis for this, would appear from Fig. 21 (Lindhard, 1931), in which a number of ordinates calculated according to Fuji's formula are inserted into the first phase of the curve

of an action current obtained by KEITH LUCAS with a capillary electrometer. The slight deviation towards the end is possibly due in part to the same cause as the deviation in FUJI's curve, and partly also to the commencement of the second phase of the action current. The so-called single-contraction curve behaves in a similar manner; in this case, however, the correspondence is not so close.

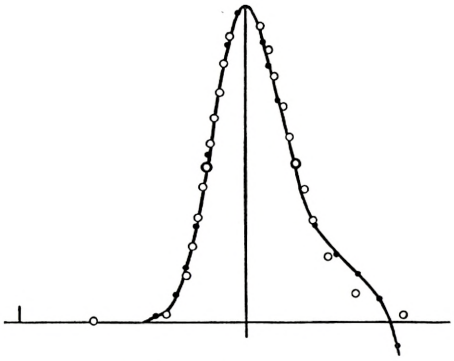


Fig. 21. First phase of action current from a frog's muscle. Circles calculated from FUJI's formula.

As the interference curves obtained from experiments on whole muscles are always difficult to interpret, ASMUSSEN & LINDHARD (1933) tried to examine the action current of single muscle fibres. Similar experiments had already been made by GELFAN & BISHOP (1933), who stimulated fibres of the frog's membrana basihyoidea mechanically. When such stimulation was applied to the fibre substance no action current appeared in spite of the occurrence of a localised contraction, though it was seen occasionally when, as the authors stated, a nerve branch had been stimulated. ASMUSSEN & LINDHARD did not employ frog's muscles but lizard's muscles (Lacerta agilis), which have the advantage of possessing localized end-plates. The superior, subcostal part of the M. obl. abd. int., which consists only of a double layer of fibres, is used, for which reason it is easy to observe end-plates in the fibres by transmitted light. In this muscle the fibres are 13—14 mm. long and 40—70 μ thick. The end-plate is of an almost circular shape with a diameter of

30—60 μ. Examination and stimulation was performed under a binocular microscope with a magnification of about $\times 125$. As it became at once apparent that both electrical and thermal stimulation affected the galvanometer the motor end-plate itself was mechanically stimulated by means of an extremely fine glass needle, which like the conducting electrodes, was adjusted in position by means of Péterfi's micromanipulator. Mechanical stimulation is not consistently effective, at any rate when used with caution so as to avoid damaging the preparation. To remedy this the preparation was sensitized to such stimuli by adding a minute amount of a strychnine or phenol solution to the drop of Ringer's solution in which it was immersed. The action currents were led off through a condenser-coupled amplifier to a string galvanometer (Boulitte), by means of fine platinum electrodes. The amplifier recorded alternating currents down to a frequency of 5 per second. For further details reference may be made to the original paper.

When both electrodes were placed on the muscle fibre and the latter was stimulated mechanically close to one of the electrodes no deflection appeared on the galvanometer, even when a local contraction of the part stimulated could be distinctly observed under the microscope. As will be shown later, this does not necessarily imply that the process of contraction in the fibre does not give rise to electric phenomena; these, however, could not be demonstrated by means of the apparatus described above. When, on the other hand, one electrode was placed on the fibre and the other immediately at the edge of an end-plate (it could not be placed on the end-plate, as it would then have interfered with the mechanical stimulation) and the end-plate then was irritated, a diphasic deflection of the galvanometer was

obtained as shown in Fig. 22. When one electrode was situated at the end-plate, the other could be placed on the same fibre or a neighbouring fibre, and moreover the muscle fibres on either side of the end-plate could be cut through by means of a fine needle, and the end-plate thus comparatively "isolated", all without changing the form of the electrical response to any considerable extent. As will be seen from Fig. 22, most of the curves are uniformly diphasic; but in a certain number of cases forms like those shown in Fig. 22 left are seen, different from the usual type though the conditions were apparently the same and there was no injury produced. A direct comparison between these curves and those obtained from experiments on whole muscles is of course difficult, because the latter must be considered an expression of interference phenomena. All things considered, it seems likely that the first phase of these curves is identical with the first phase of the action current. The second phase of the electrogram is no more symmetrical with the first than in the curves from experiments with whole muscles; and, therefore, as already pointed out by HENRIQUES & LINDHARD (1920), it is improbable that the two phases are due entirely to one and the same underlying process. The second phase of the action current, as it appears in the measurements made here, might be connected in some way with the first, rapid change in the contraction potential (BUCHTHAL 1934) referred to later. If this is so, the second phase of the action current thus has its origin partly in

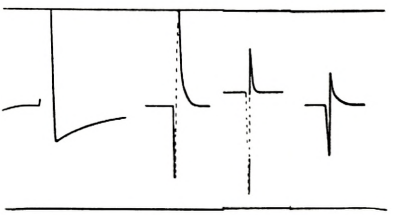


Fig. 22. Action currents from single muscle fibres (lizard). Direct mechanical stimulation of the end-plate. (ASMUSSEN & LINDHARD).

processes in the fibre-substance. Asmussen & Lindhard's failure to demonstrate electrical changes on stimulation of the fibre itself must be due to the fact that the mechanical stimulus employed by them could only produce localised contractions of the fibre.

There are, however, further difficulties involved in such a comparison, first and foremost that Asmussen & Lindhard's apparatus was entirely different from that used by Buchthal and Lindhard (see below). This does not imply, however, that the above explanation of the second phase of the action current is completely inadequate. It is worth emphasizing in this connection that the potential changes both of excitation and contraction, are monophasic and that their time relations appear to correspond to those between the two phases of the action current; but the time relations of the contraction potential and the second phase of the action current do not correspond, which may have some connection with the type of amplifier used by Asmussen and Lindhard.

Finally, it may be pointed out that the ratio between the number of the usual diphasic curves and the "monophasic" ones is about the same as the ratio between the cases in which the changes in excitation and contraction potentials are opposite, and those where they are in the same direction.

Owing to the experimental difficulties previously referred to, it can only be deduced from these experiments that there is a potential difference occurring when the endplate is stimulated which cannot be observed on direct stimulation of the fibre.

Accordingly, it appeared probable that the rapid diphasic oscillation was, as supposed by Lindhard (1924), in some

way associated with the process of excitation in the muscle fibre; this gives, however, no further insight into the mechanism of excitation. The next step towards the understanding of this was taken when measurements of current were replaced by electrostatic measurements, which have

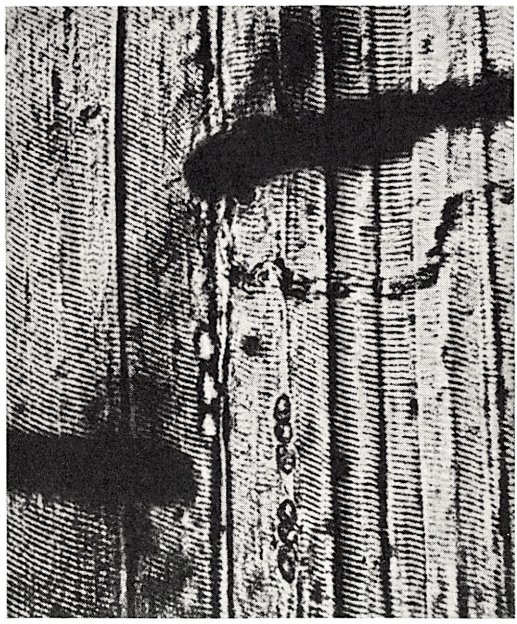


Fig. 23. Lizards muscle bundle, living unstained fibres. Upper electrode on motor end-plate, lower electrode on the same fibre. Magnification 450 ×. (BUCHTHAL & LINDHARD).

been introduced into this field by BUCHTHAL. A brief survey of the principle of this method will be found in a following chapter. For further details the original papers may be consulted.

After BUCHTHAL & PÉTERFI (1934) had shown that in frog's muscle fibres there exists a potential difference between different parts of the surface, increasing with the distance apart of the electrodes, i. e. with the number of

muscle compartments included, BUCHTHAL & LINDHARD (1934) measured the potential difference between the motor end-plate and the fibre substance by means of the same technique. For histological reasons these experiments were made with lizard's muscles. The micro-electrodes were placed 40—60 μ apart on the same fibre; the grid electrode was placed on the motor end-plate (Fig. 23). It then appeared that whilst the potential difference measured on the fibre amounted to 0.5—1.0 mV., the potential difference between fibre and end-plate, measured with the electrodes the same distance apart, was about 10 mV. Moreover, the fibre-fibre potential difference in some cases went in the same direction, in several cases however in the reverse direction, to the fibre-end-plate difference. (Fig. 24.)

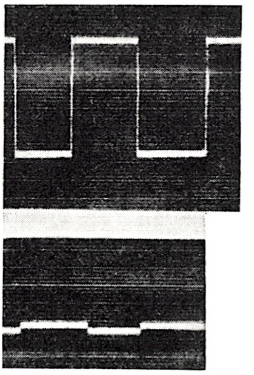


Fig. 24. Potential difference between motor end-plate-muscle fibre (upper curve) and between two points on the same fibre (lower curve). Electrodes same distance apart. (BUCHTHAL & LINDHARD).

The measurements of currents previously dealt with may give some indication that the potential difference as measured electrostatically plays a part in the excitation process. Therefore BUCHTHAL and LINDHARD (1936) examined the effect of temperature changes upon the fibre-end-plate potential difference, at first within the limits at which laboratory experiments usually take place; later it was believed that by extending the temperature range (still within physiological limits) a clue might perhaps be obtained as to the processes underlying the production of the potential difference. It appeared from these experiments that the fibre-end-plate potential difference, like the fibre-potential (which will be

dealt with in detail in a subsequent chapter) varied directly with the temperature (cf. Fig. 25). Calculation of Q_{10} gave 1.8 ± 0.08 with an average error of 23 per cent. — But there was no significant relation between Q_{10} and the general level of the temperature curve. In these experiments it was, however, striking that the fibre-end-plate potential difference[1], which often remained unaltered for at least 1 hour at a constant temperature, was unable to withstand considerable changes in temperature. Furthermore, it appeared

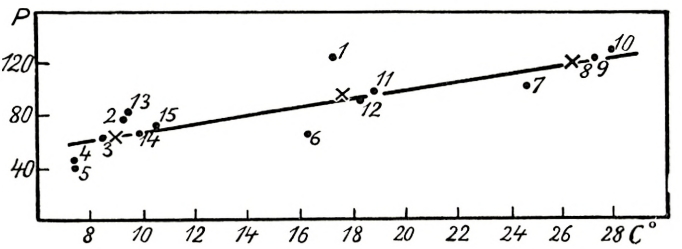


Fig. 25. Relation between end-plate-fibre potential and temperature. Numbers indicate sequence of measurements; ordinate: potential in arbitrary units; abscissa: temperature.
(Buchthal & Lindhard).

that even moderate rises of temperature might easily cause alterations of potential to become partly irreversible.

Buchthal & Lindhard (1935) have also examined the effect of curare and radium emanation on the *F*—*E* potential. Lizard's muscles were used and measurements were made by Buchthal's electrostatic technique, recording with an electron tube voltmeter. It was first ascertained that the above potential differences existed in the resting system, and it appeared once more that the *F*—*E* potential was nearly 10 times as large as the *F*—*F* potential with the same distance between the electrodes in each case. The potential difference

[1] In the following termed the *F*—*E* potential, the potential difference between two parts of the same fibre being termed the *F*—*F* potential.

in different animals often varied in size and direction, but the variations found in preparations from the same animal were small and may be due partly to a different position of the end-plate within the fibre. Although in $^2/_3$ of the cases examined the end-plate was positive in relation to the fibre, the reverse was the case in the remaining $^1/_3$. No satisfactory explanation can be offered for this fact at present, even considering the possibility that the location of the electrode in relation to the end-plate may have some effect on the direction of the potential as indicated by experiments on amoebae made by BUCHTHAL & PÉTERFI (1937).

The principal results of the experiments with curare are shown in Table VII. The greater changes found in the *F—F* potential are chiefly due to a different distance between the electrodes.

As will be seen the curare effect consists of a reduction

Table VII.

Fresh preparation potential		Curarized preparation potential	
F—E	*F—F*	*F—E*	*F—F*
— 96	— 18	— 6	— 7
+ 40	— 16	— 6	— 4
+ 31	+ 7	— 27	— 28
+ 17	— 16	+ 24	+ 24
— 80	+ 2	— 3	— 3
+ 95	— 5	— 4	— 4
— 56	— 4	— 13	— 4
— 65	— 5	+ 20	+ 22
+ 33	— 3	+ 12	+ 18
— 74	+ 8	+ 6	+ 5
— 75	— 18	+ 20	+ 7
— 78	— 11	+ 6	+ 8
— 33	+ 5	+ 20	+ 12
+ 70	+ 6	..	..
+ 38	+ 7	..	..
+ 78	+ 2	..	..

of the high F—E potentials to the order of magnitude of the F—F potentials. The first experiments were performed by measuring the F—E potential of a preparation, covering it with a 1—3 per cent. fresh curare solution and then, after 30—60 mins., when the whole muscle was poisoned, again measuring the F—E potential of the same end-plate. As a

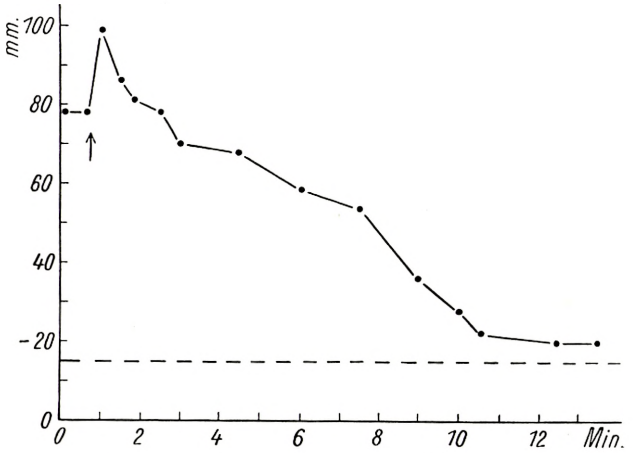


Fig. 26. Effect of curare on the potential difference between end-plate and muscle fibre.
- - - - - -: potential difference in fibre.
↑: application of curare.
Ordinate: potential in arbitrary units.
Abscissa: time in minutes. (Buchthal & Lindhard).

control the F—E potential was measured twice on unpoisoned preparations at the same intervals of time as in the poisoned ones.

Besides these preliminary experiments a number of others were performed, in which the course of the process of curarization was followed. The F—E potential was first determined in the usual manner, and a curare solution then run slowly on to the preparation, the potential difference being read simultaneously at frequent intervals; by means

of a number of varied control experiments we made sure that the addition of the curare did not in itself produce disturbances of potential that would influence significantly the experimental results. The experiments indicate that the *F—E* potential steadily diminishes, sometimes after a short initial rise, until the *F—F* potential has been reached (Fig. 26). If the F—F potential has the reverse sign of the *F—E* potential, the latter must thus change sign in the

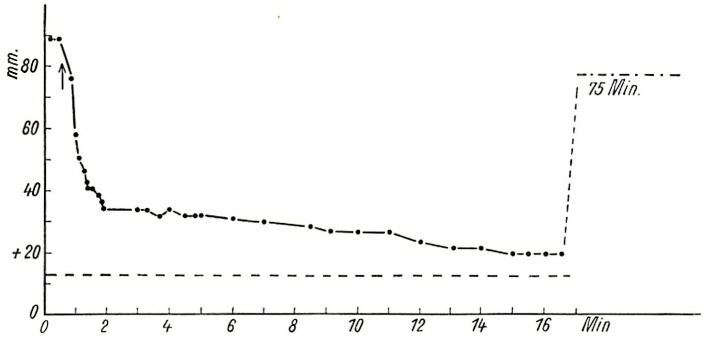


Fig. 27. Effect of curare on the end-plate-fibre potential (see Fig. 26). —•—•—•—: potential difference after removal of curare. (Buchthal & Lindhard).

course of the curare action. When curarization is complete the fibre is no longer excitable by indirect means but can still be stimulated directly[1]. If the curare is removed by rinsing with Ringer's solution, the *F—E* potential will be reestablished and simultaneously it will again be possible to stimulate the fibre indirectly. (Fig. 27.)

These experiments show that the *F—E* potential is a necessary condition for indirect excitability; when this potential difference has been abolished the end-plate is

[1] In this connection it may be emphasized that the threshold value both of mechanical and electrical stimuli is considerably higher with direct than with indirect stimulation. With electrical stimulation the effect is independent of the direction of the current.

simply a continuation of the electrode transmitting the direct stimulus. Moreover, in correspondence with Asmussen's experiments, these results show that, as regards the time course of its action, curare is a very inconstant drug, and also that the mechanical recording of the muscular contraction cannot be employed when we want to make certain that all the end-plates of a preparation are paralyzed. This can only be secured in preparations that can be controlled by observation under the microscope. Whole muscles are, therefore, quite unsuitable objects on which to examine the curare effect, and such experiments reported in the literature must be regarded with scepticism, especially where the object has been to examine the electrical changes in muscles.

As is well known, emanation emitted by radioactive substances is able to ionize gases and fluids, and is moreover able to influence boundary surfaces of living tissue and their permeability to ions. Buchthal & Lindhard (1935) have examined the effect of the radium emanation on the $F—E$ potential difference. The experimental methods were similar to those of the curare experiments referred to above. The preparation was irradiated from a small glass tube, 5 mm. long and 1.5 mm. thick, containing emanation, and fixed by means of paraffin to a thin needle, which could be moved towards or away from the preparation by means of the micro-manipulator. The tubes contained 8—50 millicurie of emanation; in order to prevent effects of possible external influences on the preparation it was also covered by a layer of liquid paraffin. These experiments were thus concerned with the effect of the β and the γ rays, especially the former. In order to avoid influencing the measuring instruments themselves the emanation tube was always

kept at the greatest possible distance from them, and careful screening was provided, which proved to be effective in control experiments. The results of these experiments were not quite as constant as those with curare, since irreversible

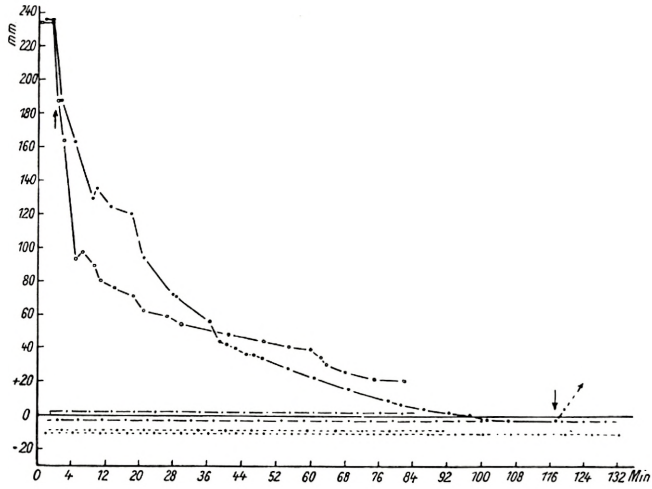


Fig. 28. Effect of β-rays on end-plate-fibre potentials. 50 millicurie radium emanation 2 mm. distant from the preparation. Grid electrode on end-plate, earthed electrode on the fibre. Electrodes 12—15 μ apart (2 experiments).

Abscissa: time in minutes.

Ordinate: potential in mm. (13 mm. = 1 mV.).

—·——··——· end-plate-fibre potential.

— · — · — · — · electrode potential.

- - - · - - - · - - - · fibre-fibre potential.

↑ application and ↓ removal of the emanation.

(Buchthal & Lindhard).

changes were liable to be produced fairly frequently. The difficulties were, however, not such as to prevent clear-cut experimental results.

As will be seen in Fig. 28 the curve falls, i. e. the *F—E* potential is abolished. After an initial fall for approximately

the first 10 min. which is more abrupt the higher the *F—E* potential, there follows a more slowly declining, almost straight part of the curve, which does not stop at the *F—F* potential but continues until there remains only the potential difference between the electrodes which existed before the experiment. When this stage has been reached the fibre is

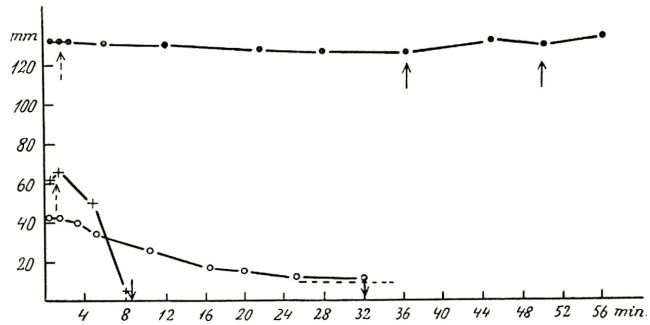


Fig. 29. Potential difference between end-plate and muscle fibre. Upper curve living fibre; Lower curves dying fibres.
↑↑: control of excitability, constant threshold.
↓: preparation unexcitable.
↑: addition of liquid paraffin.
- - - - -: fibre potential.
Ordinate: potential in mm.
Abscissa: time in minutes.
(Buchthal & Lindhard).

no longer excitable directly or indirectly. When the preparation is quite fresh and the experiment has not been of too long duration, the potential differences will reappear when the radium emanation is removed, and the excitability will also return, first the direct and then also the indirect excitability (Fig. 28).

In order to be able to assess these results properly a series of control experiments were made, in order to study the behaviour of the *F—E* potential in fresh preparations, which were covered with paraffin but otherwise undisturbed

apart from occasional testing of the excitability in order to ascertain whether the threshold value was unchanged (Fig. 29, upper curve). Moreover the phenomena seen in dying preparations were examined. In the case of these it appeared, firstly that the *F—E* potential was initially at a comparatively very low level and that as a rule it soon became as low as the electrode potential; secondly that the indirect as well as the direct excitability disappeared simultaneously; and thirdly, that all changes were irreversible in character. At the same time the microscopic picture of the fibre changed, the cross striation first becoming indistinct, then rapidly disappearing. Simultaneously there occur changes in bi-refringence previously mentioned. Finally it was again apparent in the course of the work that direct observation under the microscope is of considerable value in judging the condition of the fibres.

After the experiments reported above, the next step was to examine how the *F—E* potential was influenced by indirect stimulation of the fibre, (BUCHTHAL & LINDHARD (1937)). The solution of this problem entailed very considerable changes in the apparatus hitherto employed, as it now became necessary to register potential differences in resting systems as well as changes of rapid and slow character; and above all the possibility of an escape of the stimulus, which has vitiated innumerable experiments in this field, had to be absolutely excluded. These technical difficulties have been satisfactorily overcome by BUCHTHAL & NIELSEN (1936).

In order to avoid the possible leading off of current from the muscle cell examined, measurements must be made electrostatically, and since the potential differences that had to be measured were of the order of 10^{-4}—10^{-3} V. very

precise conditions had to be established as regards the input resistance, inertia, sensitivity, and stability of the apparatus. The outcome of this was the construction of a balanced D. C. amplifier with electrostatic input, using the cathode ray oscillograph as recording instrument. The beam of light of the oscillograph was photographed together with the time record on moving film.

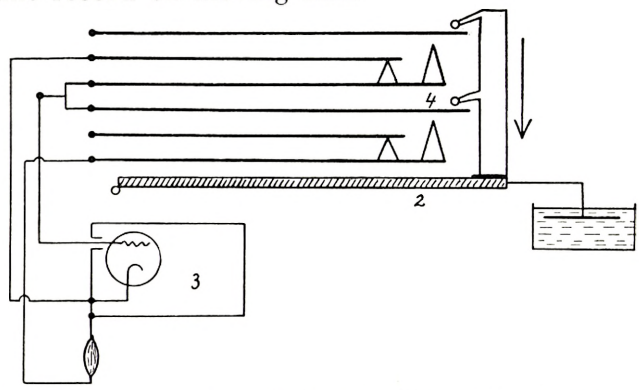
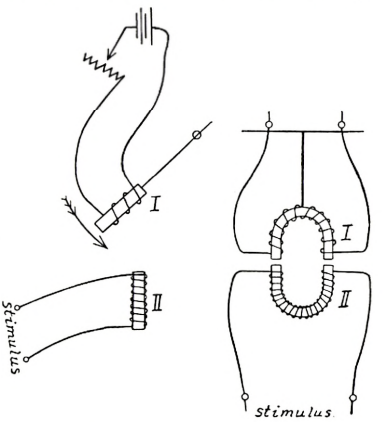


Fig. 30. Contact arrangement (relay) to disconnect and reconnect immediately before and after stimulation to avoid stimulus escape.
1. Glycerine damping arrangement
2. Moved downward by magnet.
3. First stage D. C. amplifier.
4. Contact time varied by insertion of paper here.
(Buchthal).

As the electrical stimuli are the only ones which are quantitatively reproducible this form of stimulation was employed in spite of the great difficulties involved. For if special precautions were not taken the stimulation current would be conducted by the electrodes to the input terminals of the amplifier, where, like the electric responses, it would be amplified and so distort the latter or even obscure them entirely. The difficulties were increased by the fact that the preparation had to be stimulated through the leading-off electrodes, because it was desirable to lead off from the site

of stimulation itself and also because it was inconvenient, for technical reasons, to have three electrodes on the preparation. On the basis of these requirements BUCHTHAL (1937) worked out an arrangement for stimulation which, although the leading-off electrodes were used for stimulating, avoided any spread of the stimulus. This was achieved by interrupting the connection with the measuring instrument during stimulation and re-establishing it within the latent period of the preparation. Thus it became necessary to devise a rapidly working switch system which was able to short-circuit and re-open the amplifier and measuring instruments in about 1 msec. (Fig. 30). Moreover the stimulus had to be a rather high frequency alternating current so as to reduce polarization phenomena at the electrodes.



a b

Fig. 31. Device to produce a symmetrical single cycle pulse.
a = side view; b = seen from forward.
I = electromagnet attached to the pendulum (300 turns, 0.5 mm. diam. wire). II = stationary coil (1500 turns, 0.1 mm. diam. wire). (BUCHTHAL).

Since the metal switches and electron tube generators employed gave rise to disturbances in several ways BUCHTHAL (1937) introduced a special stimulation device excluding all mechanical contacts (Fig. 31). The stimulus was a single cycle pulse of symmetrical shape (Fig. 32) generated by swinging an electromagnet attached to the Helmholtz-pendulum, past a stationary coil.

BUCHTHAL & LINDHARD's (1937) experiments, using the above methods, gave the following results. In experiments

on single fibres of the frog's semitendinosus a decrease of the resting potential of the fibre was found on direct stimulation, in agreement with Buchthal & Péterfi's results (1934), the resting potential being re-established in a period of restitution that lasted for a considerably longer period than the mechanical response. On direct stimulation when one electrode had been placed near the nerve ending, indicated by the fact that the intensity of the stimulus could be lowered considerably, two different changes in the potential occurred successively and could interfere to some extent. The first rapid potential change (the *F—E* potential) appeared 1—2 msec. after the excitation and lasted for 2—3 msec. Then followed the change in contraction potential, sometimes after a short isoelectric interval. The direction of the two potential changes relative to each other was, of course, responsible for the shape of the records. Fig. 33 represents one curve, in which the two potential changes are separated by an isoelectric interval and both go in the same direction. Both potential changes are diminutions of the *F—E* and *F—F* potentials respectively. After the experiment the fibre gradually became inexcitable, even when strong stimuli were employed; the resting potential of the fibre had then disappeared. Then a potential difference was produced between the electrodes from a voltage source in series with the earthed electrode and the dead fibre was then stimulated. Fig. 33 a shows that in these records of controls the voltage of the non-reacting fibre is completely unaffected by stimulus escape. The downward spike (shown by arrow in Fig. 33) in this,

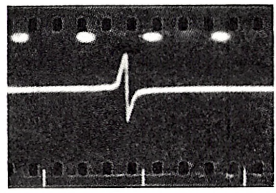


Fig. 32. Record of the single cycle pulse (cathode ray oscillograph) produced by the device shown in Fig. 31. Time in 1/50 sec. (Buchthal).

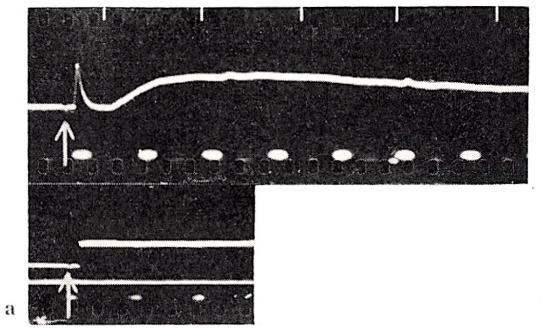


Fig. 33. Potential difference accompanying excitation and contraction of an indirectly stimulated frog's muscle fibre. Electrodes 300 μ apart. Potential (4 mV.) of the resting fibre compensated. Upward movement, grid electrode more negative. The spike over the arrow marks moment of stimulation. Distance between two light marks 20 msek. a. Control for stimulus escape by stimulation of unexcitable fibre after introducing a direct current of 0.5 mV. via the electrodes. (BUCHTHAL & LINDHARD).

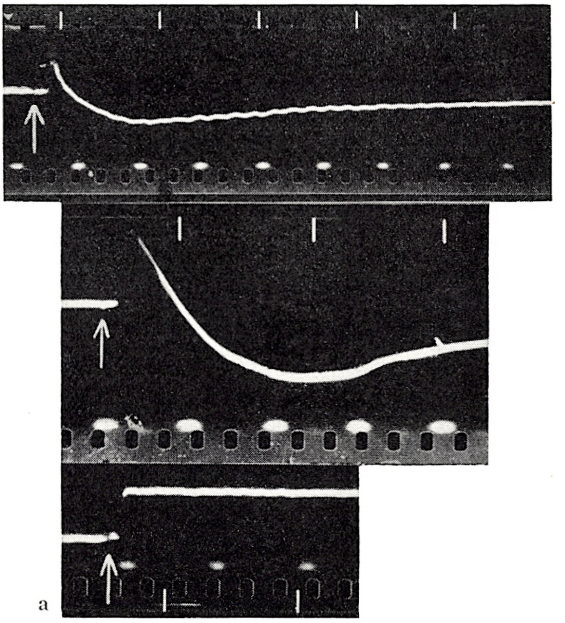


Fig. 34. Potential difference accompanying excitation and contraction. Indirectly stimulated frog's muscle fibre. Excitation and contraction potentials vary in opposite directions. Electrodes 300 μ apart. Compensated potential of the resting fibre 3.5 mV. For (a) see Fig. 33 a. Time marker 20 msek. (BUCHTHAL & LINDHARD).

as well as in all other curves indicates the moment of stimulation and not the moment of connection with the amplifier, which occurs a little later. Fig. 34 reproduces an experiment in which the two potential differences, in this case moving in opposite directions, interfere to some extent. The resting

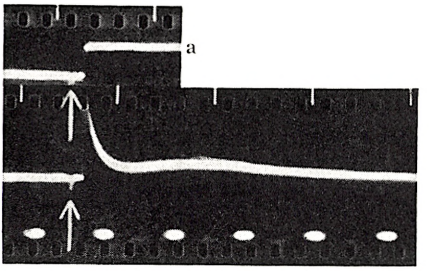


Fig. 35. Potential difference accompanying stimulation and contraction of a lizard's fibre. Grid electrode on the motor end-plate. *E*—*F*. potential compensated 35 mV. For (a) see Fig. 33 a. (Buchthal & Lindhard).

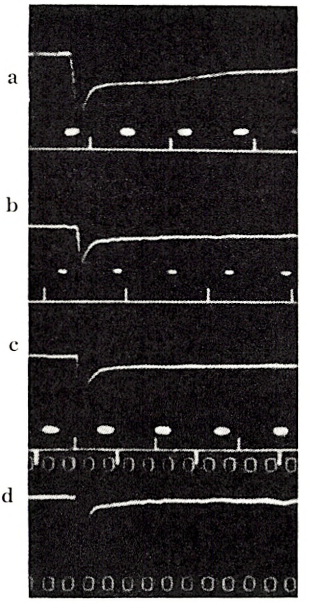
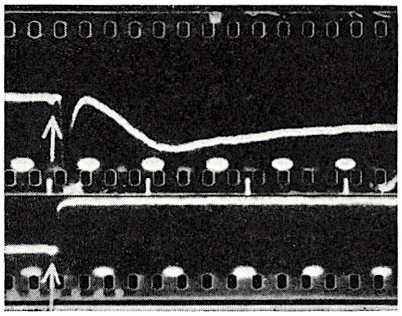


Fig. 36. Lizard's fibre. Influence of different electrode separations. One electrode remains on the end-plate. *F*—*E*. Potential 35 mV., compensated. (a) distance 400 μ. (b) distance 250 μ. (c) distance 150 μ. (d) distance 50 μ. Time marker 20 msec. (Buchthal & Lindhard).

potential in all experiments was compensated, only the changes in potential differences being registered.

It was thus possible, in the case of frog muscle fibres to distinguish between changes in excitation potential (*F*—*E* potential) and in contraction potential. Closer examination of the former made it necessary to carry out experiments on lizard's muscles, in which it is possible to lead off from and to stimulate directly the motor end-plate. The potential difference between fibre and end-plate, the *F*—*E* potential, previously referred to, was compensated so that only the

alterations of potential were registered. About 1—2 msec. after stimulation a fall in the *F—E* potential occurred, amounting to about $^1/_{10}$ of its resting value, after which in the course of 1—2 msec. the potential regained its former value, the latter part of the curve interfering with the oscillations of the *F—F* potential. Fig. 35 shows the appearance when the two potentials change in the same direction. Fig. 35 a represents a control curve on an unexcitable fibre obtained as above. In this case, too, the potential change has been reproduced without any disturbance whatever. The relation between the variations of the *F—E* and the *F—F* potentials will appear more clearly when the distance between the electrodes is varied, though the distance available is not great enough to decide whether the potential change is propagated or spread with decrement.

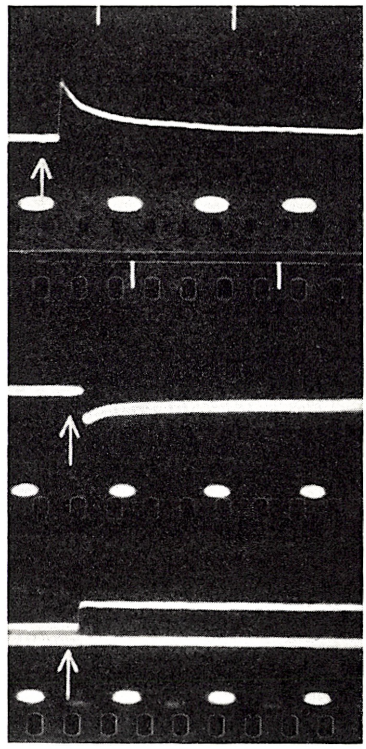


a

Fig. 37. Lizard's muscle fibre, indirectly stimulated by eddy currents, both electrodes on the muscle fibre. *F—F.* potential 2 mV. compensated. Electrodes 400 μ apart. For (a) see Fig. 33 a. Time marker 20 msec. (Buchthal & Lindhard).

The grid electrode is placed on the end-plate whilst the earthed electrode is placed on the fibre at varying distances from the end-plate, the resting potential being compensated as previously. The curves in Fig. 36 show how the potential difference varies under these conditions. If both electrodes are placed on the fibre, and one of them close to the end-plate, the latter thus being stimulated by eddy currents, a curve like that in Fig. 37 will appear, i. e. an excitation potential followed by a contraction potential corresponding in all respects to the one found in the frog's muscle fibre.

In order to secure direct stimulation of the fibre a number of experiments were performed on curarized lizard's fibres. The grid electrode was placed on an end-plate as usual, the earthed electrode on the fibre. The curare solution was applied to the preparation and it was found as previously that the *F—E* potential gradually diminished simultaneously with an increase in the threshold value of the stimulus. After 10—30 minutes, only the *F—F* potential remained, the end-plate was electrically inactive. Direct stimulation is then not accompanied by two separate changes but by a single abrupt decrease of the fibre's resting potential. The more abrupt initial fall found here is probably due to a greater density of current under the stimulating electrode. Such experiments must be very carefully controlled, because the intensity of the stimulus is necessarily many times greater than in the case of indirect stimulation. The potential changes of a curarized fibre are reproduced in Fig. 38. As usual in these experiments the resting potential was compensated, only the abrupt decrease and slow regeneration of the potential being seen in the curve.



a

Fig. 38. Variation of potential difference on stimulation of completely curarized fibres. Fibre potential, 3 mV. compensated. Electrodes 200 μ apart. For (a) see Fig. 33 a. Time marker 20 msec. (Buchthal & Lindhard).

In certain cases the *F—E* potential is found to have disappeared after 10—15 minutes, whilst the indirect excitability remains for a further 15 minutes or so. Since the potentials are led off through the sarcolemma, it is reasonable to suppose that when a potential can no longer be detected by the recording instruments, traces of the latter will still be found in the fibre. As is well known, it is possible to stimulate a single fibre without including neighbouring fibres in the process, the interpretation of which seems to be that the sarcolemma is a comparatively effective insulator. These experiments show once again what care must be taken in order to secure complete curarization of a muscle fibre. Where experimental conditions must be exactly controlled, curarization of whole muscles is a hopeless undertaking. It may be that the results published by SCHÄFER (1936) and ROSEMANN (1936), which differ from ours, are partly explained by that fact. Moreover, our experiments with curare show that the very rapid change of the *F—E* potential which takes place first of all, does have its origin within the fibre end-plate system, and thus is not an action current of nervous origin; for curare would not affect the latter.

This series of experiments confirms the opinion previously expressed, that the problems with which we are dealing, like several others in muscle and nerve physiology, can only be solved by the use of the functional units themselves. The complicated conditions presented by the whole muscle, composed as it is of fibres, end-plates, nerve twigs, blood vessels, connective tissue and tissue fluid, makes conflicting results almost inevitable.

Direct measurements on end-plates and fibres, on the other hand, give uniform results; it shows that between

these structures there is a difference in potential, which on indirect stimulation undergoes a sudden change, spreading over the fibre as a wave[1], and whose relation to the fibre potential has been recorded. The motor end-plate is morphologically and functionally a highly specialised region of the fibre, as Lindhard has pointed out (1924, 1931); and it appears probable that Keith Lucas' β-substance, which, as is well known, is characterized by high excitability, might be referred to this region. Some of the electrical changes taking place on stimulation of the fibre are thus due to changes in the F—E potential, as could be inferred from Asmussen & Lindhard's experiments already referred to; but the changes in the F—F potential could not be demonstrated with the experimental arrangement they employed. Furthermore, Bishop & Gelfan (1932) did not succeed in demonstrating electrical changes when localized contractions were produced in muscle fibres by direct stimulation. The quantitative determination of electrical changes occurring in the single fibre, and especially their time relations, only become possible when electrostatic recording is employed and the measuring device has an input resistance of at least 10 meg. ohms; if this is not the case the fall of voltage in the preparation may result in considerable errors. The experimental results reported here are not yet applicable to whole muscles; but by employing the electrostatic methods of measurement on whole muscles we can, in certain cases, obtain results which correspond qualitatively with those arrived at in experiments on single fibres. (Fig. 39.) In particular, it appears that the electric phenomena in muscle are associated with the process of excitation as well as that of contraction.

[1] Not to be confused with the so-called contraction wave observed in whole muscles.

Although the former experiments on whole muscles, for reasons previously discussed, do not permit definite conclusions to be drawn concerning the electrical changes in the muscle substance, the experiments described above on single fibres have shown beyond all doubt that besides the potential changes in the transmitting system end-plate-fibre, potential changes also occur in the muscle substance proper.

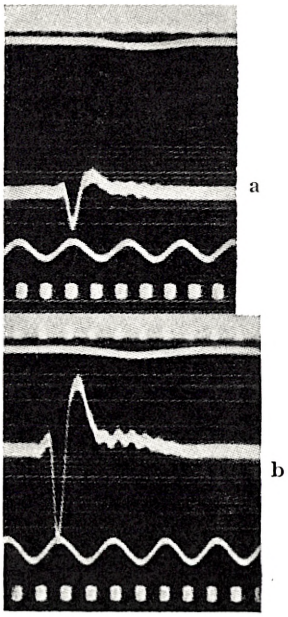


Fig. 39. Mechanogram and action current of M. semitendinosus (frog) stimulated over the nerve. (a) input resistance of the electrone tube voltmeter = 3000 Ω. (b) = 40 Meg. Ω. Time 1/50 sec. (Buchthal & Lindhard).

In the question as to the precise mechanism of the transmission of impulses to striated muscle, the rôles of acetylcholine on the one hand, and the electrical phenomena on the other, have been the subject of much discussion (Brown, Dale & Feldberg (1936), Monnier (1936) Dale (1937)).

The transmission of excitation from the nerve-ending in the end-plate to the sole of that organ need not necessarily be of the same nature as the process taking place between end-plate and fibre-substance. It seems advisable in future work to differentiate between these two boundaries. It may be mentioned that experiments in which acetylcholine was applied directly to single end-plates, indicate that the effect of this substance is more complicated than has been hitherto supposed. The process of excitation, as it is usually understood, involves several stages, in which transmission by both physical and chemical means may be involved.

The Spread of the Stimulus.

As already mentioned, the older conception was that the process of stimulation spread as a "wave of negativity", which advanced from the point where the nerve entered the whole muscle at the rate of the wave of contraction. On anatomical grounds this theory is untenable and it cannot be made the basis for further work. A more productive idea was Keith Lucas' (1905) that each fibre of a skeletal muscle reacts according to the so-called "all-or-none" principle, which for a long time had been considered to apply only to the myocardium. Keith Lucas showed that a frog's muscle, when excited by stimuli of gradually increasing strength, responded with a "stepped" contraction curve; the author's explanation was that with increasing intensity the stimulus spread more widely in the muscle and thus would successively attack one "set" of fibres after the other. And as the "steps" of the stepped curve were horizontal, this was interpreted as a maximal reaction of each new set of fibres. The latter conclusion was not entirely justified, however, as the production of a stepped curve does not necessarily result from the maximal response of the fibres, but might equally well have its origin in a constant, and not necessarily maximal, reaction of each. This might be the case, even though an increasing stimulus is applied to the muscle as a whole.

Some uncertainty existed as to the precise condition of stimulation in these experiments of Keith Lucas and his school; both he and later workers found stepped curves both on direct and indirect stimulation and on fresh and curarized muscles. Therefore it was considered necessary to define more precisely the relation between direct and

indirect stimulations as well as to examine closely the behaviour of curarized fibres.

The question of direct and indirect stimulation has been dealt with in a number of classical experiments by the German physiologist SACHS (1874) who examined the effect of stimulation of a muscle in the longitudinal and transverse directions of the fibres. These experiments have now been extended, and their results confirmed, by ASMUSSEN (1933). The procedure was as follows:— Two Gastrocnemii of the same frog were suspended in the same myograph under identical mechanical conditions and their contractions registered simultaneously. The muscles were stimulated directly and indirectly, and in the former case either along or across the direction of the fibres. It then appeared that by indirect or direct stimulation along the fibres, almost identical "stepped" curves were obtained, though with indirect stimulation the steps were somewhat more pronounced and shortening slightly less (Fig. 40). In other words, the so-called "direct" stimulation along a non-denervated muscle is chiefly of an indirect nature; it is mainly the intramuscular nerve branches which are stimulated, as HAPPEL (1926) had already shown.

In certain cases an underlying effect, due to true direct stimulation, may result in a moderate increase in shortening. If, on the other hand, the muscle is stimulated transversely it is found that firstly the strength of the stimulus must be increased very considerably before the muscle will react at all, and secondly that the curve as a rule shows no signs of "steps" (Fig. 41). In other words, in this case stimulation is mainly direct. However, by increasing the strength of the stimulus very slowly, fairly well-marked "steps" in the contraction curve produced by transverse stimulation

may sometimes be found. These are presumably due to direct effects on the motor end-plates. With gradual increase of the stimulus a perfectly smooth rising curve is obtained only when the muscle is "denervated" in some way; and, for this reason, the majority of the experiments in which "direct" stimulation of non-curarized muscles was employed must be considered of doubtful value. But this does not

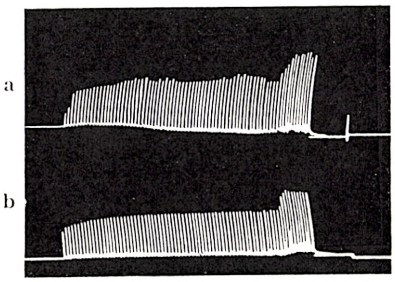


Fig. 40. Frog's muscle. (a) "direct longitudinal" stimulation. (b) indirect stimulation. (Asmussen).

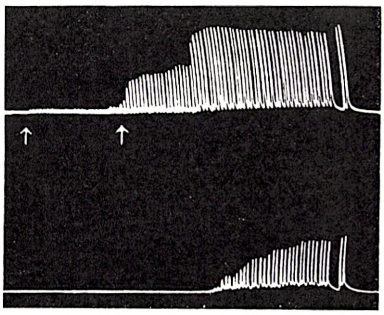


Fig. 41. Upper curve: indirect stimulation; between the arrows a variable resistance was gradually removed. Lower curve: direct transverse stimulation. (Asmussen).

imply that curarized muscles can always be used in these experiments; it has already been mentioned that a great deal of work reported in the literature can be said with certainty to suffer from the serious defect that curarization has been incomplete.

It is obvious that experiments using mixed direct and indirect stimulation on muscles only partially curarized are unsuitable for the study of the problems discussed here. Despite several notable advances in technique, e. g. Pratt & Eisenberger's pore electrodes, some confusion still existed, mainly owing to the use by many workers of unsuitable preparations (the basihyoid membrane) as at that time it had not been found possible to use isolated fibres.

BROWN & SICHEL first succeeded in doing so, and shortly afterwards ASMUSSEN and KATO. BROWN & SICHEL (1930, 1936) showed that a muscle fibre stimulated directly did not follow the "all-or-none" law but responded with contractions which increased with the intensity of the stimulus. This was in agreement with the work of GELFAN (1930). ASMUSSEN (1931, 1932), who undertook a systematic study of the mechanical properties of the isolated fibre, chiefly employed BROWN and SICHEL's technique, though he also made use of small bundles, to some extent. The procedure is illustrated in Fig. 42. As will be seen, the arrangement does not record completely isometrically, but the changes in length become so small in proportion to the fibre length that the curves obtained may be considered as isometric tension curves. The result of ASMUSSEN's work was as follows:— With direct stimulation of constant strength the tension was constant also, but sooner or later, a "contraction-remainder" developed, i. e. relaxation gradually passed off more and more slowly so that after each contraction the fibre did not return to its initial length or tension. Thus it appeared that the increase of tension was constant with each successive stimulus (Fig. 43). In other words it was not the tension but the increase of tension that varied with the strength of the stimulus. After cessation of the stimulus the tension disappeared. With the onset of fatigue, both the increase in tension and the "contraction-remainder" steadily approach zero (Fig. 44).

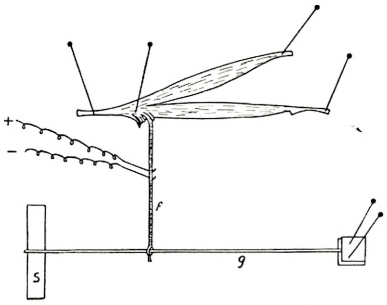


Fig. 42. Arrangement to record tension of a single fibre. Frog's M. semitendinosus. f = single fibre with electrodes. g = glass rod. s = scale. (ASMUSSEN).

If the strength of the stimulus varies, it appears, in accordance with Brown & Sichel's results, that the tension

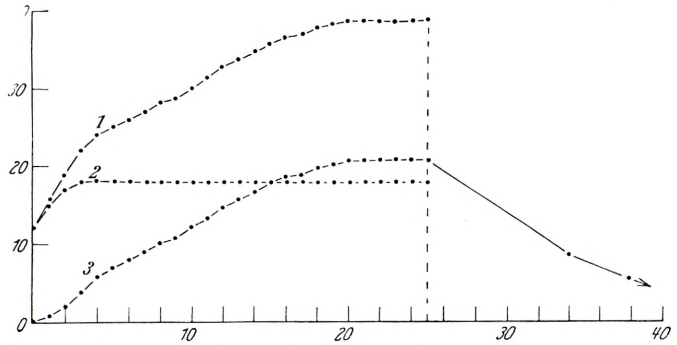


Fig. 43. Single muscle fibre, direct stimulation with constant strength and frequency. 1. Total tension. 2. Increase of tension for each stimulus. 3. Change of base line.
Abscissa: time in 0.8 sec. Ordinate: arbitrary units.
(Asmussen).

will vary accordingly. If the intensity of the stimulus is kept constant whilst its frequency changes, the response of the muscle with increasing rate of stimulation, will gradually pass from a series of single contractions into an almost

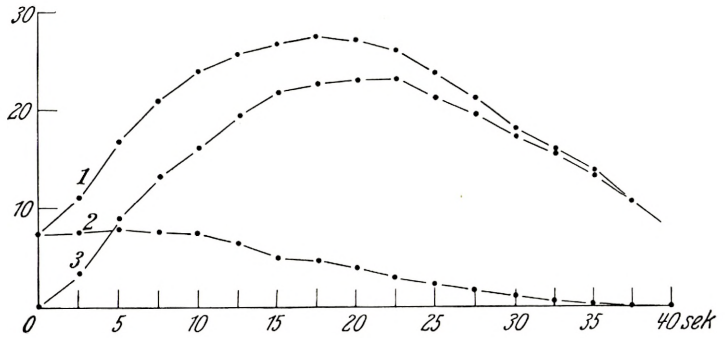


Fig. 44. Single fibre, direct stimulation continued until onset of fatigue. For 1, 2 and 3 see Fig. 43.

smooth curve of interference, the so-called tetanic curve. The latter will, however, not run parallel with the base

line but will be a rising curve. Since this curve represents the total tension of the fibre it will be obvious, from the above, that this total tension, denoted by the "height of contraction" will depend upon the frequency of stimulation. The more rapid the rhythm, the higher the total tension; for the greater will the "contraction-remainder" become in a given time. If the intensity of the tetanizing stimulus is increased, the curve of tension will rise correspondingly.

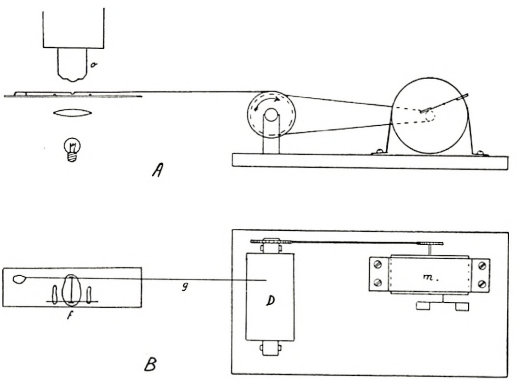


Fig. 45. Device for recording tension of single fibres. A. side view. B. seen from above. f = fibre. g = glass rod. D = drum driven by clockwork motor. o = microscope objective. (ASMUSSEN).

These experiments show that on direct stimulation the isolated fibre does not follow the "all-or-none" law.

In experiments with indirect stimulation ASMUSSEN (1934) employed a somewhat modified experimental technique (Fig. 45). The preparation employed was the same as in the experiments first referred to, namely isolated fibres or small bundles of the frog's Semitendinosus. In some experiments lizard's muscles were employed.

The experiments showed that despite large increases in the strength of the stimulus, the contraction and the increase of tension of the isolated fibre remained constant (Fig. 46 a). However, on excitation of a bundle with increasing stimulus, a stepped curve was obtained which possessed as many steps as the bundle contained fibres. KATO and his collaborators have obtained similar results. If stimulation is

continued at constant strength the bundle will become fatigued, and a curve of tension falling stepwise will then be

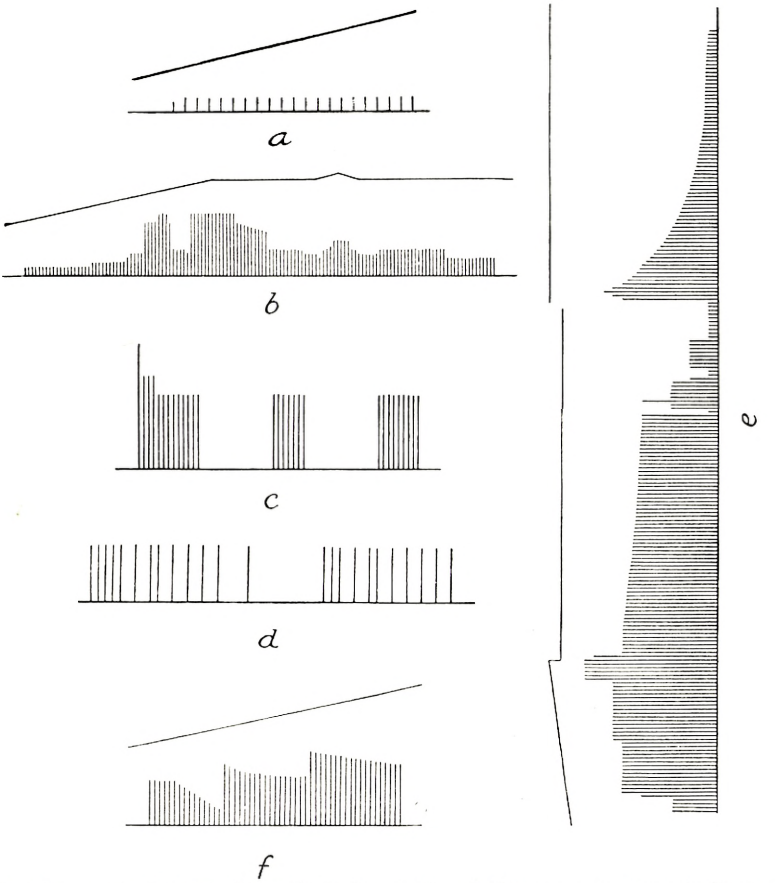


Fig. 46. a. single fibre (lizard); indirect stimulation; upper curve indicates strength of stimulus. b. small bundle of 8 fibres, indirectly stimulated; upper curve strength of stimulus. c. small bundle, continuous indirectly stimulated, strength of stimulus constant (compare Fig. 47). d. small bundle indirectly stimulated with constant frequency, threshold stimulus. e. response of muscle bundle. Upper curve strength of stimulus; left side indirect, right side direct stimulation. f. response of muscle bundle, indirect stimulation. Upper curve strength of stimulus. (Asmussen).

obtained. These stepped curves are not due to fatigue of the peripheral nerve, since as is well known this is an extremely

slow process; they are moreover not due to fatigue of the fibres themselves, since these will still respond to direct stimulation, and with the onset of fatigue show a smoothly falling curve. ASMUSSEN's explanation is that they are due to an "all-or-none" reaction of the end-plate. Fig. 46b shows how "end-plate fatigue" affects the response of the bundle. It is seen from Fig. 46 a—d that even if the tension has fallen to zero the fibre will, after some time, respond afresh to the same or a slightly increased stimulus, and then again cease to react. If the fibre has become greatly fatigued by indirect stimulation, and a change is then rapidly made to direct stimulation, the strength of the stimulus being increased simultaneously, a very powerful response may be obtained for a short time, which soon passes into a continuously declining curve of fatigue (Fig. 46e). Thus it is possible from the shape of the curve of fatigue to decide whether the site of the fatigue is the end-plate or the contractile substance.

In some cases (Fig. 46e) the two forms may be combined. The intermittent fatigue curves show that on indirect stimulation the fibre does not fatigue in the same way as on direct stimulation. If the curve of contraction in the case of direct stimulation falls to zero, the fibre has reached the stage of absolute fatigue and cannot be made to respond again by increasing the stimulus; only after a comparatively long rest will it be possible to make it react again. In the case of an intermittent fatigue curve there are, however, two possibilities. Either the motor end-plate may become fatigued, as ASMUSSEN suggests, or the excitability of the muscle fibre suddenly may decrease so much as to make the constant stimulus initiated from the end-plate "sub-threshold". It is quite impossible to decide with certainty

which of these alternatives is realized under normal conditions; but in certain cases the muscle may be fatigued without the intervention of the end-plate, so that the muscle tension decreases towards zero whilst the impulses from indirect stimulation indicated by action-currents continue with unaltered intensity (Henriques & Lindhard). This is probably the explanation of Fig. 46 f; here fatigue of the muscle fibre is manifest before any signs of this have appeared in the end-plate. An experiment such as that reproduced in Fig. 46 d, where contractions recommence as the result of an increase in indirect stimulation would appear however to indicate that in this case we might really speak of fatigue of the end-plate. Considering the very short refractory period of the end-plate, it seems likely that when the fibre ceases to act, this is due to a decreased excitability owing to fatigue (cf. e. g. Fig. 47). As previously mentioned, however, we have in all cases to reckon with the fact that the normal stimulus, as indicated by the change in the *F—E* potential, is constant under given experimental conditions within rather narrow limits. The next question will then be whether this constant stimulus is maximal as regards the fibre. Keith Lucas believed this to be so, because the steps of the stepped curve were horizontal. This fact, however, as already mentioned, proves only that the stimulus is a constant one and that the fibre is in good condition. In order to investigate this question more closely Asmussen & Lindhard (1935) compared the maximal contraction of the whole muscle and the single fibre with direct and indirect stimulation.

Fig. 48 shows the behaviour of a frog's gastrocnemius under a tetanus of short duration, produced by a very strong stimulus applied directly, and also to the motor nerve.

Though the direct stimulus might not be maximal since the arrangement employed did not permit further increase in the strength, it is nevertheless certain that the indirect

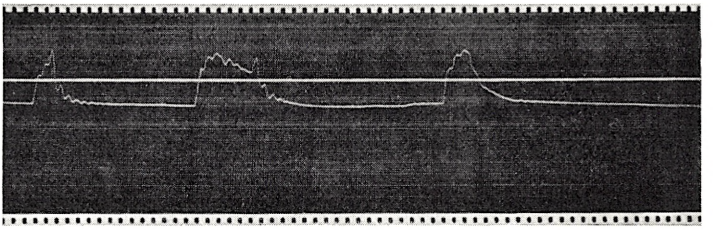


Fig. 47. Tension of a whole muscle (a) and of one of its fibres (b); indirect stimulation (compare Fig. 46 (c). (ASMUSSEN)).

stimulus employed is a maximal one, in so far as there would be no purpose in increasing it. It will be seen that the muscle tension falls rapidly, as would be expected, but further that in every case it is greater in direct than in indirect stimulation. The same result is shown in Fig. 49,

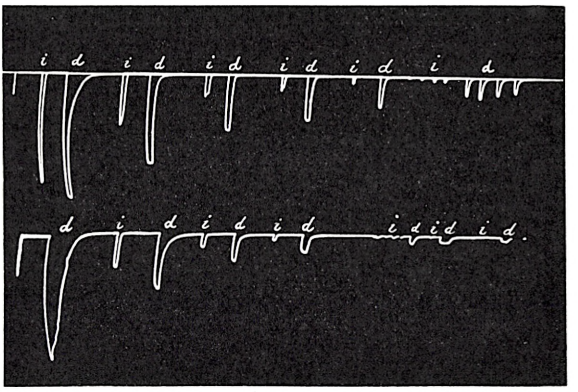


Fig. 48. Frog's muscle; indirect (i) and direct (d) stimulation. (ASMUSSEN & LINDHARD).

an experiment with an isolated fibre; the maximal tension is far greater in the case of direct than with indirect stimulation. This must mean that an indirect stimulus is not maximal

as regards the response of the fibre so that the expression "all-or-none" cannot be used at all in the case of a fibre of a skeletal muscle, but that we really have to do with a constant reaction. Nor can we speak of the all-or-none law in the case of the *F—E* potential. This term, therefore, should be discarded, the more so since it probably does not apply to the myocardium itself either but only to the excitation of this (Schütz, 1936).

As to the spread of the stimulus in the muscle we constantly find the term "wave of contraction" in the older literature. This wave was imagined as a gross alteration in shape, which could be recorded mechanically, especially when alterations of thickness were measured. This "wave of contraction" must be dismissed from consideration, at any rate when normal function of the whole muscle is concerned. It is true that, from a consideration of the experiments of Fuji and others, we may suppose that the latent period of the different fibres varies, but that the individual fibres are not so situated as to divide the muscle into separate regions, which can contract individually; on the contrary, they are situated as far as is known, so that fibres innervated from the same ganglion cell are lying scattered in the muscle and therefore a partial muscular contraction will appear as a contraction of the whole muscle of decreased amplitude. As regards the individual fibre, hundreds of microphotographs taken by Buchthal, Knappeis & Lindhard at, and immediately after, the moment of stimulation in no case give any indication that the muscle

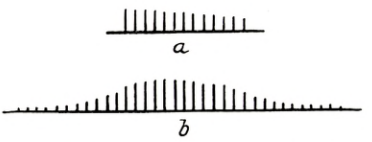


Fig. 49. a. single fibre, indirectly stimulated; commencement of fatigue. b. direct stimulation of the same fibre; strength of stimulus first increasing then decreasing. (Asmussen & Lindhard).

compartments in the field of the microscope are in different states. In this connection it may be borne in mind that from the standpoint of energetics the older conception of the contraction wave in the whole muscle would be futile as it would result in a series of useless tensions in the fibres, which during the whole of the process of contraction would be prevented from attaining a state of equilibrium.

Asmussen & Lindhard (1935) have examined the spread of the stimulus in a fibre or a small bundle by means of a modification of a method first devised by Kato (1934) and reproduced in Fig. 50. The preparation, covered by a very small quantity of Ringer's solution, is fixed to a slide in its centre with a little plaster of Paris; then to each end is attached a long thin glass-rod writing on a drum. The preparation is stimulated at one end by condenser discharges through platinum electrodes, or indirectly through its nerve branch. If the preparation was completely symmetrical and attached completely symmetrically, the glass-rods of equal thickness and the amount of their friction on the drum quite uniform, and lastly, the muscle fibre attached to the fulcrum at the same distance from the ends of the two glass-rods, then two symmetrical curves should be obtained on the drum, if the stimulation passed through the whole of the fibre. Owing to the numerous sources of error, which will not be dealt with in detail here, such symmetrical curves are rarely obtained, but on the other hand when these

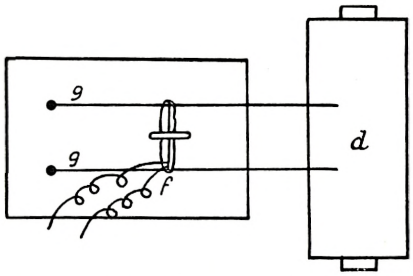
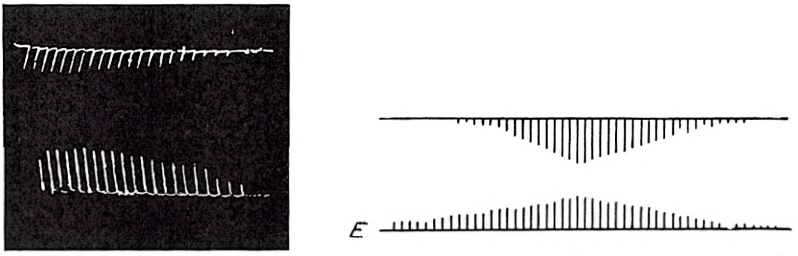


Fig. 50. Recording device for registering tension at both ends of the same fibre. gg = glass rods, d = recording drum, f = fibre fixed in the middle by plaster of Paris. Electrodes attached to the one end of the fibre.

sources of error are taken into account asymmetry will not become so great as not to allow certain conclusions to be drawn from the experiments as to the spread of the excitation in the fibre.

On indirect stimulation of a bundle the symmetry is so marked that it cannot be doubted that the spread of the stimulation is rapid and decrementless. It was necessary



a b

Fig. 51. a. Tension record from both ends of a fibre fixed at its middle. Direct stimulation of increasing strength. E denotes record from portion of fibre on which electrodes are placed. b. As (a); strength of stimulus first increasing then decreasing. a = original record, b = drawing from record. (Asmussen & Lindhard).

to assume beforehand that as stimulation from the end-plate is constant any portion of the fibre that remained uninfluenced by the stimulus would be unable to contract at all under normal conditions.

On direct stimulation some variation in the appearance of the curves is found owing to the uncertainty associated with the method employed. There seems to be a tendency for visible contractions to begin at the stimulated end of the fibre or the bundle, but this tendency is far from being so marked that it would be possible to express it numerically (Fig. 51 a & b). For further details the original paper should be consulted. It appears with certainty from the experiments that the stimulus passes throughout the fibre and that the

contraction thus becomes complete at an intensity below that of the normal stimulus which may therefore be regarded as being above the threshold. Although it does not appear certain from these experiments that a fibre can be stimulated partially, the observations nevertheless show that the contraction becomes complete a long time before it becomes maximal. By other means, however, especially by stimulation of single fibres with microelectrodes (BUCHTHAL & PÉTERFI) (GELFAN) or mechanical stimulation (ASMUSSEN & LINDHARD) under the microscope, it has been demonstrated by direct observation that in the case of weak stimulation a localised contraction may appear at the site of stimulation.

For many years a major problem of muscle physiology was the so-called "latent period" i. e., the period between stimulation of the motor nerve and the mechanical response of the muscle as measured by the curve of a single twitch. So long as we regard the whole muscle as a single unit such a viewpoint is natural; but gradually it has come to be generally realized that all curves obtained in such experiments are interference curves, often very difficult to interpret quantitatively; and for this reason, the question of the latent period of the whole muscle has lost in interest. At the beginning of this century it was realized that something took place during the latent period, fibrillary twitching of the muscle belly was observed and it was seen that the lever of the myograph fell below the base-line during the latent period indicating altered conditions of elasticity. It was observed that with improvement in the apparatus used the latent period constantly decreased. A new attempt was made in 1925 by FULTON to analyse the phenomenon; it proved unsuccessful, however, because it was not appreciated that all such curves are interference phenomena and

because the structure situated between nerve and muscle viz. the motor end-plate, was not taken into consideration. The curve of the single twitch, too, is but an inadequate statistical expression of what happens in the muscle after stimulation (cf. Fuji's experiments). This is shown, in a somewhat exaggerated form perhaps, by Fig. 52.

The impulse initiated in the nerve by the stimulus reaches the different fibres by routes of unequal length, causing the fibres to contract at slightly different moments, so that a considerable number of fibres must be set in action before the lever can be moved. The latent period of the muscle measured from this curve will thus be the period between stimulation of the nerve and the contraction of a certain unknown number of fibres. Then we must consider the "height of contraction", from which it is supposed to be possible to calculate the shortening of the muscle. In this case at any rate it cannot be done even with moderate accuracy; because owing to its kinetic energy the heavy lever must have risen further than really corresponded to the shortening of the muscle; when a sufficiently large number of fibres have relaxed it falls down to, and below, the baseline again. Even if it was really possible to learn something about the shortening of the muscle, we should learn nothing about the far more interesting phenomenon, the shortening of the fibre, as this rarely takes place in the direction of the pull of the whole muscle.

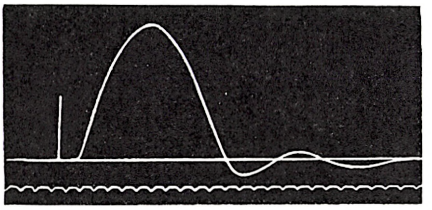


Fig. 52. Single twitch of whole muscle. (Beddard).

It will probably appear from the above that the old idea of the latent period has been played out. The process

of stimulation is a complex physico-chemical process, and at each stage of the various parts of this we may, of course, speak of a latent period, but this will be quite another thing than that previously understood by the term "latent period", something for the measurement of which quite different methods are necessary, and first and foremost, requires the use of isolated fibres. A few experiments of this nature have already been made, as BROWN & SICHEL (1936) have determined the time interval between the stimulus and the beginning of the mechanical response, which they state to be 1.5—2.5 mill. sec., whilst BUCHTHAL & LINDHARD have determined the time interval between the stimulus and the fall in the *F—E* potential, which amounts to 1—1.5 mill. sec., and the interval between the fall of the *F—E* and the *F—F* potentials, which is of the same order.

It has been mentioned several times that frequent stimulation of a fibre may bring it into a state of permanent contraction which, to use an expression borrowed from experiments with whole muscles, has been termed "tetanic contraction" or "tetanus". As already referred to, this condition is, however, labile and can only be maintained for a few seconds at the most. EISENBERGER (1918) states that sometimes it is impossible to keep a fibre tetanized long enough to photograph it in this condition, which may perhaps be due to the fact that he uses fibres in situ and not isolated, which would entail a fairly strong tension on the single, contracted fibre, and therefore presumably, fatigue of earlier onset. Here again there is a contrast between observations made on single fibres and on whole muscles, which is scarcely to be wondered at. As we have said, the so-called single contraction curve is formed through interference of

the curves of the individual fibres; the tetanic curve arises through summation of such interference curves. Therefore it is almost hopeless to attempt to analyse a tetanic curve, whether it be in mechanical or thermal problems. It is unfortunate for the progress of muscle physiology that this highly complicated phenomenon has been characterized as a special "form of contraction".

As is known, a muscle may remain in "tetanic contraction" for several minutes, provided the stimulus is of suitable intensity. When maximal stimuli are employed the curve, after rapidly reaching a maximum, immediately falls to the base-line. It might, therefore, be imagined that it was for similar reasons that the isolated fibre can only remain in tetanic contraction for a brief space of time, since the intensity of the natural stimulus lies far above the threshold value, as has already been mentioned. This fact is, however, undoubtedly only of minor importance here. The main reason why a muscle can remain in tetanic contraction for minutes at a time is that single fibres or groups of fibres alternate during the contraction. That this does actually occur has been shown by Asmussen (1934) in the following way. He dissected out a single fibre so that it was connected only by its nerve with the remaining muscle, both being arranged for isometric contractions. When stimulated by faradic current the whole muscle gave a perfectly smooth tetanic curve, parallel to the base-line. The single fibre, on the other hand, gave an intermittent curve consisting of a series of tetani of short duration (Fig. 47). These experiments are in agreement with those above on isolated fibres and explain why the tetanus is of longer duration in the whole muscle. The single fibre, either because its end-plate becomes fatigued or, what is more probable, because its

excitability is diminished, becomes irresponsive to stimulation for a short period, contracting again after this pause. The experiments also explain why in the case of maximal stimulation the muscle cannot remain long in tetanic contraction; the maximal stimulus acts on all the fibres simultaneously and, therefore, they will very soon all cease to act; there will be no possibility of alternation between different groups of fibres.

It is difficult to ascertain from the literature whether it has been realized that all these so-called contraction forms should properly be termed innervation forms, since the underlying process in the fibre or, more precisely, in the muscle compartment is the same, qualitatively, and in certain cases (indirect stimulation) quantitatively also. A muscle which, indirectly stimulated, can without alteration in length support a given weight, will if the strength of the stimulus is increased, raise the weight and so perform an amount of external work (concentric contraction); if the strength of stimulus diminishes the weight will be lowered and the muscle will perform a negative amount of work (excentric contraction). A loaded fibre stimulated indirectly will be able to contract only for a very short period; but it will not be able to raise or lower the load because the indirectly stimulated fibre has only one degree of contraction. Only if the load can be varied will the fibre be able to perform a positive as well as a negative amount of work. But the directly stimulated fibre, like the whole muscle, has an "infinite" number of degrees of contraction.

It was a long time before a contraction of normal type could be obtained from a muscle dissociated from the central nervous system. When at last it became possible (Grace Briscoe, 1927) it was not because a closer knowledge

of the nature of muscle contraction had been attained, but because it was realized that the question involved was one of innervation.

The Reaction of the Muscle Fibre to the Stimulus.

Although there exists an enormous literature on the reactions of whole muscles, only in a few cases have single fibres been studied. The results of experimental studies on the reactions of whole muscles are even more difficult to interpret than those previously referred to. The painstaking work of the past three decades on muscle chemistry, aiming at establishing a direct relation between chemical and thermal changes, and the mechanical phenomena, has proved disappointing for several reasons. This is due partly to the fact that the chemical changes cannot be limited as to time so as to make it possible to follow directly the different links of the complicated biological processes taking place in rapid succession in the muscle fibre, and moreover we cannot be certain whether the substances found in the dead fibres exist as such in the living state; lastly, the determinations have been performed on a mixture of substances from all the structural elements present, not only in the fibre but in the whole muscle. Therefore, recent investigations of the physico-chemical basis of the contraction mechanism have followed quite other lines than previously, which have been referred to briefly in the chapter on the minute structure of the muscle fibre.

As far as the thermal investigations are concerned, the problem is even more difficult, because the heat change that is measured, and which attemps are made to relate to the known chemical changes, may have no immediate

quantitative connection with the latter. It is true that a successful distinction may be made between contraction heat and recovery heat (of chemical origin), but the highly complex conditions existing in intact muscles during tetanus where we are concerned with the summation of interference curves, do not allow us to relate the various heat changes observed to definite processes in the single fibre; we know nothing at all as to the number of reacting fibres, though we do know that the fibres reacting to a given stimulus do not do so simultaneously. Complications therefore arise when attempts are made to refer the heat changes quantitatively to the mechanical processes. Apart from the fact that the mechanical phenomena dealt with in these experiments can only in part be considered as immediately associated with the process of excitation, there are several facts to be discussed below which will influence the results of the experiments.

Firstly, in most of the experiments hitherto performed, not the tension of the fibre but the tension of the terminal tendons has been measured; for tension of fibre = tension of tendon/cos v, where v is the angle of attachment. Conversely, if we measure the degree of shortening, we find the shortening of the tendon = the shortening of the fibre/cos v. The magnitude of the possible sources of error shown by this calculation cannot be stated in simple terms, but comparison of a resting and a contracted gastrocnemius will show that, at any rate in the case of this muscle, such errors may become fairly considerable.

Secondly, before the final tension is developed in the muscle, certain displacements occur which take an appreciable time and may not be completed during a single twitch. As already mentioned, the muscle comprises several structural elements, which can be displaced in relation to each

other with more or less friction. In any steady state of the muscle there exists a condition of relative equilibrium between the stroma, the active and the inactive fibres. If this equilibrium is disturbed a certain time will elapse before it is re-established, and during this interval it is impossible to obtain any information as to the tension of the fibre. Experiments made by Gasser & Hill (1924) for other purposes may give some idea of the duration of this process of adaption. Any sudden change in the length of a muscle contracting isometrically will entail a momentary positive or negative change in tension passing far beyond the level of tension that corresponds to the new length. It appears justifiable to suppose that the time from the onset of the change of length till the new tension is reached, amounting to $^1/_{10}$—$^1/_5$ second, corresponds to the re-establishment of the equilibrium between the tensions of the structural elements of the muscle, and to their alteration of shape. So long as the disturbance of equilibrium lasts it will, as already mentioned, be impossible to say anything about the tension of the individual fibres and what takes place within them.

There is, however, another source of error, which may occur in cases where the direction of the fibres of the muscle is parallel to the direction of pull of the tendons, and which may result in errors when small bundles or isolated fibres are dealt with, namely that due to the shape of the fibres[1]. Lindhard and Möller (1930) have shown that when a conical fibre contracts isometrically, the thick end of the fibre will become shorter and thicker, whilst the thin end will become longer and thinner. Somewhere in the fibre

[1] This change of shape of the individual fibre should not however be confused with the above displacements between the structural elements of the muscle or bundle (Fischer (1934), v. Muralt (1936)).

there must therefore be a cross section which will remain unaltered during contraction, and whose position can be determined when the shape and dimensions of the fibre are known. The area of this cross section will probably determine the tension which the fibre is able to develop during contraction. If a fibre be imagined having the shape of a blunt cone, the length of which is 20 mm., its diameters being $^1/_{10}$ and $^1/_{60}$ mm. respectively, and which is able to retract to $^1/_3$ of its original equilibrium length, then it may be calculated that the unchanged cross section, which is also the one whose movement is greatest, will be situated 5.8 mm. from the thinner end when the fibre is at rest, and that during isometric contraction it will move 5.6 mm. towards the thicker end. When the change of shape begins there will be the same tension per unit of cross section area everywhere in the fibre, whilst when the change of shape is fully established there will be the same tension on any total cross section of the fibre, irrespective of its area. Thus the thicker end of the fibre will shorten and so perform work, but part of this work will result in increased potential energy in the thinner end of the fibre, which thus performs a negative amount of work. If the whole of the work was transformed into potential energy, the change of shape would not entail any heat formation and would not involve any loss of tension; but that this is not so is illustrated by a simple example.

Imagine two elastic cords of the same material and the same length but with different cross sections, so that their tensions when they are drawn out to twice their length will be in a ratio of 1:2. The two cords are fixed so that their ends will meet when each is stretched to twice its original length. If they are joined together in this position and then

allowed to pull against each other, it will be seen that the thicker cord will shorten and the thinner one will be extended until the tension between the two fixed points is the same everywhere. The increase of tension, x, of the thinner cord will then be determined by the equation $1 + x = 2\ (1 - x)$, or $x = \frac{1}{3}$; at the same time the increase of length (a) in the case of the thinner cord if it is perfectly elastic, will be $a = \frac{1}{3}$. The increase in potential energy of the thinner cord will then be

$$E_1 = \int_0^a (1 + x)\, dx$$

and the loss of energy of the thicker cord

$$E_2 = 2 \int_0^a (1 - x)\, dx$$

which, when $a = \frac{1}{3}$, gives

$$E_1 = \frac{7}{18} \text{ and } E_2 = \frac{10}{18},$$

which again gives the total loss of energy of the system $= \frac{1}{6}$.

Reference may be made to the original paper (1930) for the method of calculation employed. It will suffice, in order to indicate the order of the results found, merely to state that the calculation made in three cases assuming a maximal tension of 3 kg./cm.^{2} gives an average deformation-heat $Q = 0.0109$ cal./cm.^{3}.

It is obvious that these computations of the loss of energy in deformation cannot more than approximately indicate the order of magnitude, as the fibres in situ are unable to move independently of the neighbouring ones, and because Q will vary with the shape and dimensions of the fibres.

But it is evident that the source of error referred to here may under certain circumstances make the result of the myothermal experiments performed in the usual manner untrustworthy since the ratio between tension and development of heat becomes variable.

The muscles of the living animal have partly a static, partly a dynamic function; they must either maintain a steady tension or perform an amount of positive or negative work. As maintainance of a steady tension and the performance of work are essentially different from a mechanical point of view, and as the process taking place in the muscle, or rather the muscle compartments, must be supposed to be the same in all cases, these functions should be considered to arise from an underlying, primarily mechanical change in the muscle, and the change of elasticity previously referred to then naturally suggests itself.

A. FICK first maintained that the contracting muscle was a new elastic body, but knowledge of muscular function was not sufficiently advanced at that time as to make it possible for him to prove his assertion. Later on A. V. HILL, on the basis of his myothermal experiments, maintained the same view, for he claimed to be able to demonstrate that when an isometrically contracting muscle has developed maximal tension, the muscle is able to perform an amount of positive work without further heat production. HILL therefore maintained that the primary mechanical function of the muscle was the development of tension (potential energy) and that the fully developed tension may then either be dissipated as heat or be employed for work, according to the external conditions, without influencing the consumption of energy by the muscle. Therefore HILL defined the mechanical efficiency of the muscle as:

$$\frac{\text{(Potential Energy thrown into an active Muscle by Excitation)}}{\text{(Total Chemical Energy liberated as Heat)}}$$

These ideas agree very well with the conception that, when stimulated, the muscle will assume a shorter length of equilibrium; if fixed at its original length it will thus be able to develop tension, but if allowed to shorten, it will be able to perform some work. Hill's interpretation and valuation of his previous results appear to have undergone some change. In his more recent publications it seems, however, as if Hill regards his original view as incorrect, that the muscle when it shortens and performs an amount of work must develop extra energy in addition to that released by the stimulation (Fenn Effect). The number of phases recognized in the heat production have increased in recent years and their interpretation under different experimental conditions becomes very complicated. It seems to us almost hopeless to attempt such an analysis in the case of tetanic muscular contraction, the complex nature of which we have previously made clear. Hill's conception of the mechanical function of the muscle is, however, untenable as will appear from the following investigations into tensionless contraction.

If a frog's muscle, e. g. a Gastrocnemius, is immersed in Ringer's solution so that it does not support its own weight, and is then submitted to a series of indirect stimuli, the muscle will not develop mechanical energy. The first stimulus will cause it to change its shape, but then, provided the stimulation is of a suitable frequency, it will remain almost completely at its position of equilibrium. It is unable to shorten further, and is thus unable to perform any work, and it cannot develop tension at its length of equilibrium. Thus the stimulus does not bring about develop-

ment of potential energy in the muscle. Only if some external force prevents the muscle from assuming its length of equilibrium will it develop tension. Thus the idea of efficiency as a physiological characteristic of the muscle fibre vanishes. It might perhaps be imagined that a momentary development of mechanical energy took place in the muscle and that such energy would also immediately be dissipated as heat. But this is not so. The contraction without tension involves only a very slight development of heat. A slight heat formation will take place because in practice it is impossible to make the contraction absolutely tensionless. Any muscle has a characteristic shape corresponding to its length of equilibrium when unstimulated. Near the length of equilibrium the shape is determined by the fibres, whereas in greater changes of length the stroma no doubt plays a part both in the case of extension and contraction. Thus it is not unusual to see the perimysium fold during maximal contraction. The single fibre, too, possesses a typical shape, which, apart from internal forces, is determined by the sarcolemma. The usual stage of restitution is also present only to a slight extent after tensionless contraction, if it occurs at all.

Asmussen has measured the difference between the development of heat after a series of isometric tetani and corresponding number of tensionless contractions; he employed for this purpose a thermo-couple consisting of about 250 insulated copper-constantan junctions, half of which were placed in a muscle chamber filled with Ringer's solution but not in direct contact with the muscle, and the other half placed in ice water stirred by means of ice-cold air. A Hartmann-Braun galvanometer was employed as measuring instrument. The result of a series of experiments is repro-

duced in Fig. 53. The method is only an approximate one and can hardly give absolute values of the heat production; it seems, however, sufficient for this purpose, where the object is to differentiate between the heat production in the case of contraction of a loaded muscle and of an unloaded muscle, that is, between isometric contraction and tensionless contraction. Asmussen's results correspond well with the heat measurements made on muscles under varying loads by McKeen Cattell (1932). Since, however, the theory and principle of tensionless contraction has not been

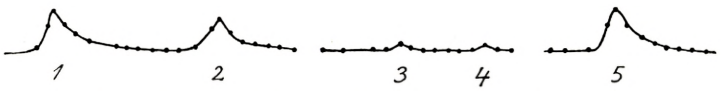


Fig. 53. Total heat production of a frog's muscle. Arbitrary units. 1 and 2 isometric tetanus. 3 and 4 tensionless contraction. 5 as 1 and 2.

previously fully discussed, and as it plays a decisive part in the theoretical interpretation of the contraction phenomena, we considered it desirable to test Asmussen's experimental results by other methods. With this end in view A. Topsøe-Jensen has made a series of determinations of the oxygen consumption of the muscle under isometric tetanus and under tensionless contraction. The experiments were made with small frog's Sartorii (weight 35—65 mgm.) by means of Krogh's micro-respiration apparatus (1914), the principle of which is well known. The muscle was placed in a chamber consisting of a glass-tube (volume about 7 cm.^{3}), one end of which was closed and which was provided with a side tube with a glass stopper, into which a couple of platinum electrodes were sealed. Beneath the latter, also attached to the glass stopper, was a small celluloid pot in which the muscle was placed in Ringer's solution saturated with oxygen. By means of two small hooks the muscle could be

fixed at its length of equilibrium when the nerve was placed across the platinum electrodes. The CO_2-absorbent (5 per cent. KOH) was spread on filter-paper covering the interior of the muscle chamber. During the experiment the muscle chamber and the control container were placed in a water-bath at room temperature. In order to avoid changes due to temperature variations, the manometer was read by means of a kathetometer, and by blank experiments it was ascertained that the stimulus alone did not influence the manometer. As it appeared that the manipulations required in making the preparation resulted in an increased and highly fluctuating basal (resting) metabolism, the mounted preparation was placed for 15—30 minutes before the experiment in oxygenated Ringer's solution. The muscle was then first stimulated without tension for 15 sec. with a maximal tetanizing current and the manometer read every 10 minutes for 30—40 minutes. The muscle was now secured isometrically and the whole experiment repeated precisely as in the case of contraction without tension. The readings of the manometer were constant in all cases after the cessation of the experiment. The results are given in Table VIII.

As will be seen, the results of these experiments agree approximately with those of ASMUSSEN'S. In the experiments of the 6th and the 13th the increase of the O_2-absorption in tensionless tetanus is strikingly large; it should, however, be borne in mind that errors of this magnitude are likely to occur, if the arrangement of the muscle is not quite successful, if for instance there is too little fluid on which the muscle may float, or if it adheres to the container and so on, though errors in the other direction can hardly occur. The lowest figures of the column must therefore be considered the correct ones. The increase of the oxygen absorption, in

Table VIII.

Date	O_2-consumption in mm.^{3} per gm./min., determined in 10 min.-periods				Total rise of O_2-consumption per gm. of muscle in 30 min.	
	Rest	Tensionless Tetanus for 15 sec.	Rest	Isometric Tetanus for 15 sec.	Tensionless Tetanus	Isometric Tetanus
6/x	1.53	1.73	1.53	3.57	5.03	31.6
8/x	1.70	1.80	1.70	3.30	1.01	30.0
9/x	1.28	1.44	1.28	3.08	1.59	27.0
12/x	1.02	1.23	1.02	2.25	2.04	26.6
13/x	1.36	1.64	1.36	2.73	4.09	19.1
14/x	1.04	1.25	1.04	2.40	2.08	26.0
15/x	1.68	1.96	1.68	2.66	2.80	15.9

the course of isometric contraction must, of course, vary according to the size and condition of the muscle. It will moreover be noticed that whilst the increase of metabolism after tensionless tetanus has ceased at the end of 10 minutes in all the experiments except the two specially referred to above, it takes much longer however after isometric tetanus.

From the results of the two preceding series of experiments, showing that the metabolism of the muscle is only slightly influenced by the tensionless contractions, it must be supposed that such contractions would less easily give rise to fatigue than contractions during which the muscle develops mechanical energy. This proves to be so. If, for instance, two gastrocnemii of the same frog are placed in a myograph and one of them is stimulated a certain number of times without loading, and then both muscles are loaded and stimulated simultaneously to fatigue, fatigue occurs practically at the same time in either case, as is shown by Table IX (unpublished experiments by A. Topsøe-Jensen).

For purposes of comparison it should be mentioned

Table IX.

Date	Shortening in arbitrary Units			Tension-less Contract-ions	Contractions under Loading		Diff. b — a	Decreased Working Capacity $\frac{(b-a)\cdot 100}{b}$
	Beginning		End					
	a	b	a and b	a	a	b		
4.12.36	40	40	3	180	500	620	120	19 Gastrocn.
14.12.36	39	44	3	300	480	580	100	17 —
18.12.36	38	38	3	360	455	470	15	3 —
19.12.36	38	38	3	420	485	495	10	2 —
23.12.36	46	47	3	180	400	500	100	20 Semitend.
23.12.36	44	43	3	240	515	505	—10	—2 —

that in 16 double experiments with symmetrical muscles which were stimulated to fatigue in the same manner as before, but without preceding tensionless contractions, an average difference between the number of contractions of 3.6 per cent. was found, with an average error of 54 per cent.

A closer examination of the table will show that at the beginning of the experiment the two muscles gave the same height of contraction; only in the experiment of Dec. 14th has the control muscle an appreciably greater height of contraction than the (a)-muscle. This experiment is therefore of less value, because we do not know whether the difference in the last column is due to fatigue of the (a)-muscle owing to the tensionless contractions, or whether it is due to different development of the two muscles. The experiments are stated chronologically and it therefore appears as though the highest values in the last column might be due to lack of experience in the experimental technique. The number of tensionless contractions preceding the fatigue experiments proper is apparently of no importance. In the experiment of Dec. 19th, in which the number of tensionless contractions almost equals the number of loaded contractions, required to bring

both muscles to the limit of absolute fatigue, the effect of these contractions is scarcely demonstrable. The result of these experiments is thus directly opposed to investigations by Riesser & Schneider (1929), who maintain that the time of onset of the fatigue is independent of the loading of the muscle.

The general impression of the three series of experiments referred to is that tensionless contractions are without appreciable effect on the muscle, which in turn implies that in the course of the tensionless contraction no irreversible processes take place to any appreciable extent. Therefore it must be supposed that the energy liberated by the stimulus forms part of a cyclic process, the last phase of which is the re-establishment of the excitation—and contraction potentials. This energy moreover must be supposed to be equal to the work which the fibre would be able to perform, or rather the potential energy it would be able to develop, if it was fixed at its original length of equilibrium at the moment when it changes from one elastic condition to the other, and which must be of the same magnitude as the electrostatic forces keeping the molecular chains of the myosin micellae (see below) extended in the "state of rest". The tensionless contraction, which is the direct result of the stimulation when the muscle is uninfluenced by external forces, should then chiefly take the course of a series of reversible processes. As a picture of the mechanism of the energy output we may imagine a pendulum that is supported above its lowest position. It will then possess potential energy. If the pendulum is released its potential energy will disappear but simultaneously it will obtain an equivalent amount of kinetic energy which reaches its maximum at the lowest position of the pendulum and disappears again

under the development of potential energy as the pendulum moves on to the end of its swing in the opposite direction. But if external resistance stops the movement the kinetic energy will be transformed into heat. This analogy may be extended to the muscle fibre. If, as a consequence of the change of elasticity, the latter performs work or develops potential energy, which is dissipated irreversibly into heat, the regeneration of the above-named potentials will require the liberation of an amount of energy equal to that which the fibre has lost in the shape of work or heat. As the tensionless contraction must no doubt be considered very rare under natural conditions, we generally find a relatively long-continued stage of recovery after the contraction, during which a number of energy-producing processes occur, which besides yielding the energy required for the regeneration of the contraction potential also dissipate heat; of these processes the essential one seems to be the oxidative destruction of carbohydrate. v. MURALT's (1935) statement that "Das Funktionsziel der Muskulatur ist die Bereitstellung mechanisch verwertbarer Energie aus chemischen Energiereserven", and that "Das Endglied des Energieumsatzes ist primär immer 'die Bereitstellung', denn der eigentliche Umsatz vollzieht sich zunächst so, dass potentielle, mechanische Energie aus chemischen Energiereserven entsteht", would appear to be theoretically untenable. It is correct that the primary function of the muscle is "Bereitstellung mechanisch verwertbarer Energie"; but this "Bereitstellung" is expressed by the change of equilibrium length, the only mechanical process taking place in the muscle fibre as a direct consequence of stimulation. All other mechanical phenomena, work, development of tension etc., are secondary, a consequence of the interference of external forces.

If, on the basis of the investigations into tensionless contraction, the histology of muscle, its minute structure, and into the electrical phenomena of the fibre, (supported especially by H. H. Weber's, Kurt H. Meyer's and our own experiments), we try to form a picture of the internal condition of the "resting" and the "contracted" fibre, to establish a contraction hypotheses, we arrive at the following result:—

The rod-shaped formations in the *A*-segments of the muscle fibrils consist of myosin micellae composed of long, flexible but firmly connected molecular chains, which in a state of equilibrium are completely deranged, coiled or collapsed. K. H. Meyer attaches great importance to the fact that the individual links of the molecular chains are connected by means of very firm valencies; if these are lacking the orientated atoms and molecules may become disorientated through a rotation that does not change the shape of the object, as is the case with dipoles orientated in a field when the activity of the field ceases. In the unstimulated resting muscle fibre these molecular chains are, however, not in their state of equilibrium, but are more or less straightened in an electric field. They cannot be completely straightened, for, if so, the *A*-segment of the fibre would behave very much like a tendon, i. e. it would be near its limit of elasticity and, therefore, unable to withstand further stretching without the occurrence of irreversible structural changes; the micellae cannot be completely coiled either, for the *A*-segments would then be unable to shorten. K. H. Meyer does not take the electrostatic forces in the fibre into account and assumes that when a muscle is at its state of elastic equilibrium the contractile elements, the myosin micellae, must be in a similar condition. He maintains that the molecular chains that are stretched in the case

of passive extension will coil up when the latter ceases, whereas in fact though the stretched molecular chains do become folded to some extent, they do not completely reach the coiled-up state of equilibrium which they would assume if left alone.

When the muscle fibre is stimulated a sudden large change in the excitation potential (i. e. the potential difference between end-plate and fibre substance) is seen, as referred to above, which is followed by a fall in the fibre potential; and thus a sudden, marked decrease might occur of the electric field keeping the molecular chains of the myosin micellae stretched, so causing them to "collapse". The completely coiled-up or collapsed state of the molecular chains is, however, unstable, as the electric potentials are soon re-established. This, as we have seen, causes the molecular chains to become more or less straightened again. According to the investigations into the minute structure of the myosin previously referred to, compared with similar work on rubber and elastic connective tissue, it must be supposed that when the molecular chains are straightened to the state of longitudinal orientation a decrease of the entropy of the system will occur, whilst collapse is accompanied by an increase of the entropy. Therefore it is conceivable that the work required for the re-establishment of the electric field originates from the increase of entropy. The condition of the myosin micellae in the "resting" fibre is thus determined by an interaction between elastic and electrostatic forces in the fibre, and corresponds to the condition of the molecular chains in the passively extended rubber cord. The stability of the condition depends on the stability of the electric field. On the other hand the maximally contracted muscle fibre corresponds to the resting rubber

cord, the molecular chains of which are completely coiled up; but in the case of the muscle fibre this condition is unstable owing to the rapid re-establishment of the electric field.

It is not known what substances determine the presence of the electric field. There is, however, reason to believe that somewhere at a boundary surface in the muscle compartment an ion production and exchange takes place. The strength of the field may vary, partly as the result of the rate at which the ions are produced, partly owing to variations in the permeability of the "membrane" itself, and partly owing to variations in the rate of the process by which the ions are removed. It is known that the strength of the field may remain practically constant for 60—70 minutes; but it is probably not always of the same intensity; on the contrary no doubt like all other natural phenomena it varies under changing conditions. But for the time being, we can only advance hypotheses on this point.

The mechanism of contraction outlined above applies to tensionless contraction. If external forces prevent or hinder the collapse of the myosin micellae the muscle fibre will develop tension, also termed potential mechanical energy, and this development of energy will continue till the field strength, decreased as a consequence of the stimulus, has regained its former intensity. In this case the regeneration of the electric field must take place by means of other forces than in the case first considered, the reversible process now being transformed into an irreversible one, which entails the occurrence of oxidation processes in the fibre; but the mechanism involved is at present unknown. These processes, whose purpose must be the regeneration of the potentials of stimulation and contraction, are not reversible

but entail a loss of energy to the fibre, which is manifest as dissipated heat corresponding to the mechanical energy developed.

Tension will arise in a muscle when the contractile elements are prevented from assuming their length of equilibrium, and only in that manner. In the living organism the muscles are usually extended beyond their length of equilibrium and, therefore, they possess tension; this tension is termed muscle tonus. The tonus may vary either as a consequence of external forces causing changes in length of the muscle, or more especially owing to variations of intensity of the electric field in the muscle fibre produced by stimulation, and finally owing to variations in the intensity of the field of the unstimulated fibre. The last-named possibility is for the time being purely hypothetical, for variations in muscle tonus can be explained on the basis of the somatic innervation alone.

If, on the other hand, the individual fibre is considered, it has, as referred to above, only one degree of contraction in indirect stimulation and therefore its tonus cannot be graded as a consequence of somatic innervation, less so because this innervation can produce changes in one direction only. If the tonus of the individual fibre could be graded and vary in either direction these variations might be due to changes of intensity of the electric field, which controls the length of the myosin micellae and thus the length of equilibrium of the unstimulated fibre. Variation of the field not caused by the somatic innervation has not been demonstrated directly but must be considered probable; and the extent of such variation may become very large, covering the whole scale from completely coiled-up to perfectly straight myosin micellae. This aspect of the mechanism

of contraction will not, however, be dealt with in detail here, since there is insufficient experimental work to substantiate it thoroughly.

In this connection mention may be made of the considerable number of experiments and arguments on the basis of which it has been maintained that muscular tonus is regulated through the autonomic nervous system; whilst according to another view the tonus is regulated exclusively by means of the somatic innervation. The latter has been demonstrated in resting muscles. Neither of these two attempts of an explanation is, however, complete, but on the basis of the hypothesis of contraction advanced above it is possible to combine the two viewpoints so as to enable us to explain the existing experimental results if it is supposed that the equilibrium length of the unstimulated fibre is regulated through autonomic nerves, whilst it is the somatic innervation which determines the tension superimposed on this background. For the time being such a hypothesis lacks experimental support.

If the working hypothesis outlined here is to be actually applied to the process of muscular contraction it is, of course, necessary that the electrostatic forces existing in the muscle compartment are of the order necessary to keep the myosin micellae extended to the degree they possess in the *A*-substance of the resting, unstimulated fibre. As the latter may be stretched on extension and resume its original length by relaxation, the molecular chains cannot be completely straight when the fibre is at rest, as is the case for example in a tendon; complete straightening must be supposed to correspond to a degree of extension of about 1.4 × the resting length, or to about 50 per cent. elongation of the *A*-substance. Further extension gives rise to irreversible

changes in the structure of the fibre, as mentioned above. On the other hand we may expect the maximal degree of shortening of the *A*-substance to be to about $^1/_4$ of its resting length. As the electrostatic forces correspond only to the degree of contraction of the muscle fibre at its resting equilibrium length and not to the maximal contraction of the muscle, an idea of the relations of mechanical and electrical energy can be arrived at, if we examine the tension-length curves previously referred to. Before dealing with this question we shall, however, see what can be learned about the electrical forces by examination of the electrostatic conditions of the fibre.

The electrostatic forces acting on the contractile elements of the muscle fibre play a prominent part in excitation and contraction processes; but direct measurement of these is so untrustworthy at present that we must be content with relative values of their sizes in different parts of the fibre. Estimation of the order of magnitude of the electrostatic energy of the system involves not only a knowledge of the potentials, but also determination of the resistance and capacity existing in the fibre.

Considerable changes in experimental technique resulted from the realization that in the understanding of electrical phenomena in muscle fibres, the electrostatic potentials were at least as important as the currents arising from them. The electrostatic methods of measurement in this field have been developed by BUCHTHAL, who has stated their principles and limitations, and has examined the sources of error which may arise under different conditions. There is no need, however, to follow this development in detail here; if required, reference may be made to the published accounts, but a brief description will be given of the apparatus

which at present is considered suitable for this work and of the precautions that must be taken if reliable results are to be obtained by its use.

With the introduction of amplifying valves into electrophysiology it became possible to detect far smaller potentials and potential changes than previous methods allowed. By combination of the amplifying valve with the oscillograph as a recording instrument, more rapid changes in potential could be followed and their correct time-relations preserved. For an account of the comprehensive literature on the subject reference may be made to Buchthal & Nielsen (1936).

Examination of the electric phenomena in the individual cell makes great demands on the sensitivity of the measuring instrument, but moreover other precautions are required which can be ignored in experiments on nerves and whole muscles. Thus, when single cells are used, care must be taken to prevent any appreciable flow of current in the tissue, or to the measuring instruments employed. In order to determine the potentials actually existing, care must be taken that the measuring instrument has a resistance that is very large in proportion to the preparation, and since this and the leading-off electrodes have an internal resistance of several million ohms, the amplifier must have an input resistance of at least 100 meg. ohm, if we are to be certain not to take any appreciable current from the preparation.

The apparatus employed must be able to reproduce exactly not only potential differences of resting systems (e. g. the resting potential of the muscle fibre) but also rapid and slow changes of potential. All these requirements can be fulfilled only by the use of a direct current amplifier. As recording instrument either a mirror oscillograph can be employed, or—as we have done—a cathode ray oscillograph.

Owing to its high input resistance this instrument is especially suited for use in conjunction with valve amplifiers; moreover it possesses negligible inertia and is not damaged by overcharging, a property that makes it especially well-suited for measurements on tissues stimulated electrically.

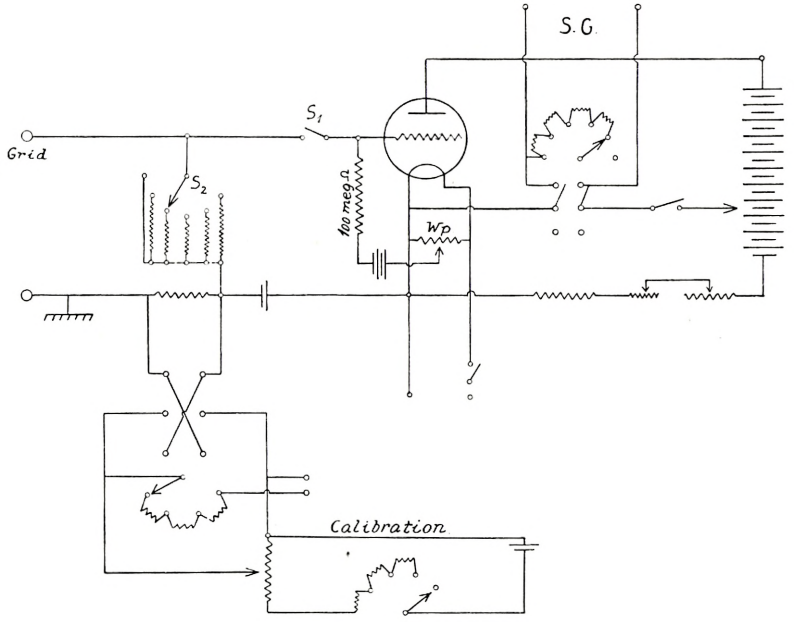


Fig. 54. Electrostatic electron tube voltmeter. Wp = Compensation of the constant component of grid current. S_1 = Control of grid current compensation. S_2 = Variable shunt resistance to the preparation, 1 meg. Ω —100 meg. Ω. *S. G.* = String galvanometer. (BUCHTHAL).

In their first experiments BUCHTHAL & PÉTERFI (1934) worked with a Binant electrometer; and in recent experiments, in order to follow more rapid potential changes, BUCHTHAL has also employed an electrostatic valve-voltmeter in connection with a string-galvanometer for recording. In this arrangement (Fig. 54) the disturbing effect of the grid current was eliminated. The grid current of the input

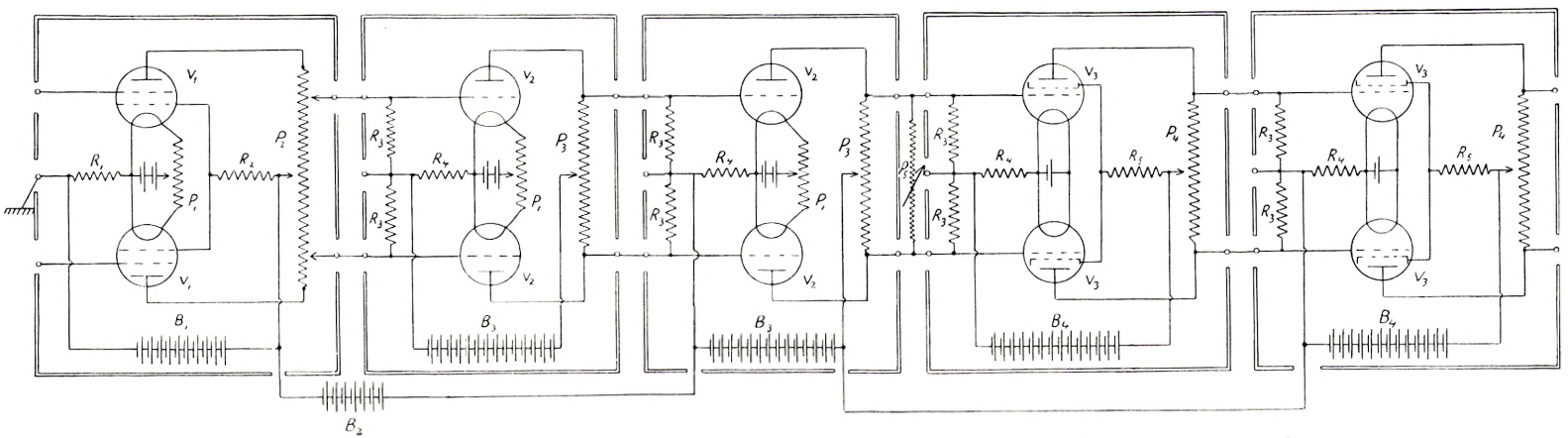


Fig. 55. Electrostatic balanced D. C. amplifier. (BUCHTHAL & NIELSEN).

$R_1 = 1000\ \Omega.$
$R_2 = 3000\ \Omega.$
$R_3 = 2$ Meg. $\Omega.$
$R_4 = 500\ \Omega.$
$R_5 = 0.2$ Meg. $\Omega.$

$P_1 = 25\ \Omega.$
$P_2 = 40000 + 1000 + 40000\ \Omega.$
$P_3 = 100000 + 10000 + 100000\ \Omega.$
$P_4 = 200000 + 50000 + 200000\ \Omega.$
$P_5 = 5000\ \Omega - 1$ Meg. $\Omega.$

$V_1 =$ Osram T 113.
$V_2 =$ Telefunken RE 034.
$V_3 =$ Philips KF 1.

$B_1 = 20$ Volt.
$B_2 = 70$ Volt.
$B_3 = 120$ Volt.
$B_4 = 180$ Volt.

valve makes it impossible to determine the actual rise of potential differences and may cause changes in the resistance of the preparation to be mistaken for potential changes. By employing a special valve with a carefully isolated grid coil it was possible to eliminate the inconstant compo-

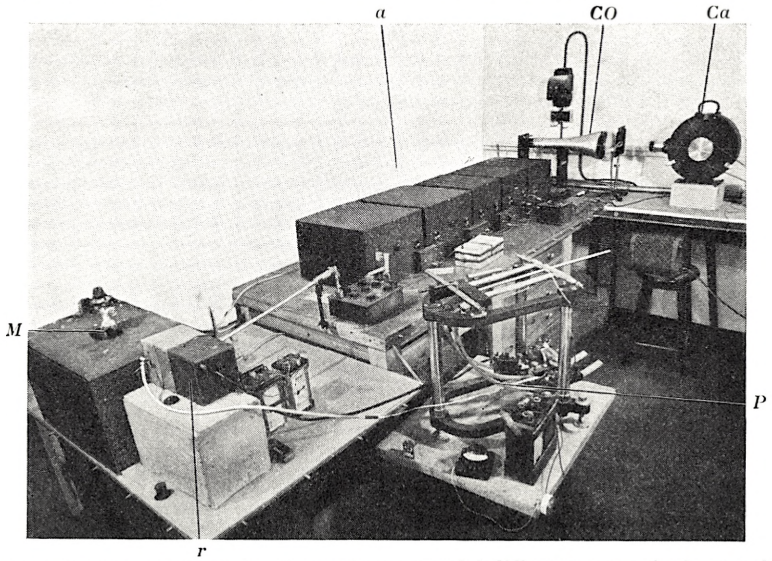


Fig. 56. Arrangement for measuring potential differences on single muscle fibres. *M* = microscope, micromanipulator with micro-electrodes and preparation. *r* = relay to prevent stimulus escape. *P* = Helmholtz-pendulum. *a* = D. C. amplifier. *CO* = Cathode ray oscillograph. *Ca* = camera.

nent of the grid current and to compensate the relatively constant ion-current to such an extent as to make measurements without current-flow possible. Compared with the "electrometer" valves usually employed, this arrangement has the advantage that it gives greater amplification, so that in many cases such an arrangement with a string galvanometer as recording instrument will be adequate. The use of the string galvanometer will, however, cause the period of inertia to become comparatively long. Therefore, in order

to record very rapid potential changes e. g., change of the excitation potential and its time relations with the contraction potential, it was necessary to build a balanced direct current amplifier with electrostatic input (Fig. 55 and 56). The variations in voltage of the batteries was reduced to a minimum by balancing of the stages individually and also with respect to each other (negative direct current feed-back). This arrangement proved to be extremely stable both to sudden and to slow variations of voltage in the current.

After "warming up" the displacement of the base line was only 10 mm. (= 100 microvolt) in the course of three hours. Such stability is necessary in many biological measurements because we are often forced to work near the limits of the amplifying arrangement. The theoretical limit of potential measurements in a conducting system is where the potential changes which result from the heat-motion of the electrons become of the same order of magnitude as the potential differences which it is desired to measure. The size of these disturbing voltages depends on the temperature and the resistance of the conducting elements, whilst the disturbances in the output circuit of the amplifier depend on the degree of amplification and the range of frequency response of the arrangement. The latter disturbances can be calculated according to the formula

$$V = 2\sqrt{K \cdot T} \cdot \sqrt{R \cdot F} \cdot G$$

where V denotes the magnitude of the disturbing voltage at the output terminals of the amplifier, K Boltzmann's gas constant ($1.37 \cdot 10^{-23}$ Ws per degree of absolute temperature), T the absolute temperature, R the resistance of the object, F the range of frequency covered by the measuring arrangement, and G the degree of amplification.

In the case of a frequency range for instance of 10,000 cycles and a resistance of the object of 2 meg. ohm, the active "disturbance-level" will become 18 microvolts. The frequency response curve of the amplification arrangement employed by us will appear from Fig. 57. It will be seen from the figure that the degree of amplification is constant up to frequencies of 10,000 cycles. Knowledge of the theo-

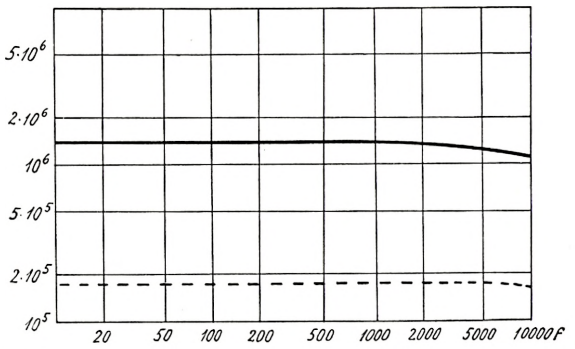


Fig. 57. Measured amplification for different frequencies. Upper curve 5 stage amplification. Lower curve 3 stage amplification. Ordinate: amplification in volts. Abscissa: frequency cycles per sec. (Buchthal & Nielsen).

retical and practical limitations of the method employed is, however, not sufficient to safeguard against error in biological work; it is constantly necessary by means of control experiments to safeguard against potential changes occurring from various sources of error such as injury to the preparation, mechanical displacement of the electrodes, disturbances of capacity, "stimulus escape" and so on as referred to in detail in the various papers.

Finally, attention should be called to the fact that the potentials that are measured even under the best possible conditions are only summation phenomena originating, among other things, from diffusion potentials, absorption

potentials, oxido-reduction potentials which at present cannot be analyzed, but which may be considered as indicators of what is taking place in the cell. Such potentials may well be important factors in biological processes. The difficulty of measurement and of reproducing the experimental conditions of experiments, when whole muscles are used, has often resulted in the formation of poorly supported hypotheses, because it has been impossible to establish the real connection between the measurements and the structural and functional properties of the tissue and this has caused more cautious observers even to question the existence of potential differences in resting systems, which however is now beyond doubt. The potential differences observed must, however, as already mentioned, be considered a reflection of a "dynamic equilibrium" between formation and removal of ions.

In the first publication on the electrostatics of the muscle fibre Buchthal & Péterfi (1934), used frog's muscles as experimental objects, especially the broad abdominal muscles, which were examined mostly in situ, the circulation being intact, sometimes in isolated preparations of thin muscle plates. It proved to be of considerable importance that the muscle fibre should be actually exposed; if the current was led off to the electrometer through the peritoneum or other connective tissue, the fibre potentials obtained were smaller than if leading off took place from the sarcolemma. On the muscles of the extremities the potential differences could not be demonstrated at all if the electrodes were placed on the fascia of the muscle. The sarcolemma, too, has insulating properties. The potentials measured become far less when the electrodes are placed on the sarcolemma than if they are inserted into the fibre substance itself

(Fig. 58). It appeared, however, that the potential differences measured through the sarcolemma were of the same type as those obtained by leading off from the fibre substance itself; they gave a quantitatively diminished but qualitatively correct expression of the resting potential of the fibre. It became preferable in later work to use the relative (epilemmal) potentials because the unavoidable injury to the fibre in the case of sublemmal measurements entails considerable uncertainty, as unless the injury to the tissue is quite the same at both electrodes, unbalanced injury potentials will be produced. As moreover the insertion of the electrodes into the fibre substance brings about a microscopically demonstrable disturbance of the cross-striation, it will be impossible to obtain normal values, even though an average figure might be arrived at by increasing the number of experiments performed. However, the potentials determined will always be too small.

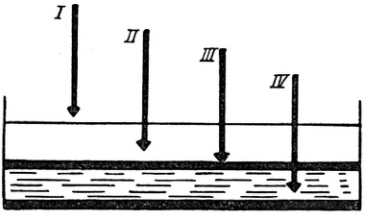


Fig. 58. Different positions of electrodes. I. Above the preparation. II. In Ringer solution. III. On the sarcolemma. IV. Inside the fibre. (BUCHTHAL & PÉTERFI).

The principal result of the experiments was that when both electrodes were placed on the surface of the same fibre, a potential difference was found which, in contradistinction to that found in similar examinations of other organs, varied in a fairly regular manner with the mutual distance of the electrodes. The potential increases with the distance between the electrodes to a maximum (Fig. 59), the latter presumably implying that the electrode moved has either passed the place where the fibre ends, or has slipped on to another fibre. Owing to the difficulties which may be associ-

ated with the following of a single fibre in situ, the distance between the electrodes should not be greater than about 500 μ. Fairly large individual variations are, however, found in different fibres. Fig. 59 and Table X give some examples of the experiments.

The first column of the table shows the distance between

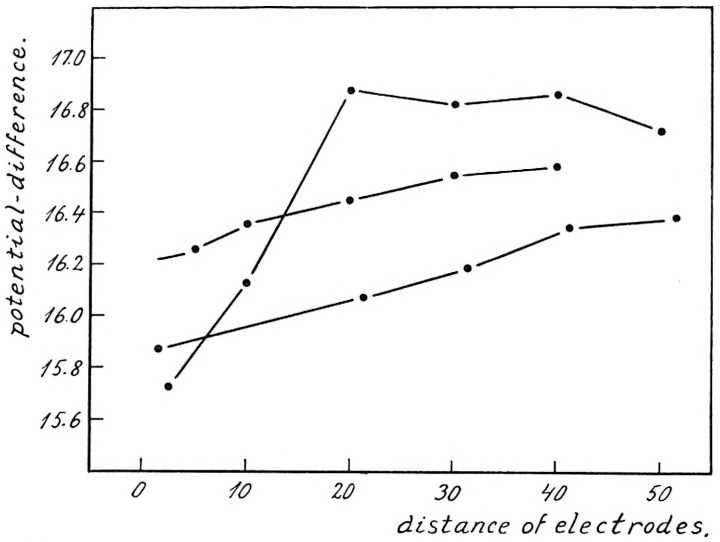


Fig. 59. Potential differences in the frog's muscle fibre with electrodes different distances apart. (Buchthal & Péterfi).

the electrodes, the figures representing units of 100—150 μ, 0 indicating that both electrodes are in Ringer's solution. The next 9 columns show the variation of the potential with the distance between the electrodes. The direction of the variation depends on whether the earthed or the non-earthed electrode has been moved. The figures given represent scale divisions, each division corresponding to 0.5—1 mV. For purposes of comparison it may be stated that the average of 16 sublemmal measurements is 45 $\pm$ 8.4 mV, corresponding to a distance between the electrodes

Table X.

Dependence of the Resting Potential on the Distance between the Electrodes.

Distance between Electrodes	Experiments								
	I	II	III	IV	V	VI	VII	VIII	IX
0	16.00	..	16.00	16.15	14.20	15.72	16.90	16.00	..
1	15.98	16.10	17.00	16.35	14.70	16.73	16.70	17.90	16.00
2	15.90	15.97	17.20	17.16	16.30	16.80	16.50	17.95	17.90
3	15.85	15.65	17.30	..	16.55	16.82	16.65	16.55	17.95
4	15.80	15.45	16.10	..	..	16.87	17.65	15.93	16.55
5	15.60	15.50	..	..	..	16.72	18.10	..	15.93

of 100 μ, which shows that these measurements are subject to considerable errors, as already stated. When the fibre looses its excitability the fibre potential will disappear; in dead fibres no definite potential differences are found, no matter what the distance between the electrodes. In muscles poisoned by chloroform the potential will decrease gradually as the intensity of narcotisation increases. As previously mentioned, it will also disappear when influenced by radium, whilst it remains uninfluenced by curare.

It was to be expected that potential differences would be found between the different structural elements, for example, between the sarcolemma and the contractile substance or between the nuclei and the sarcoplasm of the muscular cell, but such potential differences do not change with the distance between the electrodes. This variation of the fibre potential cannot be due to movements of the electrodes as shown by control experiments, nor can it be explained by conduction through the surroundings, since it is the same whether the preparation is covered with Ringer's solution or with paraffin. Therefore it must be considered

that the variation of the potential difference with the distance between the electrodes is associated with the segmental structure of the fibre. This view is further corroborated by the fact that when the electrodes are placed on unstriated muscle cells a potential difference may be demonstrated, but it does not vary with the mutual distance between the electrodes. For this reason we must believe that the individual muscle compartments are electrically isolated from each other, and that the boundary may perhaps be Krause's membrane. It is this membrane which determines the cross-striation; if it is disturbed, the cross-striation is also disturbed; in that case the excitability of the fibre will cease and the resting potential will disappear. Thus it plays an all-important part in the life and function of the fibre, but apart from the above, the underlying histological structure and its histo-chemical, or physico-chemical properties are unknown. On stimulation, either the rapid change of the excitation potential or the direct application of an electric current, results in a change of the fibre potential which is re-formed in the following recovery period. Buchthal & Péterfi showed that during contraction the fibre potential was diminished by 2—5 millivolt (Fig. 60) and that it was only slowly re-established after cessation of contraction; but with the apparatus then employed the authors were unable to follow the change itself at the moment of stimulation. Buchthal later succeeded in doing so (1934). It was first ascertained that the fibre potentials and their fluctuations could be measured directly, or that the resting potential

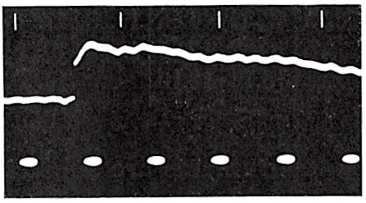


Fig. 60. Frog's muscle fibre directly stimulated. Decrease of the *F*—*F* potential Time marker 20 msec. (Buchthal).

could be compensated and then possible fluctuations of it recorded. According to the position of the electrodes on the fibre and the distance between them, the resting potential is from 4 to 10 mV, and decreases when the circulation in the muscle ceases, e. g. in decapitated frogs in which the spinal cord has been destroyed. As in such cases the circulation will cease first in the abdominal muscles, these will at a certain stage have a lower potential than the muscles of the limbs. The resting potential is also diminished in poisoning with cyanide and on continued stimulation. When the fibre is stimulated the resting potential will suddenly fall 1—3 mV, usually returning to its original value in 0.5—2 sec.

Sometimes the regeneration may, however, take much longer. The time course of the potential change shows that it has nothing to do directly with the excitation-potential; the latter change may sometimes be observed as a slight irregularity of the first abrupt part of the contraction potential. The combined curves obtained by simultaneous recording of both changes have previously been referred to.

In order to obtain further information as to the nature of the fibre potential, Buchthal & Lindhard (1936) examined the potential changes occurring with variations of temperature. The potential was measured and recorded as already described. The preparation was placed on a vulcanite capsule resting on a very thin cover-glass (Fig. 61). The capsule was provided with two short, vulcanite tubes by means of which water was led through the interior of the capsule from a Mariotte's flask. There were three of these, one containing water at 5—10° C., the second water at 12—18° C., and the third water at 23—28° C. As it was found that the temperatures inside the capsule, and of the preparation, might differ to a considerable extent from

each other, a thermo-couple consisting of a carefully insulated copper-constantan junction was placed on the preparation, in the immediate neighbourhood of the micro-electrodes; the other junction was placed in a Dewar-flask

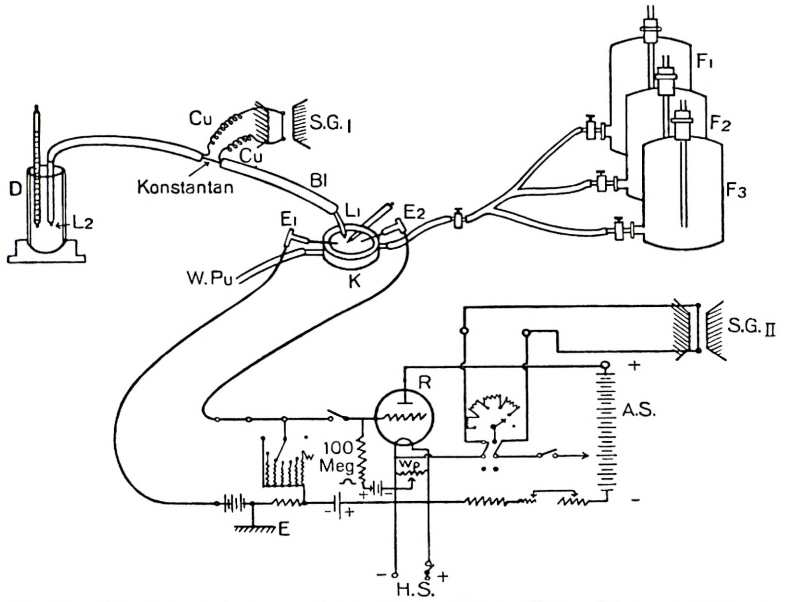


Fig. 61. Arrangement for measurement of the effect of temperature on the potential differences.

F_1, F_2, F_3 = Reservoire with water at different temperature. K = Capsule with electrodes E_1 and E_2 and thermo-couple L_1 to measure temperature of the preparation. SG_1 = String galvanometer to record thermo-currents. D = Dewar flask containing junction L_2. SG_2 = String galvanometer registering potential differences. R = Special electron tube with high insulation of grid. AS = Anode volts. HS = Heating current. (Buchthal & Lindhard).

containing liquid paraffin, the temperature of which could be kept constant within 0.01° C. In most of the experiments the temperature variations were recorded from one string of a double-string galvanometer, the other being used for recording the potentials. The arrangement was calibrated for each experiment. A difference of temperature of 1° C.,

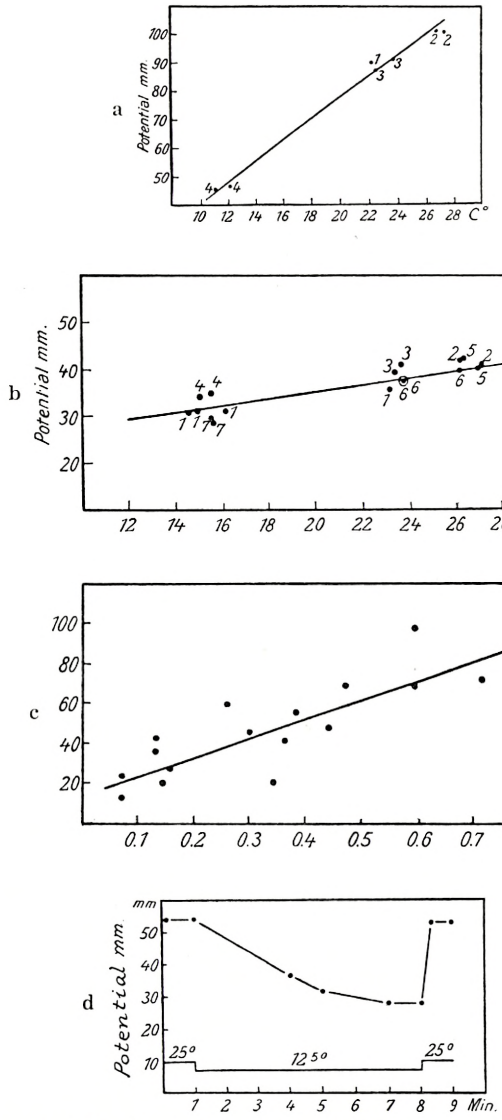


Fig. 62. a. and b. Fibre potential at different temperatures. Numbers indicate sequence of measurements. Ordinate: potential in mm. Abscissa: Temperature. c. Potential level and slope of potential/temperature curves. Ordinate: potential level. Abscissa: slope. d. Rate of adaption of potential with decrease and increase of temperature.
(Buchthal & Lindhard).

gave a deflection of 2—3 mm. on the string galvanometer. The whole of the experimental arrangement functioned satisfactorily in the tests performed; the greatest difficulty appeared to be that of obtaining absolute tightness between cover-glass and vulcanite capsule, but it was managed to secure this by leading the water through the capsule at a slight negative pressure. The slightest leakage, however, was shown at once by great disturbances of the potentials. The readings were not taken until both the thermo-element and the thermometer in the capsule had attained to constant values.

The result of this examination was that within the range examined the fibre potential rose and fell with the temperature (Figs. 62 a & b), provided this did not reach a level higher than 26—28° C. At temperatures as high as this, irreversible changes of the fibre usually occurred, so that only very rarely did the potential fall again with decreasing temperature. Otherwise these potential variations were reversible and reproduceable. It was, however, difficult to find a formula covering all the experimental results. Calculation of the temperature coefficient according to the formula

$$Q_{10} = \left(\frac{p_1}{p_2}\right)^{\frac{10}{t_1 - t_2}}$$

where p denotes the potential difference and t the temperature, gave as a mean figure 1.4 ± 0.03 with an average error for a single determination of 22 per cent. A systematic variation of Q_{10} with temperature could not be demonstrated. Calculation of b according to Bělehrádek's (1935) formula,

$$b = \frac{\log p_1 - \log p_2}{\log (t_1 - \alpha) - \log (t_2 - \alpha)}$$

where α is the difference between the "biological zero" and the zero of the thermometric scale, gave $b = 1.08 \pm 0.05$ with an average error of 45 per cent. Lastly, a comparison of the percental variation of temperature with the percental alteration of the potentials did not suggest any connection between the results. The difficulty of finding a general formula for the results of the experiments is probably due to the fact that these experiments cannot be considered comparable in simple terms, for the slopes of the potential-temperature curves were different for each experiment (Figs. 62 a & b) proportional to the level of the potential, a more abrupt temperature curve corresponding to a higher initial value of the potential level. Fig. 62 c shows the relation between the level of the potential and the value for the slope of the temperature curve. The cause of this inter-dependence is unknown.

The potential change on cooling was fairly slow, taking place in about 6 minutes, in the case of a fall in temperature from 25 to 12.5° C., whilst a subsequent increase to 25° C., resulted in increase of the potential to its former level in the course of a few seconds (Fig. 62 d). A similar phenomenon is seen in the variations of viscosity of hydrophilic colloids with temperature (Loeb 1922).

Finally it may be mentioned that the influence of temperature changes on both fibre-fibre and fibre-end-plate potentials, is much greater than if they were proportional to the absolute temperature, as Bernstein (1902) has found for injured preparations.

The resistance of the individual muscle fibre in situ as well as of the isolated fibre was measured by Buchthal (1934, 1935) in two different ways. Only experiments in which the isolated fibre was used will be discussed here,

since they at the present time afford the best information as to the resistance of the contractile elements.

In these experiments, fibres of the M. obl. abd. int. and limb muscles of the frog were used in situ and fibres of isolated muscles of frogs and lizards. For examination of fibres in situ the animal under urethane was placed on a paraffined glass-plate on the stage of the microscope. As potential source the fibre potential referred to above was used; non-polarizable micro-electrodes were employed for leading off. Electrodes were selected whose openings, as far as possible, were of equal size, and their resistance was determined before and after each experiment. The possible potential difference between the electrodes never exceeded 0.5 mV.

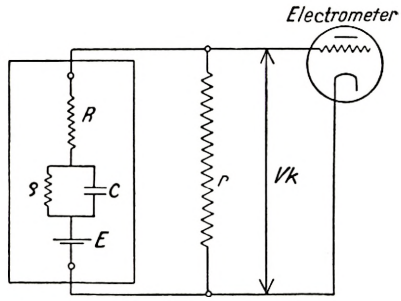


Fig. 63. Diagram of model. E = voltage, R = resistance and C = capacity; ϱ = shunt resistance to C. r = input resistance of electrometer, the variation of which permits measurements of $R+\varrho$. (Buchthal).

The method of measurement consisted in an electrostatic determination of the voltage, E, which was then loaded with a resistance, r, of 40, 10 and 6 meg. ohms in turn, and the voltage V_k then measured (Fig. 63). When E, V_k and r are determined, the total resistance can be calculated according to the formula

$$R+\varrho = r\,\frac{E-V_k}{V_k}.$$

The resistance determined in this manner was independent of variations in the resting potential of the fibre. The latter was constant for long periods when the preparation

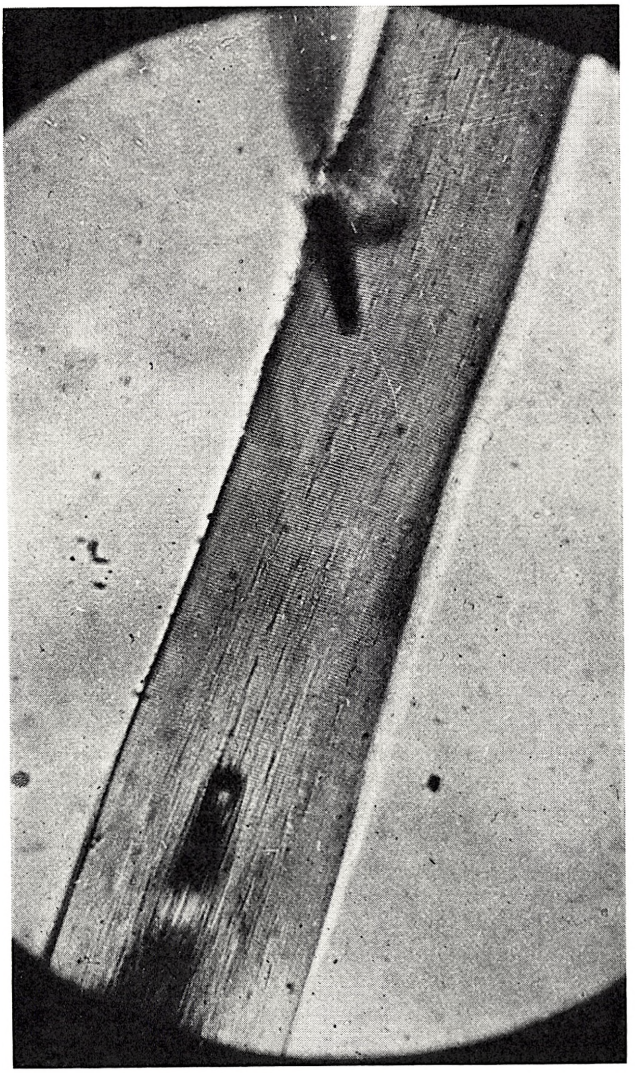


Fig. 64. Single frog's muscle fibre with micro-electrodes in position. (BUCHTHAL).

was covered with liquid paraffin. As the measurement took a short time only, the temperature could also be kept constant during the experiment. By means of control experiments

on dead muscles it was made certain that there was no significant resistance between preparation and electrodes. As the method requires both electrodes to be placed on the same fibre (Fig. 64), some measurements of fibre potentials with varying distances between the electrodes were made before each experiment; if these fibre potentials varied regularly with the distance between the electrodes, this was considered to indicate that the electrodes were on the same fibre. For the same reason comparatively thick fibres were usually selected for the experiment. As the resistance of fibres depends upon their thickness, the diameter of the fibre must be measured, and, as has previously been mentioned, this can only be done approximately on fibres in situ; accurate measurements can, however, be made on isolated fibres; Table XI, shows the results of a number of such measurements on living, resting muscle fibres.

As the principal result of the experiments BUCHTHAL found that the resistance of a resting fibre of about 70 μ diameter with a distance between the electrodes of 400 μ, was about 3 meg. ohm. For purposes of comparison it can be stated that a column of 0.6 per cent. NaCl solution of the same dimensions has a resistance of 100,000 ohm. The specific resistance of the fibre will thus be 3000 ohm/cm. against that of the salt solution of 109 ohm/cm.

Whilst the resistance of fibres of different frogs might vary by 20 to 30 per cent., it was fairly constant in different fibres of the same animal, when the thickness of the fibres was taken into consideration. Measurements on isolated fibres generally gave a somewhat higher result than on those in situ, which is probably due to the fact that the single paraffin-covered fibres are better isolated from their sur-

Table XI.

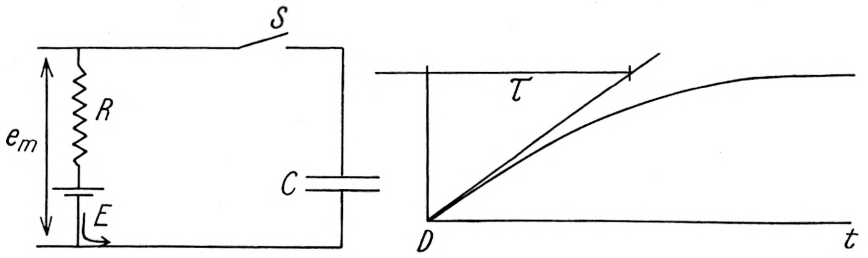
Preparation Nr.	Fibre Diameter in μ	Resistance of resting fibre in meg. Ω. Distance between electrodes, μ.		
		600—400	400—200	200—50
1	115	3.81	2.04	1.24
	110	—	2.27	—
	110	—	2.27	0.92
	80—82	—	2.26	—
	70	—	3.17	2.82
2	90	..	2.50	0.82
	90—95	..	2.84	—
3	120	..	1.24	—
	120	..	1.48	0.80
	120	..	1.25	2.95
	130	..	1.88	1.15
4	80—100	2.86	2.11	..
		3.20	2.12	..
		2.97	2.30	..
		3.34	—	..
		3.64	2.50	..
5	80	—	2.21	1.47
	75—80	—	1.77	1.67
	80	—	2.22	1.50
	100	2.12	1.64	1.42
6	120	..	2.10	1.85
	100—110	..	1.95	—
7	90	2.97	2.12	—
	95—100	2.50	2.85	—
	55	4.54	3.48	1.98
8	120	..	1.70	1.10
	90	..	2.00	1.84
9	90	2.65	—	..
	90	2.40	2.03	..
	80	—	2.05	..
	80	—	2.12	..
10	70	3.60	2.70	..
	70	—	2.90	..
	60—80	—	2.73	..
	60—80	—	2.70	..
	60—80	—	2.10	..

Table XI (continued).

Preparation Nr.	Fibre Diameter in μ	Resistance of resting fibre in meg. Ω. Distance between electrodes, μ.		
		600—400	400—200	200—50
11	90	3.43	3.04	..
	100	3.51	—	..
	100	3.14	—	..
	60	4.75	—	..
	60	4.81	4.43	..
	65	4.63	4.00	..
	65	4.90	—	..
12	110	2.20	..	1.58
	about 45	4.95	..	—
13	90—115	..	2.97	—
			3.02	2.65
			2.85	—
			2.72	—
			2.92	—
			2.22	—
14	70	5.22	—	..
	70	—	3.42	..
	70	—	4.30	..

roundings than are fibres in contact with others and with capillaries.

The results arrived at by means of the method outlined



a b

Fig. 65. (a.) Measurement of the resistance R by recording time course of e_m, after closure of S. (b). exponential course of e_m. Abscissa: time. Ordinate: potential difference. τ = time constant. (Buchthal).

above were controlled by experiments using another method. The resting potential of the fibre was again used as source of voltage, which from the point of view of measurement is an advantage because, if appreciable voltages are introduced from without, a new and incalculable source of error thus arises. The internal resistance was determined by recording the time-curve of the potential e_m (Fig. 65), a known capacity C being placed in parallel with the fibre. The condenser must be at zero voltage at the moment of connection. Then the required resistance, R, can be determined from the time constant, τ, and the capacity of the condenser, C, i. e. $R = \tau/C$. For details of the calculations reference may be made to the original paper. The reliability of the method was ascertained by a number of controls; otherwise the preparation of the tissue and the recording arrangements

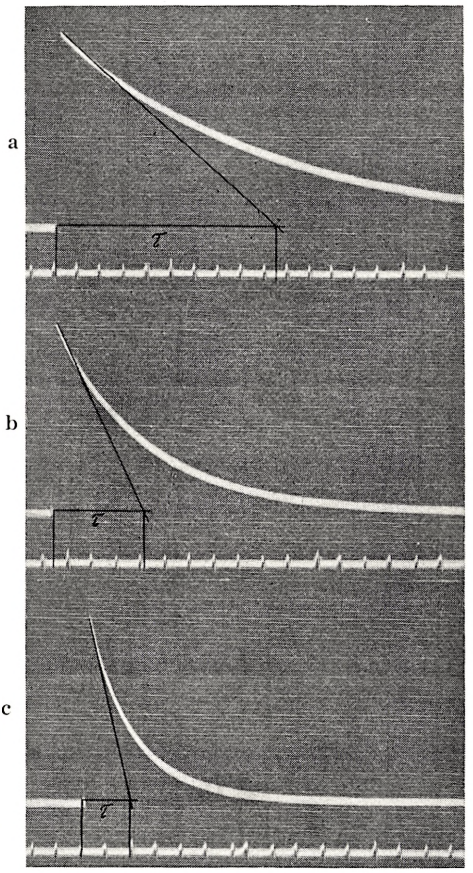


Fig. 66. Decrease of potential after shunting with different capacities. Time 1/5 sec.

	Capacity	sec.	Resistance
a	0.5 μ F	1.9	3.8 meg. Ω.
b	0.2 μ F	0.24	3.7 meg. Ω.
c	0.1 μ F	0.38	3.8 meg. Ω.

(Buchthal).

were the same as in the series of experiments first reported. Fig. 66 is reproduced as an example of the measurements. As shown by Fig. 67, the charging of the condenser takes place according to a purely exponential curve, proving that appreciable disturbances as a consequence of polarization counter-forces do not occur.

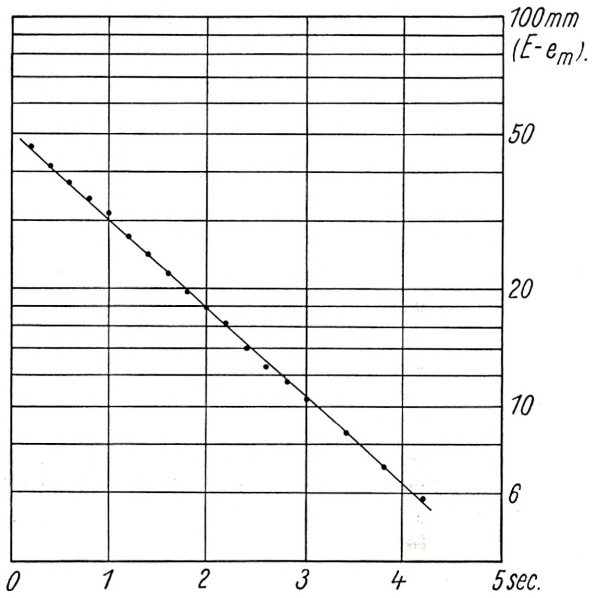


Fig. 67. Curve 66a plotted on semilogarithmic paper. Ordinate: $E-e_m$. Abscissa: time in sec. (BUCHTHAL).

By means of the method first referred to, BUCHTHAL also examined the change of resistance when the fibre was stimulated. Though indirect stimulation of isolated fibres entails some experimental difficulties, it was still considered necessary to employ this procedure besides direct stimulation. The preparations employed were similar to those previously described. In these experiments the nerve was exposed just at its exit from the spinal canal, and then prepared according to the method of ADRIAN & BRONK (1928) so that stimulation

produced contraction of a few fibres in the field of vision; in two cases one fibre only responded. In order to safeguard as far as possible against external influences in the cases where more than one fibre reacted, the distance between the electrodes was made comparatively short, only 150—200 μ. High frequency currents were used for stimulation. The terms in the formulae stated above for calculation of the

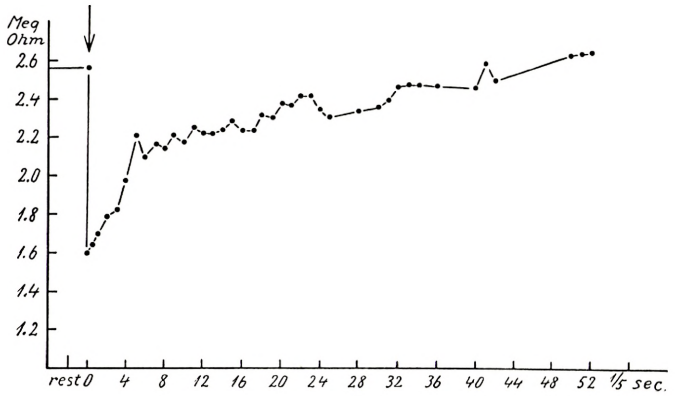


Fig. 68. Variation of the resistance of a single fibre after stimulation. Abscissa: time in $^1/_5$ sec.; ↓ Stimulus. (Buchthal).

resistance must of course have been determined at the same time in relation to the moment of stimulation.

The results of these measurements show that immediately after a direct or indirect stimulus the resistance suddenly decreases by 30—50 per cent., resuming its resting value in 4—8 seconds (Fig. 68, Table XII). The recovery curve is of exponential form comprising two parts, the first having a rapid course, which then passes into the slower almost straight second part. In about one third of the cases examined the transition between the two parts is distinctly marked by a sudden increase of short duration in the resistance. These changes of resistance cannot be due to

Table XII.

Experiment Nr.	Resistance of resting fibre in meg. Ω	Resistance during contraction in meg. Ω	Time of recovery in 1/5 sec.
1	6.6	3.6	6
2	2.3	1.8	13
3	1.5	0.6	16
4	2.55	1.6	50
5	4.6	2.9	13
6	2.3	1.95	8
7	2.1	1.7	13
8	1.7	0.6	6
9	3.6	2.2	12
10	1.9	0.6	—
11	4.0	2.6	6
12	3.6	2.6	incomplete recovery
13	2.2	0.9	6
14	3.5	3.7	6
15	1.9	1.1	12
16	5.7	5.1	5
17	2.3	1.8	5

changes in shape of the fibres, as the latter will only be able to change their thickness, and then not throughout their length. As demonstrated by LINDHARD & MÖLLER, the fibre when it contracts becomes thicker in one part and thinner in another. This, however, could result in an increased resistance in one region of the fibre, which has never been observed though measurements have been made in many different parts of these. It must also be borne in mind that possible mechanical changes after stimulation take a much more rapid course than do the changes of resistance, even if possible elastic after-effects are taken into consideration. Impedance measurements on the whole muscle have shown that the A.C. resistance of the muscle as a whole increases after stimulation (BOZLER & COLE (1935) DUBUISSON (1937)). These experiments, however, are not directly comparable to those referred to above, firstly because they

were made on the whole muscle, and secondly because the resistance measurements were made transversely and not longitudinally as in the present experiments. The resistance changes found in the single fibre may have their origin in permeability changes, in variation of the number of ions or finally in an altered distribution in the conductive and

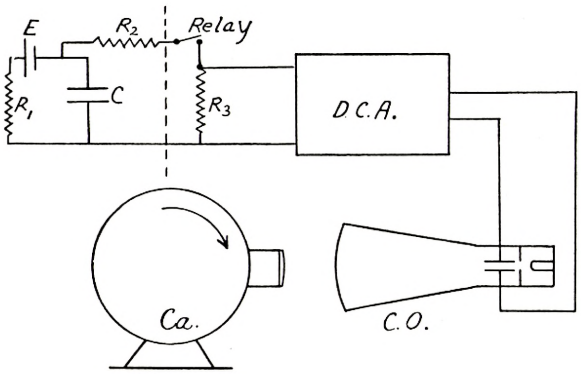


Fig. 69. Device for measuring electrostatic capacity.

E = voltage
R_1 = internal resistance of E } model.
R_2 = resistance in series
R_3 = input resistance.
D. C. A. = direct current amplifier.
CO = cathode ray oscillograph.
ca = camera. (Buchthal & Nielsen).

non-conductive elements of the fibre. For the time being it is impossible to assess the relative importance of these factors.

The capacity of the muscle fibre has yet to be discussed. It was determined by Buchthal and Nielsen, who tried to reduce the complicated conditions of the fibre to a relatively simple model. They assumed the presence of a condenser, which was charged by the voltage E (fibre potential) through a resistance R_1 (see Fig. 69). Besides reckoning with

this internal resistance, there is also a leading off resistance, R_2. For calculation of the capacity use was made of the time variation of the voltage E, when a resistance, R_3, is inserted in parallel with the condenser. Fig. 70 shows the course of the curve. When R_3 is inserted the voltage suddenly increases, falling again according to an exponential curve to its previous value. The connection with R_3 was effected by means of the relay referred to above, and the change of the potential was registered through a D.C. amplifier by means of a cathode ray oscillograph. It appeared that the course of the curve in experiments on living muscles was the same as in the model experiments, whilst dead muscles, or an artificially produced potential difference between the two electrodes gave curves that were deflected almost at right angles. We may suppose, therefore, that the course of the curve in experiments with living fibres implies that they possess accumulated electrostatic energy, which is related in some way to the normal structure of the fibre.

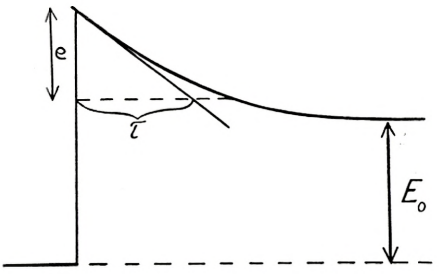


Fig 70. Variation of fibre potential after shunting with R_3 (see text). (Buchthal & Nielsen).

Calculation of the capacity is made according to the formulae below, the quantities τ, E_0, E, e, and R_3 being known, or measured from the curves, R_1, R_2, and C being unknown.

$$E_0 = \frac{E \cdot R_3}{R_1 + R_2 + R_3}$$

$$E_0 + e = \frac{E \cdot R_3}{R_2 + R_3}$$

$$\tau = \frac{R_1 (R_2 + R_3)}{R_1 + R_2 + R_3} \cdot C.$$

When these equations are solved we arrive at the following results:—

$$R_1 = \frac{e}{E_0} \cdot \frac{E}{E_0 + e} \cdot R_3$$

$$R_2 = R_3 \left(\frac{E}{E_0 + e} - 1 \right)$$

$$C = \frac{\tau (R_1 + R_2 + R_3)}{R_1 (R_2 + R_3)}.$$

E is stated in mV, R in meg. ohm, C in μF, and τ in seconds.

Experiments on muscles of different animals give some variation in the results, all of which, however, are of the same order of magnitude. The result of a single experiment will be stated here; with $R_3 = 6$ meg. ohm it was

$$R_1 = 0.2 \text{ meg. ohm}, \quad R_2 = 1.3 \text{ meg. ohm}, \quad \tau = \frac{3.5}{50} \text{ sec. and}$$
$$C = 0.36 \ \mu\text{F}/100 \ \mu$$

The measurements of potential and capacity referred to above make it possible to form a rough idea of the size of the electrostatic forces in the muscle fibre. It must, however, be realized that it cannot be more than an estimate, because it is not yet possible to determine the potential within the sarcolemma of the undamaged fibre under physiological conditions. The potentials hitherto measured are lower than the physiological potentials.

The electrostatic potential energy of the fibre can be determined according to the formula

$$\text{Energy} = \frac{1}{2}\ 10^{-5} \cdot C\mu\text{F} \cdot E^2\text{mV}\ \text{Erg}/100\ \mu.$$

If the capacity calculated from the above experiment and the value stated above (p. 154) for the potentials measured intralemmally are introduced into this equation we find

$$\text{The energy} = \frac{1}{2}\ 10^{-5} \cdot 0.36 \cdot 45^2 \cdot 10^2 = 0.37\ \text{Erg/cm}.$$

Similar difficulties and uncertainties are encountered when it is desired to determine how great a mechanical tension a muscle fibre is able to develop at its equilibrium length. In reality there are very few suitable determinations of tension. Exact determination of the tension of a single isolated fibre in the case of a so-called single contraction has not yet been made. As mentioned previously, ASMUSSEN has determined the tension of small bundles during a tetanus of short duration; but measurements of the thickness of the fibres, were not made. This would have been desirable because the tensions were measured on fibres of limb-muscles, whilst potentials and capacity were measured on abdominal muscles. On the existing basis, therefore, we cannot get further than to an approximate determination of the order of magnitude of mechanical energy. In an experiment with a bundle of 12 fibres ASMUSSEN states the tension at equilibrium length to be about 60 mg. which, when incorporated in the formula for the potential mechanical energy, $^1/_5$ Tl (p. 68), gives about 1 Erg/cm., which is about 3 times the electrical energy calculated above. Both quantities are, however, based on factors so many of which are at present uncertain, that in view of the numerous, in part uncontrollable sources of error, we cannot expect more at present; on the other hand it appears to be justifi-

able, all things considered, to maintain that the existing results confirm rather than invalidate the hypothesis of contraction here advanced. Further investigations will comprise determination of the magnitude of the potential difference in the undamaged fibre and measurement of the mechanical tension of individual fibres under physiological conditions.

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DIE GATTUNG *POLYCHELES*, IHRE VERWANDTSCHAFTLICHE STELLUNG UND IHRE POSTEMBRYONALE ENTWICKLUNG

VON

J. E. V. BOAS

KØBENHAVN
EJNAR MUNKSGAARD
1939

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VORWORT

Professor J. E. V. Boas hinterliess bei seinem Tode im Jahre 1935 ein Manuskript zu einer Arbeit über die systematisch und morphologisch bedeutsame Dekapodengattung *Polycheles*. Ausser dem von Boas selbst auf Deutsch geschriebenen Manuskript fanden sich auch eine reichliche Anzahl von Noten und Konzepten und weiter sämtliche Figuren, die von Frau Bodil Strubberg unter Leitung des Verfassers gezeichnet worden waren. Auch das untersuchte Material war alles noch vorhanden. Es war deshalb keine schwierige Aufgabe, die Arbeit druckfertig zu machen; es fehlte nur die Einfügung der Figurenhinweise und die Verfassung der Unterschriften zu den Abbildungen sowie eine Revision der Literaturangaben. Es zeigte sich zwar notwendig einige rein redaktionelle Änderungen des Textes vorzunehmen, sonst wurde im Manuskript nichts geändert.

Auf den Figuren fanden sich keine Angaben über die Species-Zugehörigkeit des abgebildeten Exemplars oder Organs. Mein Mitarbeiter Dr. Henning Lemche hat versucht mit Hilfe der in einschlägigen Arbeiten (s. Literaturverzeichnis) gegebenen Artdiagnosen diese Lücke auszufüllen, wofür ich ihm zu grossem Danke verpflichtet bin. Seine Ergebnisse lauten wie folgt:

Von den recht vielen Arten, die sich in dem von Boas untersuchten Material vorfanden, sind nur zwei Arten von

Polycheles und zwar *P. grimaldii* und *P. sculptus* für eine mikroskopische und submikroskopische Untersuchung dissekiert worden. Sämtliche Detailfiguren dürfen sich also auf diese beiden Arten hinführen lassen. Die untersuchten Exemplare sind folgende:

1) *Polycheles grimaldii.* Mehrere Exemplare von der Ingolf-Expedition, Station 83. Die Formel für die Reihe der Mitteldorne auf dem Schild ist die für diese Art typische: 2—1—1—2—1—c—2—2——2.

2) Ein etwas grösseres Exemplar, das ebenfalls der Art *P. grimaldii* angehören muss, aber durch seine Dornenformel etwas abweichend ist: 2—1—1—1—2—1—c—2—2——2. Übrigens scheint es im Bau mit den unter 1) erwähnten Stücken gänzlich übereinstimmend zu sein. Etikette fehlte, und möglicherweise ist das Tier aus einem der anderen Gläser herausgenommen.

3) *Polycheles sculptus.* Ein mittelgrosses Exemplar aus Frederikssted (auf den früher dänischen westindischen Inseln, jetzt Virgin Islands). Das Stück war nicht artbestimmt, stimmt aber vollständig mit den Beschreibungen von *P. sculptus* überein. Dornenformel: 2—1—2—1—c—2—2——2.

4) *Polycheles sculptus.* Sehr grosses Exemplar aus Südafrika (Nr. 6471—6473) von Dr. Th. Mortensens Expedition 1929—30 stammend. Das Stück stimmt genau — auch in der Mandibelgestalt — mit dem Exemplar 3) überein und weicht in denselben Merkmalen wie dieses von den unter 1) und 2) aufgeführten Exemplaren ab. Auch dieses Stück war nicht früher bestimmt, aber die Richtigkeit der Artbestimmung kann kaum bezweifelt werden. Diesem Individuum liegen die Abbildungen Fig. 7 und 8, und wahrscheinlich auch Fig. 3 zugrunde.

5) Das in Fig. 1 gezeichnete ganze Exemplar von *Pentacheles phosphorus* war nicht dissekiert, weshalb keine der Detailfiguren von diesem Tier stammen können.

Ausserdem waren zwei Exemplare von »*Eryoneicus kempi*« von Boas dissekiert, aber von diesen liegen keine Figuren vor.

Die Abbildungen in der vorliegenden Abhandlung sind in Übereinstimmung mit den obenstehenden Daten von uns zu den erwähnten *Polycheles*-Spezies hingeführt worden.

Professor Boas ist mit dieser letzten Arbeit zu einer Tiergruppe — den dekapoden Crustaceen — zurückgekehrt, die ihm seit vielen Jahren besonders lieb war. Seine bekannte Jugendarbeit »Studier over Dekapodernes Slægtskabsforhold« (1880) ist für die Systematik und vergleichende Morphologie dieser Tiere grundlegend geworden, und seine späteren Arbeiten über Lithodes, Paguriden etc., sind grösstenteils als Ergänzungen zu dieser Hauptarbeit zu betrachten. Auch die hier vorgelegte Behandlung der interessanten Form *Polycheles* soll als eine Ergänzung zu den Ausführungen in der alten Abhandlung betrachtet werden.

Professor Boas sollte nicht selbst die Drucklegung dieser Arbeit erleben. Möge sie jetzt seinen Fachgenossen und Freunden als Erinnerung an den verstorbenen Forscher dienen.

Zoologisches Laboratorium der kgl. Landwirtschaftlichen und Tierärztlichen Hochschule, Kopenhagen im November 1938.

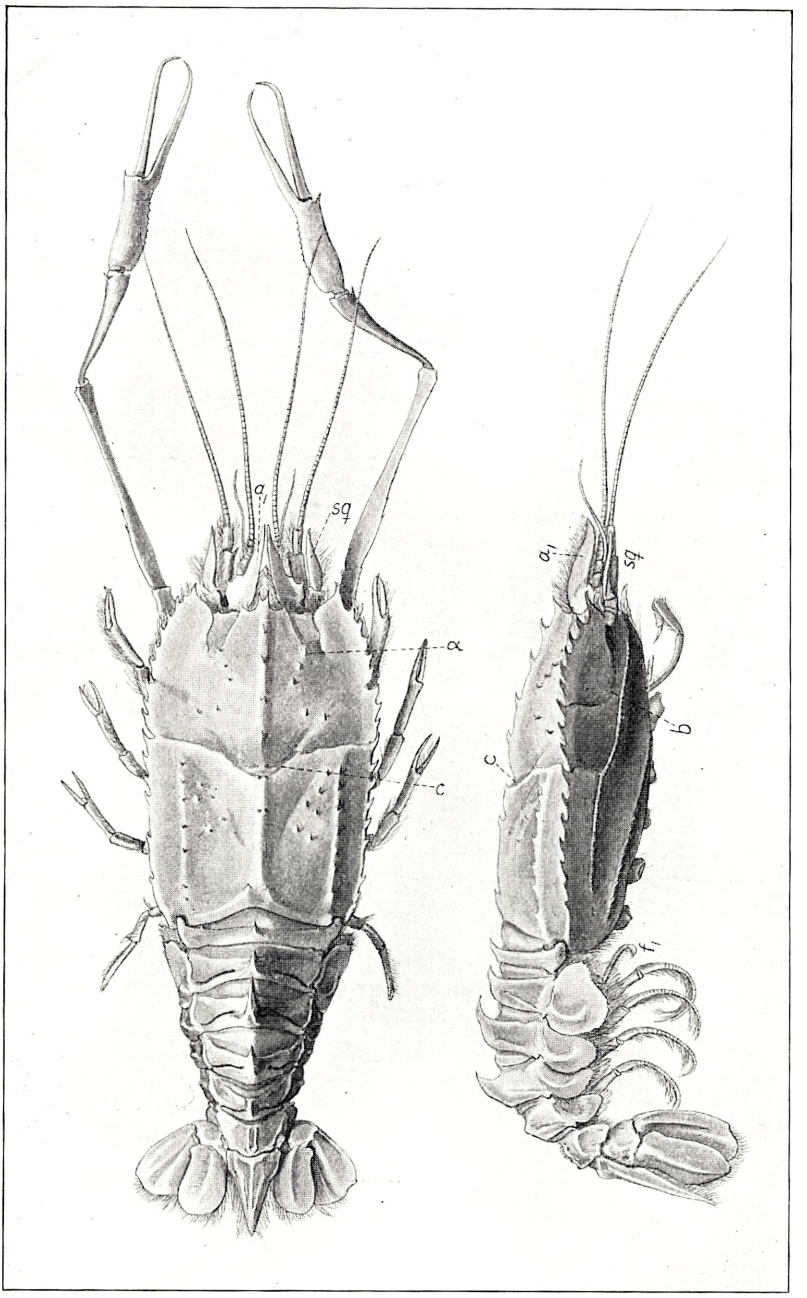
Mathias Thomsen

Morphologie des erwachsenen *Polycheles*.

Als ich vor vielen Jahren meine Arbeit über die Verwandtschaftsverhältnisse der Dekapoden ausarbeitete (Boas 1880), hatte ich kein Material von der wichtigen Gattung *Polycheles* zu meiner Disposition und war deshalb darauf hingewiesen, dieselbe auf der Basis der damals vorliegenden sehr unvollkommenen Angaben zu beurteilen, was natürlich ganz ungenügend war. Jetzt habe ich ein recht grosses Material zu meiner Verfügung, das ich hauptsächtlich der Liberalität der Herren Dr. K. Henriksen und K. Stephensen aus dem Zoologischen Museum in Kopenhagen verdanke, und welches mich im Stande setzt, die Gattung eine erneute Behandlung zu geben. Ich behandle den Stoff nach denselben Prinzipien wie die Formen in jener Arbeit.

Ich bemerke im Voraus, dass ich von der Teilung des *Polycheles* in mehrere Gattungen, wie sie von Einigen praktiziert ist, ganz absehe (vergl. Selbie 1914 p. 9); ich betrachte sämtliche jetztlebenden Arten als einer Gattung angehörig, und wenn ich von *Polycheles* rede, verstehe ich also die Gattung in ihrem vollsten Umfang.

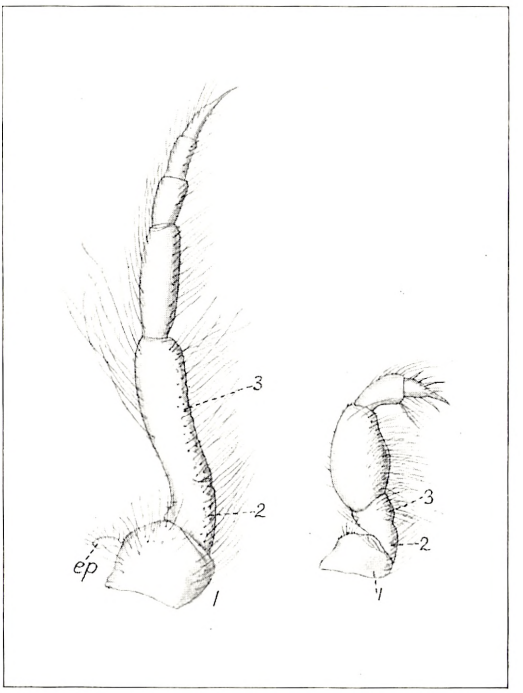
Thoraxfüsse. Es bieten bekanntlich die *Polycheles*-Arten, was sonst bei keinem anderen Reptant der Fall ist, das merkwürdige, dass die vier ersten Thoraxfüsse mit einer Schere versehen sind (Fig. 1 a). Wie ich schon in meiner älteren Arbeit bemerkt habe, liegt der bewegliche Finger (7. Glied) auch am 1. Thoraxfuss ausserhalb des unbeweglichen wie bei *Penaeus* und bei *Palinurus*, im Gegensatz zu den Homariden, wo er innerhalb desselben liegt. Bei den



a b

Fig. 1. *Pentacheles phosphorus*, Weibchen a) von der Oberseite, b) von rechts In b sind die Thoraxfüsse entfernt. a_1 Antennula. α Dorn am inneren Ende des Augenstieles. *b* und *c* Furchen. f_1 erster Schwanzfuss. *sq* Squama.

Reptantia ist dagegen häufig am 5. Thoraxfuss eine kleine, meist unvollkommene Schere vorhanden, die entweder nur beim Weibchen, oder aber bei beiden Geschlechtern vorhanden ist; bei *Polycheles* ist bald das eine bald das an-



a b

Fig. 2. *Polycheles grimaldii,* a) dritter, b) zweiter Kieferfuss. *ep* Epipodit. *1, 2, 3,* Nummern der Glieder.

dere der Fall. Das 2. und 3. Glied sind an sämtlichen Thoraxfüssen mit einander unbeweglich verbunden. Der 1. Thoraxfuss ist wie gewöhnlich stärker als die übrigen, auch länger, ist aber entschieden schwach zu nennen (Fig. 1 a). Die Thoraxfüsse sind meist mit einem Epipodit versehen, der gewöhnlich schwach entwickelt ist, selten eine ansehnliche Grösse erreicht (Bate 1888 Pl. XX m).

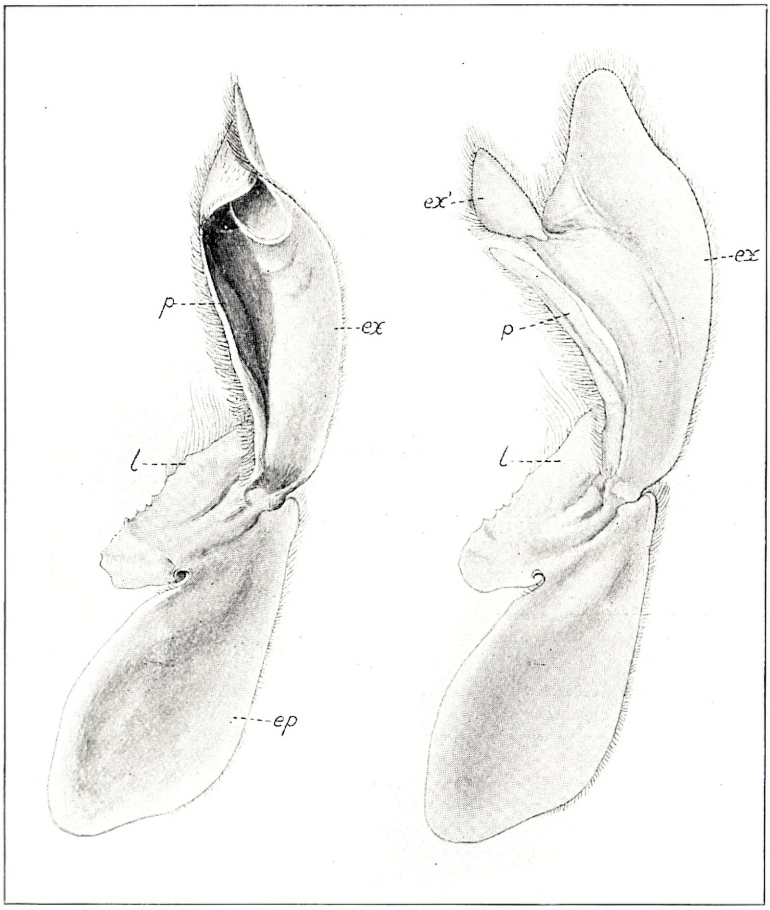
Der dritte Kieferfuss (Fig. 2 a) besteht aus den gewöhnlichen 7 Gliedern, von denen das 2. und 3. wie bei den Homariden und Loricaten unbeweglich verbunden sind. Das Endglied endet mit einem starken Dorn. An dem 3. Glied ist im Gegensatz zu den Homariden und Loricaten keine Crista dentata entwickelt. Der Epipodit ist gewöhnlich ein kleiner Lappen, sehr schwach entwickelt, kann aber (Selbie 1914 p. 10) »of fair size« sein (*Polycheles maculatus*, Selbie 1914 Pl. III, Fig. 11). Ein Exopodit fehlt völlig.

Der zweite Kieferfuss (Fig. 2 b) besteht nur aus 6 Gliedern, von denen das zweite und dritte verschmolzen sind und nur eine sehr schwache Andeutung der Verwachsungsstelle zeigten. Was für ein Glied das fehlende ist, oder welche weitere Verwachsung zweier Glieder stattgehabt hat, kann ich nicht sagen. Spuren von Verwachsungen ausser der schon erwähnten, sind nicht zu finden.

Der erste Kieferfuss (Fig. 3 a und b) ist merkwürdig gestaltet und eigentlich dem entsprechenden aller anderen Decapoden unähnlich. Die zwei Kieferfüsse dieses Paares sind stark seitlich gedrückt, so dass von einem Zusammenwirken derselben (bei der Behandlung des Futters) keine Rede sein kann. Der erste Kieferfuss scheint ausschliesslich in den Dienst der Atmung getreten zu sein (vergl. unten).

Der betreffende Kieferfuss besteht aus den gewöhnlichen Teilen: Endo-, Exo- und Epipodit. Der Endopodit zeigt nur eine deutliche Lacinie, die ich als die proximale des typischen ersten Kieferfusses gedeutet habe; dieselbe ist mit langen, sehr dünnen, unbefiederten Borsten am Innenrand besetzt. Der Palpus ist dünn, am Innenrande stark mit ziemlich weichen, befiederten, kurzen Haaren besetzt, und liegt dem Innenrande des Exopodits dicht an. Der Exopodit ist seitlich ausgebreitet und auf der dorsad wendenden

Seite ausgehöhlt. Das distale Ende desselben ist fester verkalkt als das übrige, und trägt einen platten, ebenfalls stark

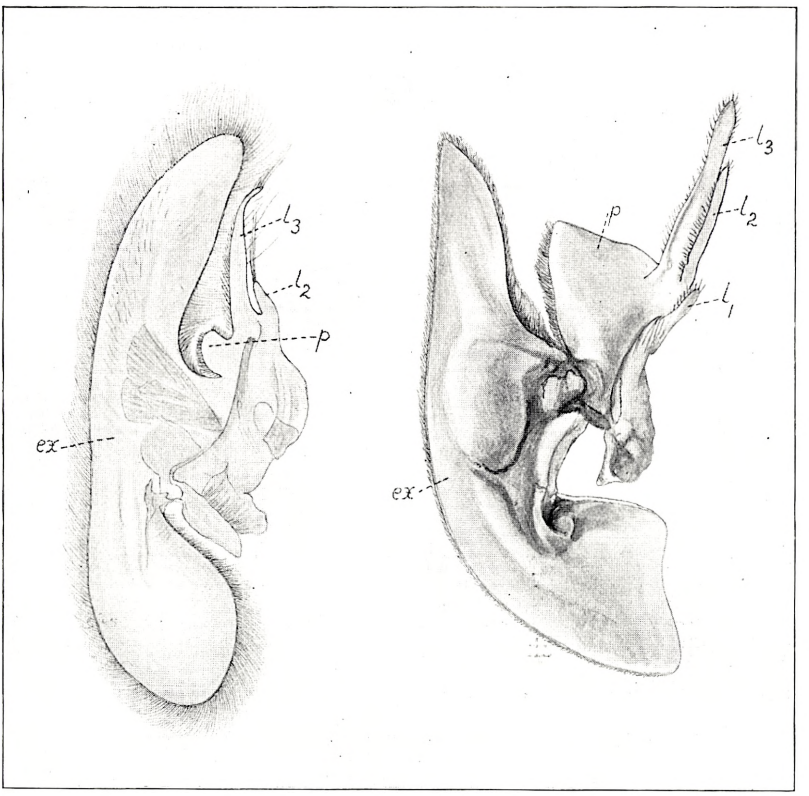


a b

Fig. 3. *Polycheles sculptus*, rechter erster Kieferfuss von oben gesehen, a) in gewöhnlicher Stellung, b) ausgebreitet. *ep* Epipodit. *ex* Exopodit. *ex'* zweites Glied des Exopodites. *l* Lacinie. *p* Palpus.

verkalkten Anhang (Fig. 3 b, ex'), der wohl der Geissel entspricht, aber ganz anders aussieht als die Geissel sonst (also eine ähnliche Umbildung wie die, welche bei der

Antennen-Geissel der Scyllariden stattgefunden hat). Die Aushöhlung des Exopodites bildet wahrscheinlich mit dem oberhalb desselben liegenden Exopodit der zweiten Maxille



a b

Fig. 4. Zweite Maxille von a) *Polycheles grimaldii*, b) *Palinurus vulgaris*. *ex* Exopodit. l_1—l_3 Lacinien. *p* Palpus.

zusammen eine Röhre, die zur Ableitung des Atemwassers dient. — Es ist ein stark entwickelter Epipodit vorhanden, der dem Epipodit des 1. Kieferfusses mancher anderen Decapoden ähnlich ist.

Die zweite Maxille (Fig. 4 a) bietet eine unverkennbare Ähnlichkeit mit der von *Palinurus* dar, die in Fig. 4 b

abgebildet ist. Die gewöhnlichen Lacinien der zweiten Maxille, die bekanntlich vier Lappen ausmachen, sind bei *Palinurus* auf drei rückgebildet, die sparsam beborstet sind und eine sehr abweichende, gestreckte Form haben. Bei *Polycheles* sind sie weiter auf zwei reduziert, die noch sparsamer beborstet sind, aber dieselbe Form wie bei *Palinurus* haben, und auch ungefähr dieselbe Richtung — hinten-vorn — nicht querüber. Wie bei *Palinurus* sind die Maxillen

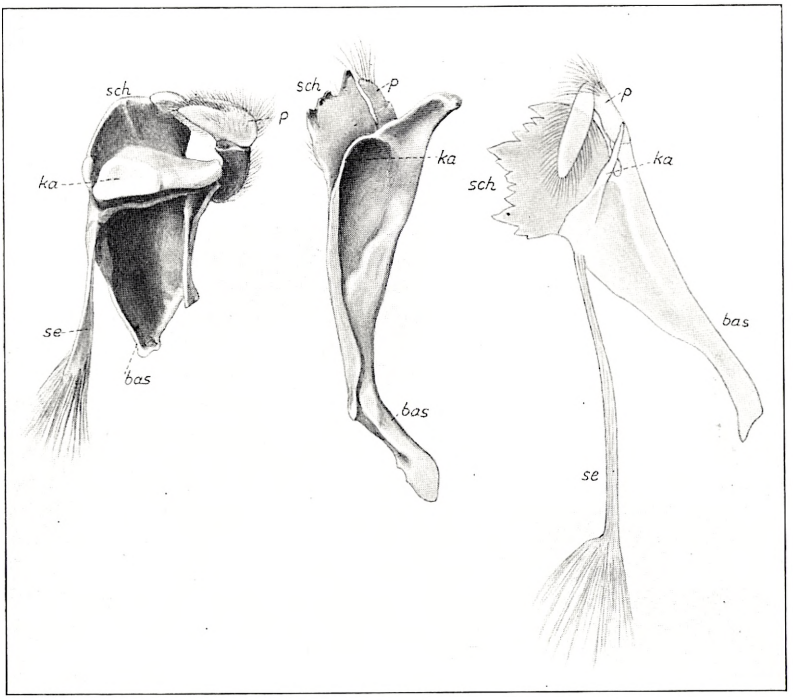
a b

Fig. 5. Erste Maxille von a) *Polycheles grimaldii*, b) *Palinurus vulgaris*. l_1—l_2 Lacinien. *p* Palpus.

weit seitlich gerückt und haben sicher keine Bedeutung für die Behandlung des Futters, deshalb die von anderen Decapoden abweichende Richtung der Lacinien und das Fehlen von Kaubborsten. Der Palpus ist bei *Palinurus* sehr kurz, so lang wie breit. Ganz ähnlich verhält sich auch der Palpus bei *Polycheles*, er ist sehr kurz, zwar nicht dreieckig wie bei *Palinurus*, sondern am Ende abgerundet. Auch die Behaarung ist ähnlich. — Es ist ein grosser Epipodit vorhanden von der gewöhnlichen Form, der keine Bemerkungen veranlasst.

Die erste Maxille von *Palinurus* (Fig. 5 b) hat in der Hauptsache die für diese Maxille gewöhnliche Ausbildung, nur ist der Palpus klein, eingliedrig; bei *Ibacus* (Bate 1888

Pl. VII, Fig. 2 e) fehlt er sogar völlig und ist durch eine Gruppe Haare vertreten. Ähnliches ist bei *Polycheles* (Fig. 5 a) der Fall, auch hier fehlt der Palpus und an seiner Stelle findet man eine Gruppe Haare. Das Ende der Laci-



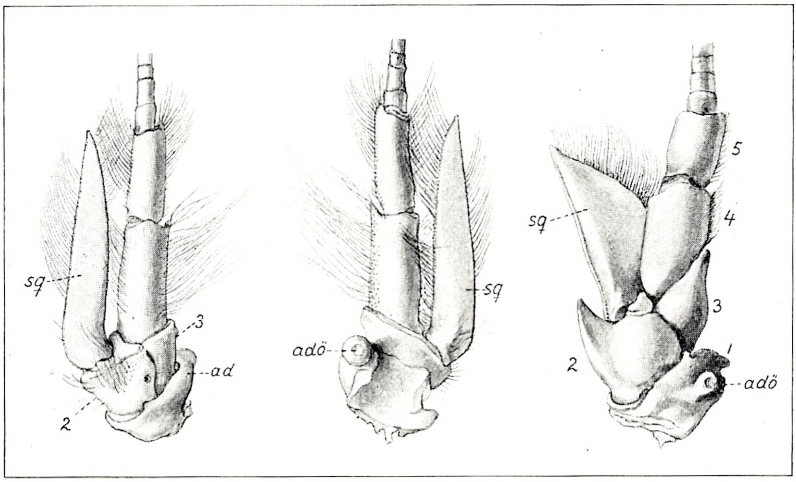
a b c

Fig. 6. Mandibel von a) *Homarus vulgaris*, b) *Parribacus* sp., c) *Polycheles grimaldii*. *bas* Basilarstück der Mandibel. *ka* Kaupartie. *p* Palpus. *sch* Schneiderand. *se* Sehne.

nien ist zugespitzter als bei *Palinurus*, und die Kauborsten setzen sich am Vorderrande der Lacinien mit einer Reihe starker Borsten fort.

Die Mandibel (Fig. 6 c) ist dadurch ausgezeichnet, dass der Kauteil nur eine schwache Kante ist, als Kauwerkzeug also ganz unbrauchbar. Ähnliches ist auch bei den Scyllariden

(Fig. 6 b) der Fall (vergl. Boas 1880). Der schneidende Teil ist mit zahlreichen Zähnen versehen, ganz abweichend von den meisten Reptantien. Der Palpus ist wohlentwickelt, nicht wie bei den meisten Loricaten mehr oder weniger rückgebildet. Diejenige Reptant-Mandibel, die am meisten mit der von *Polycheles* übereinstimmt, ist die von der ab-



a b c

Fig. 7. Basale Partie der Antenne von a) *Polycheles sculptus* von unten gesehen, b) dieselbe von oben, c) *Nephrops norvegicus*, von unten. *ad* Ausfuhrgang der Antennendrüse. *adö* Öffnung derselben. *sq* Squama. *1–5* Glieder der Antenne.

weichenden Homaridengattung *Phoberus* (Bate 1888 Pl. XXII, Fig. d), die der von *Polycheles* ungemein ähnlich ist, einen ähnlichen gezähnten Schneiderand besitzt und auch einen wohlentwickelten Palpus.

Die Antennen (Fig. 7 a und b) sind von denen der Loricaten darin abweichend, dass eine Zusammenwachsung des Basalgliedes mit dem gegenüberstehenden nicht stattfindet. An dem recht grossen Basalglied ist die Ausführungsöffnung der Antennendrüse sehr verlängert (Fig. 7 a,

ad) und mündet nicht wie sonst (Fig. 7 c) auf der Unterseite des Gliedes, sondern biegt sich um und öffnet sich auf der Dorsalseite desselben (Fig. 7 b, adö). Das zweite und dritte Glied ist wie bei den Loricaten mit einander verwachsen, aber auf der Ventralseite durch eine Längsfurche von einander abgegrenzt, was bei den Loricaten nicht der Fall ist. Die Squama fehlt bekanntlich bei den

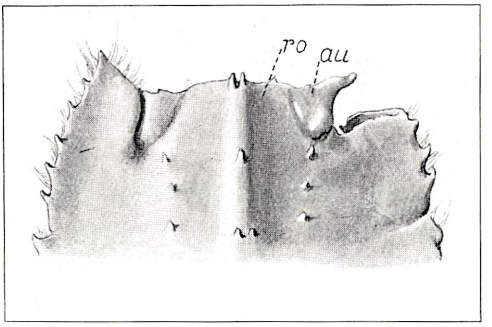


Fig. 8. Augenstiele und vordere Partie des Rostrums von *Polycheles sculptus*. *au* Augenstiel. *ro* Rostrum. (An der rechten Seite ist ein Teil des Schildes entfernt).

Loricaten, sie ist aber hier vorhanden und zeichnet sich dadurch aus, dass der Stachel, der sonst auf dem Aussenrande sitzt, hier fehlt. Das übrige ist ohne Interesse; die Geissel ist nicht lang und kräftig wie bei *Palinurus*.

Die Antennulen haben an der Innenseite des Basalgliedes einen grossen, platten, nach vorne gerichteten Fortsatz (Fig. 1 a), der den übrigen Schaft weit überragt, was den Reptantien sonst fremd ist (vergl. später).

Die Augenstiele. Diese Tiere sind bekanntlich blind. Die Augenstiele sind etwas verschieden bei den verschiedenen Arten ausgebildet, bei dem von mir in diesem Punkte näher untersuchten Exemplar war der Augenstiel (Fig. 8, au), und so ist es gewiss gewöhnlich, an seinem proximalen

Ende an die Basis des Rostrums, oder wohl richtiger an das mit dem Rostrum verwachsene Augensegment angewachsen, am distalen Ende läuft er in eine dünne Partie aus, die am Ende leicht abgerundet ist, offenbar die Stelle, wo das Auge bei den Vorfahren von *Polycheles* gesessen hat. Diese schmälere Partie ist von einem lateralen Fortsatz des Schildes gedeckt, der in Fig. 8 rechts abgebrochen ist, während er links übrig gelassen ist; dieser Fortsatz ist nicht mit dem Augenstiel verwachsen, sondern ganz frei von diesem.

Die fossile Gattung *Eryon*, die dem *Polycheles* sehr nahe stand, war offenbar nicht blind, siehe z. B. Oppel (1862) Tab. 3, Fig. 3, die grosse offene Augenhöhlen auf jeder Seite des breiten Rostrums aufweist, und Tab. 2, Fig. 4, in derselben Arbeit, in welcher augenähnliche Gebilde diese Höhlen ausfüllen.

Die Schwanzfüsse des 2.—5. Schwanzsegments des Weibchens und die des 3.—5. Segments des Männchens (Fig. 9a) sind kräftiger als sonst bei den Reptantia; die einzige mir bekannte Form, bei welcher ich eine ähnliche Ausbildung gesehen habe, ist *Nephropsis*. Die Ausbildung derselben erinnert an die von Natantia; der Schaft ist vielleicht etwas schwächer als bei diesen. In der Tat hat *Polycheles* durch die Schwanzfüsse das Vermögen kurze Strecken zu schwimmen (Santucci 1932): »Ihre Bewegungen sind gering, sie liegen meist mit dem Abdomen im Sand vergraben ..., gereizt schwimmen sie durch rhytmische Bewegungen der Pleopoden kurze Strecken«[1]. An jedem dieser Füsse sind zwei Blätter vorhanden, von denen das innere eine Appendix interna besitzt, die am Ende eine Anzahl Haken trägt (Fig. 9b). Eine solche hakenbesetzte Appendix interna findet

[1] Zitat eines Referates in: Zool. Bericht Bd. 35, p. 129, 1934.

sich selten bei den Reptantia, nämlich nur bei einem Teil der Thalassiniden und bei *Nephropsis* (Fig. 9 c, d). Dagegen fehlt sie bei *Homarus* und *Nephrops*. Bei den Loricaten ist die Appendix interna zwar vorhanden, die Haken fehlen aber, doch habe ich ausnahmsweise bei einem Exemplar

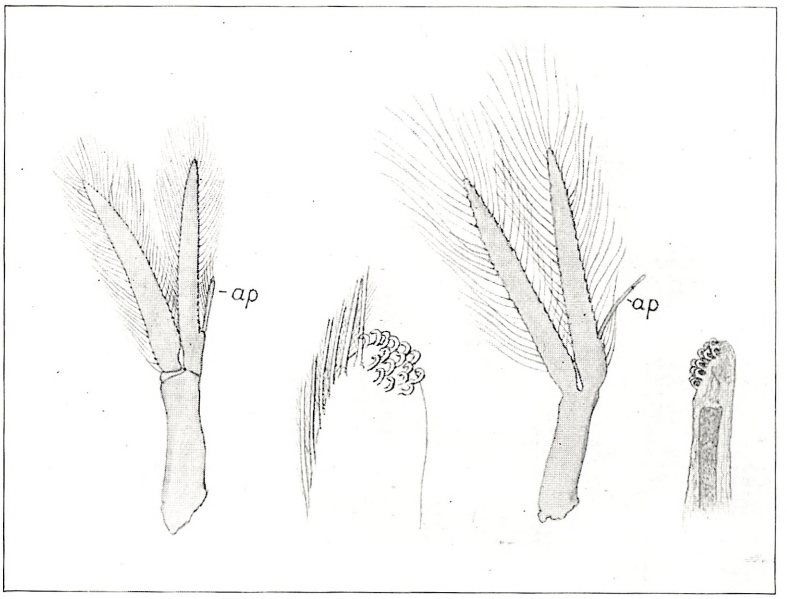
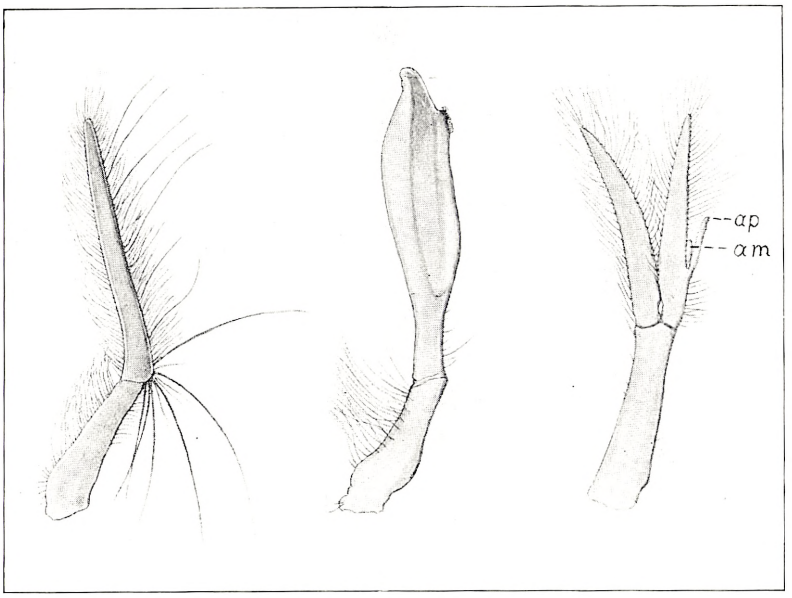


Fig. 9. Vierter Schwanzfuss von a) *Polycheles grimaldii* und c) *Nephropsis* sp. b) und d) zeigen die respectiven Appendices internae von *a*) und c). *ap* Appendix interna.

von *Scyllarus* eine hakenbesetzte Appendix interna am rechten Fuss des 5. Paares gefunden, während Haken an den übrigen Füssen fehlten (Boas 1880, p. 86). Vergl. auch Bate 1888 Pl. X, Fig. 4 q, wo eine Appendix interna von *Arctus* abgebildet ist, die scheinbar Haken besitzt.

Von den anderen Schwanzfüssen ist folgendes zu bemerken: Beim Weibchen ist am 1. Schwanzringe ein Paar einblättrige, schwache Füsschen vorhanden (Fig. 10a). Beim

Männchen ist ebenfalls ein Paar einblättrige Füsschen vorhanden, das Blatt ist aber borstenlos und etwas ausgehöhlt und am Innenrand mit einer kurzen Reihe von Haken ausgestattet (Fig. 10 b). Das 2. Paar Schwanzfüsse ist beim



a b c

Fig. 10. *Polycheles grimaldii*, a) erster Schwanzfuss eines Weibchens, b) derselbe eines Männchens, c) zweiter Schwanzfuss eines Männchens. *am* Appendix masculina. *ap* Appendix interna.

Männchen ebenso wie beim Weibchen zweiblättrig und mit einer hakentragenden Appendix interna versehen, dazu aber noch mit einer Appendix masculina, die im Winkel zwischen der Appendix interna und dem Blatte selbst sitzt (Fig. 10 c). Die Blätter des letzten Schwanzfuss-Paares (Schwanzfächer) sind ganz einfache Platten, die weder die Eigentümlichkeiten dieser Füsse bei den Homariden (Quer-Gelenk des äusseren Blattes und Stachel des Aussenrandes

beider Blätter) noch die der Loricaten aufweisen (weiche Beschaffenheit der distalen Partie).

Die Eier, die recht klein sind (c. 0,5 mm), sind nur an den 1.—3. Paar Schwanzfüsschen des Weibchens angeheftet. Am 2. und 3. Paar sind sie fast nur an den Haaren des Schaftes angeheftet, sehr wenig ausserdem an den proximalen Haaren des inneren Blattes. Am 1. Schwanzfuss sind sie dagegen sowohl an den Haaren des Schaftes wie an denen des Blattes vorhanden. Alle Eier zusammen bilden einen grossen Klumpen auf der Unterseite der ersten Schwanzsegmente.

Der Schild (Fig. 1a und b) ist am meisten dem der Scyllariden und gewissen Palinuriden (vergl. BATE 1888 Pl. XI, Fig. 2 und 4) ähnlich, in erster Linie durch das Vorhandensein zweier starker gezähnter Längskanten, die den Schild in drei Abteilungen teilen, eine mittlere ungefähr horizontale und jederseits eine seitliche, die ventral-mediad gerichtet ist. Auch das Rostrum ist dem der Scyllariden am ähnlichsten; ebenso wie bei diesen ist es sehr kurz und breit und in den Vorderrand des übrigen Schildes eingesenkt; es endigt vorne nicht mit einem, sondern mit einem Paar Dorne. Diese Dorne sind die ersten von einer Reihe von Dornen, die sich fast an den Hinterrand des Schildes erstreckten und die teilweise paarig, teilweise alleinstehend sind. Sie sitzen auf einer niedrigen, mittleren Längskante des Schildes. Die Furche c und die Furche b (Fig. 1, siehe auch BOAS 1880 Tab. IV), die sich an dieselbe schliesst, sind sehr deutlich und tief eingeschnitten. Wie bei den Loricaten ist der Schild mit zahlreichen Stacheln versehen, deren nähere Beschreibung für uns keine weitere Bedeutung hat.

Der Schwanz ist wie der Thorax ziemlich breit, und die Form ist ganz die der Reptantia. Das Epimer des ersten

Schwanzsegmentes ist ganz obsolet. Das des zweiten Segmentes ist wie auch z. B. bei *Nephrops* gross, viel grösser als die folgenden, die successiv kleiner werden, am kleinsten ist das letzte am sechsten Segment. Die Epimeren setzen sich auf die Oberseite der Segmente mit einer etwas erhöhten Partie fort, die abgegrenzt ist von der übrigen Oberseite; am 2.—5. Segment findet sich an diesen Partien der Oberseite je ein Paar behaarte Querfurchen, die nicht die Mitte erreichen. Diese Furchen finden sich auch bei *Nephrops* und bei *Palinurus*, die auch dieselben am ersten Segmente haben (wo sie sich in der Mitte vereinigen); auch bei einigen Scyllariden (*Arctus*) habe ich Furchen gefunden, die wie es scheint dieselben sind. — Das 1.—4. Schwanzsegment hat auf der Mitte des Rückens einen grossen, starken, nach vorne gerichteten Dorn. — Das letzte Schwanzsegment ist eine dreieckige Platte, die mit einer Spitze endigt.

Das erste Schwanzsegment bietet einen Charakter dar, den ich bei keinem anderen Decapoden gefunden habe (Fig 1). Vorne auf der Dorsalseite, neben demjenigen Teil des Ringes, der sich unter den Hinterrand des Schildes bei der Flexion hineinschiebt, ist jederseits ein kurzer Fortsatz entwickelt, der in eine Grube am Hinterrand des Schildes hineinpasst; es scheint, dass beide zusammen, Fortsatz und Grube, eine Art Gelenk bilden: Es wird somit hier eine besondere Verbindung zwischen Schwanz und Thorax ausgebildet, die sonst meines Wissens bei den Decapoden nie gefunden wird.

Die Kiemen bestehen jede aus einem Stamm mit zahlreichen in Querreihen geordneten Fäden, also wie bei den Homariden und Loricaten. Ihre Zahl ist aber geringer als bei diesen (sowohl die Homariden als die Loricaten haben

jederseits 21 Kiemen); hier bei *Polycheles* ist nach BATE (1888) nur 16 vorhanden, die folgendermassen entspringen:

V. Thoraxfuss:	1	von	Pleuron.										
IV.	—	: 1	-	—	; 2	von	der	Gelenkkant;	1	vom	Epipodit.		
III.	—	: 1	-	—	; 2	-	-	—	; 1	-	—		
II.	—	: 1	-	—	; 2	-	-	—	; 1	-	—		
I.	—	: 0	-	—	; 2	-	-	—	; 1	-	—		

Mp_3, Mp_2 } Keine Kiemen.

Wenn meine Angaben über die Kiemenzahl nicht auf Selbstuntersuchung beruhen, stammt es daher, dass es mir an den Exemplaren, die ich darauf untersuchte, nicht gelang, eine sichere Beantwortung der Frage über den Ursprung der Kiemen zu erhalten, und ich nicht wünschte, mehr Exemplare auf diese für meine Aufgabe nicht sehr wichtige Frage zu opfern.

Verwandtschaftliche Stellung.

In meiner älteren Arbeit (BOAS 1880 p. 95) habe ich ausgesprochen, dass *Polycheles* zweifellos den Reptantia angehört, massgebend sind namentlich die Ausbildung des 3. Kieferfusses, die Antenne, der 1. Schwanzfuss (einblättrig), die Form des Schwanzes, namentlig das Epimer des 1. Schwanzsegments. Daran halte ich noch immer fest, muss aber hervorheben, dass ich jetzt in einigen Punkten an die Natantia Anknüpfungen finde, die meist oder immer den Reptantia fremd sind, also dass *Polycheles* in einigen Punkten Charaktere von den Natantia bewahrt hat und sich in diesen Punkten ursprünglicher verhält als andere (oder die meisten) Reptantia. Es sind dies folgende: der grosse Fortsatz am Innenrand des Basalgliedes der Antennule entspricht offenbar dem grossen Fortsatz an derselben

Stelle bei den Natantia. Die Reihe nach vorn gerichteter Stacheln am Rostrum und deren Fortsetzung an der Mittellinie des Schildes erinnert lebhaft an die Natantia. Auch dürften die nach vorne gerichteten Stacheln an der Dorsalseite des 2.-4. Schwanzsegments den ebenso gerichteten Stacheln an der Dorsalseite des Schwanzes bei der Penaeide *Sicyonia* (Bate 1888 Pl. XLIII) entsprechen. Bei *Sicyonia* (ibid.) finde ich auch ähnliche Furchen wie die bei *Polycheles* erwähnten, die allerdings wie erwähnt auch bei einigen anderen Reptantien als *Polycheles* vorhanden sind. Am 1. Schwanzfuss des Männchens ist am Innenrande des Endblattes eine Reihe von kleinen Hafthaken vorhanden, die offenbar denjenigen entsprechen, die bei *Penaeus* am Innenrande des inneren Blattes desselben Schwanzfusses vorhanden sind. Bei *Nephrops thomsoni* (Bate 1888 Pl. XXVI, Fig. p), bei *Axius* und *Thalassinus* finden sich dieselben Haken. Wichtig ist auch das zugespitzte Endglied des Schwanzes, ganz wie das der Penaeiden und anderer Natantia, aber in direktem Gegensatze zu dem der Reptantia. Ich erwähne auch, dass *Polycheles* ein Hautskelett hat, das für das eines Reptantes ungewöhnlich schwach verkalkt ist, wenn auch stärker als bei den Natantia.

Von den Familien der Reptantia dürften die Homariden und die Loricaten diejenigen sein, die *Polycheles* am nächsten stehen. Der Bau der Kiemen ist der der Homariden und Loricaten, während andere Reptantgruppen mehr oder weniger von denselben abweichen. Das letzte Thoraxsegment ist bei ihnen wie bei *Polycheles* angewachsen (im Gegensatz zu *Astacus*, allen Thalassiniden und allen Anomalen).

Wenn wir übrigens sämtliche Data über *Polycheles*, die wir gefunden haben, zusammenstellen, meine ich, dass die Stellung desselben folgendermassen bestimmt werden kann.

Polycheles und die Loricaten stammen von einer gemeinsamen, unbekannten Form ab, die den Homariden angehörte, und zwar einer Form, die noch von den Natantien einige Charaktere bewahrt hatte, nämlich kräftige Schwanzfüsse mit hakenbesetzter Appendix interna, einige Schwimmfähigkeit mittels der Schwanzfüsse, und anderes mehr; andererseits waren schon das 2. und 3. Glied der Thoraxfüsse unbeweglich verbunden, die zweite Maxille hatte etwas rückgebildete Lacinien und war nur als Atmungsventil tätig, indem die zwei Maxillen dieses Paares weit von einander seitlich gerückt waren; das Rostrum des Schildes war sehr verkürzt. Der Schild war etwas abgeplattet, mit schwachen Seitenkanten.

Von dieser Urform ist die Ausbildung in zwei verschiedenen Richtungen gegangen, die eine resultierte in *Polycheles*, die andere in die Loricaten. Der *Polycheles*-Zweig ist ausgezeichnet durch das völlige Verschwinden der Exopoditen an dem 2. und 3. Kieferfuss, durch die Reduktion der meisten Epipoditen, durch die Ausbildung einer Chela am vierten Thoraxfuss (einzig unter den Reptantia), durch die Bewahrung der ursprünglichen Form und Funktion der Schwanzfüsse, durch die Bewahrung des spitzen Schwanzendes, durch das schwache Hautskelett, und anderes mehr.

Die andere Richtung, die der Loricaten, wurde dadurch ausgezeichnet, dass sie die Chela an den 1.—3. Thoraxfüssen verlor, dass die Schwimmfähigkeit der Schwanzfüsse und die Hafthaken der Appendix interna verloren gingen. Die Basalglieder der Antennen verwuchsen und die Squama der Antenne ging verloren. Das letzte Schwanzsegment hat die Spitze verloren. Das Hautskelett ist schwer geworden.

Postembryonale Entwicklung.

Vor vielen Jahren habe ich (Boas 1879 p. 256 u. 1880 p. 96) es wahrscheinlich zu machen versucht, dass die Larven-»Gattung« *Amphion* die Larve von *Polycheles* ist. Ich begründete dies mit der deutliche Verwandtschaft, die *Amphion* mit den Phyllosomen zeigt, und andererseits mit der deutlichen Verwandtschaft von *Polycheles* mit den Loricaten (deren Larven ja die Phyllosomen sind). Da davon nicht die Rede sein könnte, dass *Amphion* eine Loricaten-Larve wäre, war nur die Möglichkeit übrig, dass es sich um eine *Polycheles*-Larve handelte. *Amphion* ist ein so allgemeines pelagisches Tier, dass er nicht einer unbekannten seltenen Decapodenform angehören kann, er muss einer einigermassen häufig vorkommenden Tierform angehören (*Polycheles* ist weit verbreitet).

Die Ähnlichkeiten von *Amphion* (Fig. 11) mit den Phyllosomen sind hauptsächlich die folgenden: In dem ungemein platten Schild ist dieselbe platte Leber wie bei den Phyllosomen vorhanden, ich lege hierauf besonders grosses Gewicht, da keine andere Decapodenlarve als die Phyllosomen sonst meines Wissens eine solche Entwicklung zeigt. Ebenso wie bei den Phyllosomen ist das 2. Glied der Thoraxfüsse ungemein lang, der Exopodit derselben dagegen im Verhältnis zu dem Endopodit ungemein kurz. Wenn 6 Paar Schwimmfüsse vorhanden sind, sind es der 2. und der 3. Kieferfuss und die Thoraxfüsse 1—4, und der 5. Thoraxfuss ist dann eine Zeit lang ganz kurz. Alles wie bei den Phyllosomen.

Vergleichen wir den *Amphion* mit der Larve anderer Reptantia ist es offenbar, dass er ebenso wie die Phyllosomen dem Zoëa- und dem Mysisstadium und allerlei Übergangs-

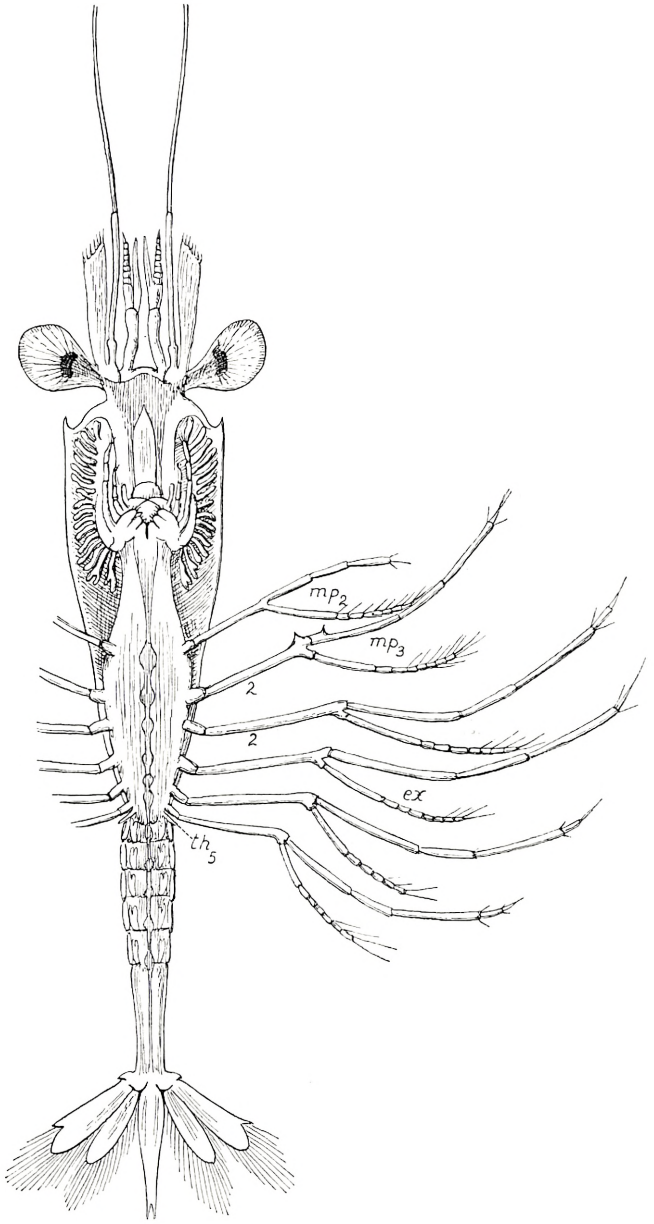


Fig. 11. *Amphion*. *ex* Exopodit. mp_2 und mp_3 zweiter und dritter Kieferfuss. th_5 fünfter Thoraxfuss. *2* zweites Glied der Rumpffüsse.

stadien zwischen beiden entspricht. Die jüngsten beschriebenen *Amphion* stehen noch auf dem Zoëa-Stadium, sie besitzen nur zwei Paare von Schwimmfüssen (Bate 1888 Pl. CXLVI Fig. 1), die offenbar der 2. und 3. Kieferfuss sind.

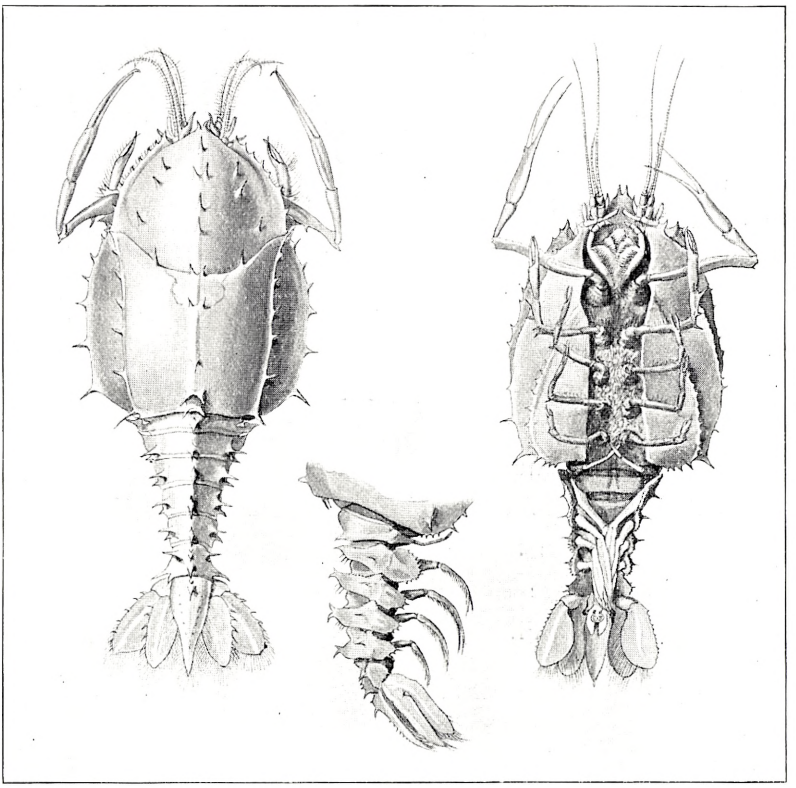
Nach allem vorliegenden kann ich nicht bezweifeln, dass *Amphion* die Larve von *Polycheles* ist, wenn ich auch voraussehe, dass einige Kollegen einen solchen »Indicien«-Beweis nicht für genügend beweiskräftig halten werden.

Weiter ist hervorzuheben, dass wir schon längst Bekanntschaft gemacht haben mit einem späteren Entwicklungsstadium von *Polycheles*, nämlich dem Natant-Stadium desselben. Ich habe (Boas 1880) auf ein Entwicklungsstadium der Loricaten aufmerksam gemacht, das schon fast in allen Stücken mit dem ausgebildeten Tier übereinstimmt, aber von diesem sich namentlich dadurch unterscheidet, dass es noch durchsichtig ist, und dadurch, dass die Schwanzfüsse 2—5 recht kräftig und mit einer hakenbesetzten Appendix interna versehen sind, offenbar ein Stadium, in welchem der Krebs wie eine Garnele sich schwimmend umherbewegen kann. Dasselbe Stadium kommt noch bei *Homarus* und bei vielen anderen Reptantia vor.

Auch bei *Polycheles* haben wir dieses Stadium (eigentümlich entwickelt), wie Sund (1915) richtig behauptet hat[1]. Er braucht aber von demselben nicht den Namen Natant-Stadium, sondern nennt das betreffende Tier, das früher unrichtig als vermeintlich ausgebildetes Tier unter den Namen *Eryoneicus* beschrieben wurde, eine Larve. Dass Sund das Tier als Larve bezeichnet hat, ist vielleicht daran Schuld, dass seine Auffassung wenig Anschluss gewonnen

[1] Der erste, der die Möglichkeit ausgesprochen hat, dass *Eryoneicus* ein Entwicklungsstadium von *Polycheles* sein könnte, ist übrigens Bate (1888 p. 125).

hat; es handelt sich ja im Natant-Stadium um Tiere, die nur in wenigen Verhältnissen noch von den Adulten abweichend sind; so ist es auch hier (Fig. 12). Sund (1915)



a c b

Fig. 12. *Eryoneicus spinoculatus*, a) von oben (nach Bouvier 1917), b) von unten (Orig.), c) Schwanz von der Seite (Orig.).

hat mit grosser Energie die Sache aufgenommen. Sein Hauptargument ist folgendes: »To begin with, it must be mentioned, that the best specific characters both in *Polycheles* and *Erion[e]icus* are derived from the arrangement of the spines on the carapace. Now a peculiar correspondance is observed

in several species of the two »genera«, making it possible to »pair« several of the *Eryon[e]icus* species each with one of the *Polycheles* species«, z. B. die atlantische *Polycheles sculptus* Smith mit *Eryoneicus Faxoni* Bouvier von demselben Meer. Bouvier, der den Gedanken Sund's sehr scharf entgegengetreten ist (Bouvier 1917 p. 54 ff.), macht diesen Daten gegenüber die interessante Bemerkung: »Il faut convenir, que ces ressemblances extraordinaires sont troublantes et l'on comprend que M. Sund ait cru devoir les signaler« (op. cit. p. 56). Er fügt hinzu, dass er trotzdem glaubt, dass sie zufällig sind, aber diesem kann als eine rein willkürliche Bemerkung keine wissenschaftliche Bedeutung zugelegt werden. Mit Recht hebt Sund (1915) auch hervor, dass: »If *Erion[e]icus* were adult animals, it would be rather remarkable that not a single egg-bearing female has been found among the fifty-nine specimens known to have been captured«. Jetzt liegen viel mehr Exemplare vor!

Ausser den von Sund hervorgehobenen Punkten spricht auch die schwache Entwicklung der drei letzten Thoraxfüsse (Bate 1888 Pl. XII E, Fig. 2) dafür, dass es sich nicht um ein ausgebildetes Tier handelt. Dass es kein völlig ausgebildetes Tier ist, passt auch zu dem ganzen gallertartigen Habitus des Tieres, der dem eines erwachsenen Decapods ganz fremd ist. Geht man dem Tier näher nach, findet man, wie es einem Natant-Stadium gebührt, fast überall dieselben Charaktere wie bei dem Adulten: Die Mandibel sind dieselben, auch die zweite Maxille und den merkwürdigen ersten Kieferfuss von *Polycheles* finden wir wieder. Das ist alles, wie man es von einem Natant-Stadium erwarten kann. Dass bei einigen *Eryoneicus*-Exemplaren der 1. und 2. Schwanzfuss schon in männlicher Richtung

ausgebildet sind, sagt nichts. Das Natant-Stadium ist ja das unmittelbar dem Geschlechtsstadium angrenzende, und es ist sehr natürlich, dass diese Charaktere hier schon ausgebildet werden können.

Nach alledem halte ich es als ganz überwiegend wahrscheinlich, dass *Eryoneicus* das Natant-Stadium von *Polycheles* repräsentiert.

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WITH 2 CHARTS

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INTRODUCTORY REMARKS

During recent decades the hydrospheric and atmospheric changes in arctic and subarctic regions, from Greenland in the west to Eurasia in the east, have been so great, that the attention of various branches of science gradually has been directed to a closer study of the conditions; this applies not only to the hydrology and meteorology of these regions, but also to the biology and glaciology.

For my own part I have had opportunities of following certain phases of the phenomenon in the case of Greenland. To begin with, I imagined that the changes might be something special for that country; but as time went on, reports also came in from other northern areas, describing similar phenomena. Consequently, there could be no talk of special or peculiar changes in Greenland alone; these had a wider distribution. I have now decided to bring together the notes I have been making for a number of years and prepare them for publication, so that they combined with my own experiences may be of some use to others who are interested in these problems and are seeking information regarding them.

As Greenland was the original starting point of my studies, I may begin with this country before dealing with other areas.

Greenland.

In the years 1908 and 1909 I was commissioned by the Danish Home Office, then in charge of the administration of Greenland, to undertake fisheries investigations both practical and scientific in the waters of West Greenland, with the object of determining, whether the fisheries there could not be developed into an industry for the benefit of the native population, to balance the decrease of the seal fishing. During these investigations, which were carried out with the brig "Tjalfe" and which extended from Cape Farewell to Umanak (ca. 60°—71° N. L.) including the coast, fjords, offlying banks and deep sea, I obtained a good insight into the conditions. And ever since then I have kept in close touch with the fishery, inaugurated by these investigations, and have been able to see — partly from the reports forwarded yearly from the fishing stations to the Greenland Administration, partly through the practical-scientific investigations, which the Administration has directed for a number of years in Greenland — how deep-going changes have occurred in the fish fauna of Greenland in the course of the years.

Most remarkable have been changes in the occurrence of the common cod.

In the later years of last century and beginning of the present it had been well known from experience, that the Cod *(Gadus callarias)* only occurred at a few places in the

waters of South-west Greenland and only in scattered quantities. During my expedition we confirmed these experiences; the cod were only met with in number at Fiskenaesset and in the sounds near Cape Farewell. But since the year 1917 the cod has gradually appeared over much wider areas and in far greater numbers, first in the southern districts, Julianehaab and to some extent also Frederikshaab, since 1919 in the area of Godthaab Fjord, since 1922 in the Sukkertoppen district and from 1927 also in the district of Holsteinsborg. We thus see, that the distribution of the cod has gradually expanded in the direction from south to north.

This northerly trend in the occurrence of the cod was continued in the following years. In 1927 already the cod reached the southern part of the Egedesminde district, to Iginiarfik (ca. 68° 10′ N. L.), but only in small numbers. In 1928 towards the end of July shoals of cod appeared in the Egedesminde district, along the coast from Agto to Hunde Eiland in Disko Bay and Akunak (a little to the east of Egedesminde). In 1929 the cod was found everywhere in the Egedesminde district at the beginning of July; large numbers remained there during the whole summer and similarly in 1930. In 1931 the cod extended even further into Disko Bay, to Ikamiut, Akugdlet, Christianshaab and Claushavn in the Christianshaab district, and to the Ritenbenk district, even reached the Umanak district (N. of 70° N. L.).

In 1932 the following dates can be stated for the gradual advance of the cod into these northern districts: In the Egedesminde district it arrived at most places about the middle of July, at Christianshaab on July 22nd, Ritenbenk on August 24th and to the Umanak district not before the beginning of September. In the years 1933, 1934, 1935

and 1936 the cod has persisted in coming to these northern districts and in point of time earliest (middle of July) at the southernmost (Egedesminde district) and latest to the northernmost (Umanak district).

In more recent years a few isolated individuals have reached as far north as the south district of Upernavik, including the settlement of Upernavik at $72^{3}/_{4}°$ N. L., but not further north.

This increasing extension of the cod had the result, that the Greenland Administration were able to set up year by year an increasing number of stations along the west coast of Greenland, where the native population could sell and prepare the cod taken as split fish or salt fish for export; at present there are 53 of these stations, the most northerly being Umanak. Some idea of the increasing abundance of the cod can be obtained most easily from a study of the graphic representation in Fig. 1, which illustrates the great upward movement in the returns since the cod fishery began in 1911; in the years 1911—1916 the amount rose from 18 to 125 tons, 1917 to 1925 from 250 tons to 1000 tons, 1926—29 from 2000 tons to 5600 tons, culminating in 1930 with 8160 tons. In more recent years the quantity has oscillated up and down, yet always considerable, between 6125 and 8000 tons[1].

As striking evidence of the difference between the earlier conditions and the present I may give the following additional examples.

[1] The decrease in recent years need not be taken to indicate, that the cod period is beginning to ebb, it may be due to other causes than decreasing numbers of cod, e. g. sickness among the native population during the fishing season; both in 1935 and 1936, to some extent also in 1937, the people of Greenland in the cod-fishing districts were visited by a devastating form of influenza epidemic.

In 1908 during the period 11.—21. June we fished in the Godthaab Fjord with lines, hand-lines and nets, both in the inner and outer parts of the fjord, from Kornok to off the Kook Islands and took only 3 cod altogether; in 1930, when the fishery reached its culmination, the Green-

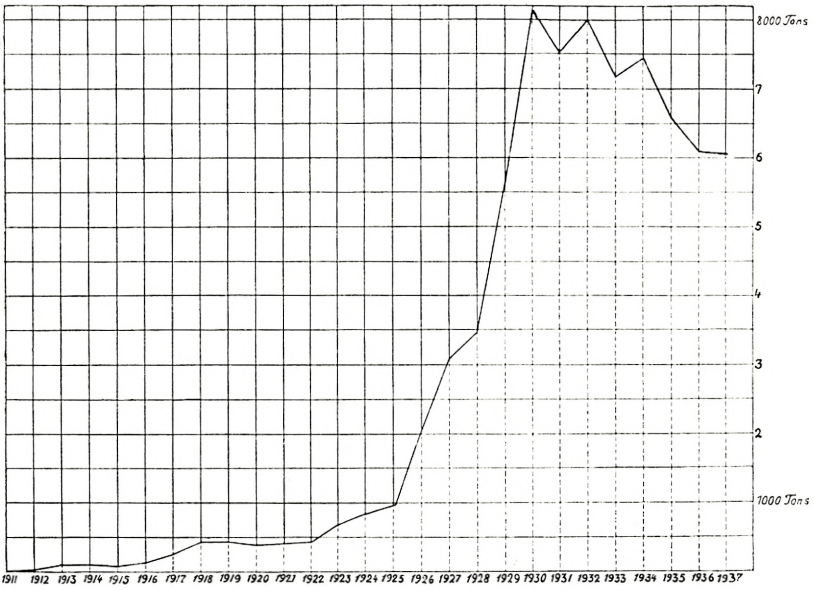

Fig. 1. Product of the cod fishery of the Greenlanders, from 1911 to 1937. Note the great increase after and including 1926 (cf. p. 34).

landers fished in this same fjord about 750,000 kg. of cod. And in Agdluitsok, also called Lichtenau Fjord (Julianehaab south district), in 1909 from 9. August to 2. September we had numerous long-lines out over the whole fjord, from the mouth right to the heads of the two arms of the fjord, without catching any cod, nor could the Greenlanders dwelling in the fjord, in spite of offers of rich rewards, obtain a single cod; in 1932, when the fishery in this fjord culminated, the Greenlanders caught 548,000 kg. of cod.

On the banks off the coast, where in poor years the cod are practically absent, the presence of this fish was noticed for the first time in 1921, some being taken in that year on Fyllas Bank off Godthaab. In the following years the numbers greatly increased on Fyllas Bank and the southern part of Lille Hellefiske Bank off Sukkertoppen and gave rise to a fishery by fishing boats from Europe, especially from the Faeroes, but also from Norway, England, France and Portugal. Good catches were made. Gradually the cod also spread more to the north and the Greenlanders, who were accustomed to carry on a halibut fishery with motor boats on the Store Hellefiske Bank off Holsteinsborg and never before had taken cod on their hooks, now caught the cod on their long lines, for the first time in 1924, later obtaining more cod than halibut. Thus, on the banks also there was a north-going movement of the cod.

With regard to East Greenland, in 1931 Johs. Schmidt published a paper[1] in which, among other things, he discussed the information he had been able to gather regarding the occurrence of the cod in the Angmagssalik District, the settlement managers Johan Petersen and A. Hedegaard being his informants. From this information and the written reports to the Greenland Administration by the latter we find, that the cod was unknown before 1912, both to the Danes and Eskimos at Angmagssalik. The cod was first observed there in 1912 and subsequently some few thin and wasted specimens were caught. It may be mentioned also, that a number of fishing experiments carried out under instructions of the Greenland Administration in 1916

[1] Johs. Schmidt: On the Occurrence of the Cod (*Gadus callarias* L.) at East Greenland. Conseil Internat. pour l'Explor. de la Mer, Rapports et Procès-Verbaux des Réunions, Vol. LXXII, V. Copenhague, 1931.

and 1917 did not find a single cod (though quantities of sharks, rays, sea-cats, Greenland halibut and Norway haddock). But about 1920 the cod began to appear in small shoals; from 1923 on it was fairly common and in the years towards 1930 it occurred almost everywhere in the district, especially in the interior of the many fjord branches, where the glaciers are not too close, and in the sounds at Cape Dan as well as round the islands there.

It must certainly, wrote HEDEGAARD to me from Angmagssalik on 11. August 1931, be regarded as a fact, that the cod has only come to this district in recent years. Since it seems but little probable, that no one among the population would have detected the fairly considerable quantities, shoals indeed, which are now reported as occurring at many places in shallow waters near the mouth of rivers, where the natives had always been accustomed to fish in kajaks, *inter alia*, for the char.

SCHMIDT concludes this part of his paper with the following summary (l. c. pp. 4—5): "In the last 5 years the cod has thus occurred in quantities in the Angmagssalik district, and it has been met with in smaller quantities since about 1912. This is the outstanding fact, but we can scarcely doubt also, that the phenomenon is of recent date in these parts of Greenland, at any rate as far as large quantities are concerned".

With this conclusion of SCHMIDT I quite agree.

As to the state of things in the Angmagssalik district since 1930, I am able to give the following information: In 1931—32 the Greenland Administration commissioned A. HEDEGAARD to carry out fishing experiments, with long-lines, hand-lines and jigger. It was then found, that in the autumn of 1931 the cod were present in some numbers at

most places in Tasiusak and in Angmagssalik Fjord, much to the domestic benefit of the natives, but nowhere in such large quantities that worth-while export could be made. From the Angmagssalik reports also for the years 1932 to 1937 it appears, that in all these years the cod fishery in the autumn has contributed to the food supplies of the population; and since seal blubber is often lacking, the oil from cod liver has been used instead in the lamps which are so important in the heating of the houses.

Thus, since 1930, there has also been cod in the Angmagssalik district and the conditions in this part of East Greenland have been in complete agreement with those in West Greenland.

It may be added, that the cod in the Angmagssalik waters occurs in summer and autumn as a rule, but according to uniform statements from the dwellers in the district the cod were present in large quantities at the settlement during the whole winter of 1930—31, as well as at most dwelling places in Angmagssalik Fjord, round Cape Dan and to some extent in Sermilik Fjord.

The question then arises: Where did these cod come from, which since 1917 have appeared in increasing quantities in the fjords and along the coasts of West Greenland and since 1926 have practically overrun the whole west coast from Cape Farewell to Holsteinsborg and even further north, right up to Disko Bay? Are these cod which have grown up at Greenland, or have they come from somewhere else?

That the cod may spawn in Greenland waters was proved by the "Dana" investigations in 1925, when the eggs and tiny fry were found near the surface over the offlying banks, from the Fiskenaes Bank off Godthaab south district to the south part of the Store Hellefiske Bank, and also in Godt-

haab Fjord[1]. Later, the fisheries biologist PAUL HANSEN[2] discovered, that the cod has a scattered spawning along the west coast in the Godthaab, Sukkertoppen and Holsteinsborg districts and good spawning grounds occur at a few places in the fjords of the same districts, thus at Kapisigdlit in Godthaab Fjord, at Atangmik in Angmagssivik Fjord and in Ikertok Fjord. One example may be cited. In the beginning of May 1936 PAUL HANSEN obtained 12,000 cod eggs in one haul of half an hour's duration with the 1m. ring trawl; this was in the mouth of the Angmagssivik Fjord (Sukkertoppen district).

The small, 1-year old cod are also frequently seen in shoals right in on the beaches, not only in the districts mentioned, but further north also (in 1935 many of the I-group were caught at Godhavn, Christianshaab and Rodebay) where they are borne in the pelagic stage by the north-going current, and also to the south (in 1937 there were unusually many of them in the districts of Julianehaab and Frederikshaab) though here not through the action of any south-going current but possibly new arrivals from Iceland. The "Dana" investigations in the waters between Iceland and Greenland have shown, that in certain years large quantities of tiny cod fry are carried from Iceland over towards East Greenland in the westgoing branch of the Irminger Current[3].

[1] AD. S. JENSEN: Investigations of the "Dana" in West-Greenland Waters, 1925. Rapports et Procès-Verbaux du Conseil Intern. pour l'Explor. de la Mer. Vol. XXXIX (pp. 85—91). Copenhague, 1926.

[2] PAUL M. HANSEN: Fiskeriundersøgelser ved Grønland. Beretninger og Kundgørelser vedrørende Styrelsen af Grønland. København, 1931—1938. — Idem: Oversigt over Fiskeriundersøgelserne ved Grønland. Det Grønlandske Selskabs Aarsskrift. København, 1937.

[3] Å. VEDEL TÅNING: Some Features in the Migration of Cod. Journal du Conseil Internat. pour l'Explor. de la Mer. Vol. XII, No. 1 (pp. 16—17). Copenhague, 1937.

That the Greenland cod has in recent years been in active reciprocal communication with the cod of Iceland, is now a well-known fact demonstrated by marking experiments. Cod taken at Iceland and liberated after marking have been recaught again in the waters of Greenland (food wanderings) and conversely (spawning migration[1]). There is every reason to believe, that these wanderings have been of very considerable dimensions, comprising many millions of cod, and that they have been of great service to the Greenland fishery[2].

If we glance back over the history of Greenland, we come upon the interesting fact, that in the previous century there were two periods of cod abundance at West Greenland, resembling greatly the present period. Dr. H. Rink, who rendered good service to the natural history of Greenland, reports for the years round 1820, that cod were present in enormous numbers in the Julianehaab district and reached as far north as Disko Bay. Thereafter cod were absent for a long series of years. The second period, according to Rink began in the forties; then also there was an abundance of cod from the southernmost parts of the west coast, shoals extending right up to Disko Bay; this gave rise from the Danish side to a commercial fishery, and English fishing vessels also took part, the results of the fishing being good for a time in the fjords, along the

[1] The principal spawning places of the cod are as is well known situated in the North Atlantic, an eastern at Norway (Lofotens), a central at the south and south-west of Iceland and a western at Newfoundland.

[2] Paul M. Hansen, Ad. S. Jensen & Å. Vedel Tåning: Cod Marking Experiments in the Waters of Greenland 1924—33. Medd. fra Kommiss. for Danmarks Fiskeri- og Havundersøgelser. Serie: Fiskeri. Bd. X, Nr. 1. København, 1935. — Å Vedel Tåning l. c. 1937.

coast and on the banks; but by 1850 the cod had become so scarce that the fishery ceased to pay. For the remainder of the nineteenth and beginning of the present century we have no certain information regarding good cod periods until we come to the present, as outlined here[1].

Other species of the cod family which have appeared at Greenland during this cod period, include the well-known European species Coalfish or Green Cod (*Gadus virens*). It had not been noticed in Greenland waters since far back in the preceding century[2], but in recent years—since 1924—it has not rarely been found among the cod shoals, in the districts of Julianehaab, Frederikshaab, Godthaab, Sukkertoppen and Holsteinsborg. The coalfish seems even to have spawned at Greenland; in addition to adult specimens of about 1 metre in length, small specimens 21—34 cm. long, belonging to the I-group, have been taken at Sukkertoppen and especially in the Frederikshaab and Julianehaab districts; at Arsuk (Frederikshaab south district) these 2-year old coalfish appeared near the shore in very great shoals in 1930 and 1932.

Then in October 1929 a 61 cm. long, thus adult, specimen of the Haddock (*Gadus aeglefinus*) was caught off Sydprøven (south district of Julianehaab). This species has never previously been known from West Greenland. And in August, September and October 1931 not a few were caught in the Sukkertoppen district (at Agpamiut and Kangamiut). In 1932 a specimen was caught at Nanor-

[1] Cf. Section: "History of the Greenland Cod Fisheries" in AD. S. JENSEN and PAUL M. HANSEN: Investigations on the Greenland Cod (*Gadus callarias*). Rapports et Procès-Verbaux du Conseil Internat. pour l'Explor. de la Mer, Vol. LXXII, 1. Copenhague, 1931.

[2] In the Copenhagen Zoological Museum are two small specimens sent down, preserved in salt, from Frederikshaab in the year 1831.

talik and one at Itivdlek in the district of Holsteinsborg. Further, in the first half of May 1937, a 77 cm. long specimen was taken in a cod-net at Holsteinsborg harbour. Lastly, 2 specimens, 58 and 75 cm. long, were taken on 29. 7. 1937 in an eel hand-net in shallow water (5—8 m.) in Sangmissok, a branch of the Kangerdluarsorujuk Fjord at Sardlok south of Julianehaab.

Previously unknown at West Greenland also was the Brosme (*Brosmius brosme*), one specimen of which was caught at the end of July 1936 in Ikertok Fjord in the district of Holsteinsborg and another on 11. 9. 1937 at Narssalik (Julianehaab district) taken on a long-line, at a depth of ca. 150 m.

The Ling (*Molva vulgaris*) was likewise unknown, but one specimen was caught in September 1928 just off the outermost islands at the outpost Narssalik in the district of Frederikshaab.

The Halibut (*Hippoglossus vulgaris*), which during the summer comes into the shallow water from the depths out in the Davis Strait, had its northern limit previously along the north edge of the Store Hellefiske Bank; in recent years it has penetrated further north and is now fished during summer and autumn in no small numbers off the Lakse Bugt at Disko (ca. 10 miles west of Godhavn), in Disko Fjord and right up to Nugssuak, Niakornat, Umanak, Ikerasak, Satut and Sermiarssuit in Umanak Bay. In recent years some few specimens have reached as far north as the Upernavik district (up to ca. $73^1/_2°$ N. L.)

The Herring (*Clupea harengus*) was formerly a rare fish in the southernmost parts of West Greenland, where in fact it only occurred in small shoals in the northern part of the Frederikshaab district (Sarfâ near Avigait). In 1909 I here

carried out fairly extensive investigations for the herring between 22. 6. and 1. 7. but the results were hardly worth mentioning, the whole catch consisting only of about a score of specimens. And in other places it was not taken at all in 1908—09. In recent years, however, the herring has appeared over more extensive areas and to some extent in greater quantities. It may be mentioned, in comparison, that on 24. July 1932 one haul with the coastal drag-seine at the just-mentioned place Sarfâ yielded over 2000 herring, on 30. June 1933 ca. 5000 herring and on 9.—10. 6. 1937 over 9000 herring in two hauls with the eel-seine. In the years 1928—1936 the herring were taken not only at several places in the Julianehaab district (specially numerous in the neighbourhood of Sardlok, where a single haul on 28. 7. 1937 yielded 4500 specimens) and noticed in Igaliko Fjord, Kangerdluarssuk and Tunugdliarfik Fjord and the Frederikshaab district (Ivigtut Fjord, Avigait and Sarfâ), but also in the Godthaab district (Fiskenaes Fjord and Godthaab Fjord). Even right up in the Sukkertoppen district, where herring had never been known before, it has occurred yearly since 1930 during the summer at Ikerasak, in southern Isortok and at the dwelling place Kangerdluarssuk, at the colony settlement and at Ikamiut as also in Evigheds Fjord; to show that the herring could occur in numbers it may be mentioned, that 3228 were taken in one haul with the eel-hand-net on 9. August 1937.

The northern movement of the herring does not stop however at Sukkertoppen district. Assistant Magnus Jensen in Upernavik informs me, that in the end of August 1934 two quite large herring were caught in a char net north of Prøven, at the head of Lakse Fjord ($72^1/_2°$ N. L.)

In 1932 (18. 8.) herring eggs were found in Tasiussak near Igdlokasik in the Julianehaab district; they were attached to algae in shallow water and the young were ready to hatch out. In the neighbourhood of Sardlok the herring were in full spawning on 8. August 1934; on hauling in the seine became full of large clumps of eggs and the water in the bay was tinged milky white with the floating sperms. It would appear, that the spawning of the herring extends throughout the whole of August and into September.

One peculiarity about the occurrence of the herring at Greenland is remarked upon by Paul Hansen, namely, that it comes right in on the beach in quite shallow water to lay its eggs, the highest temperature being found there[1].

Quite tiny, 1-year old herring of 5—6 cm. were taken on 4. 8. 1930 in the Tunugdliarfik Fjord (Julianehaab district). At Avigait in the Frederikshaab district large quantities of the 2- and 3-year old herring have several times been taken. We may conclude, that the herring in recent years both spawns at southern West Greenland and grows up there. At present the herring also spawns further north along the coast; among a quantity of herring taken in the beginning of August 1937 in the Sukkertoppen district (Kangerdluarssuk Fjord) there were many with ripe eggs and milt.

Paul Hansen undertook the counting of the vertebrae in samples of herring from Julianehaab, Frederikshaab and Sukkertoppen districts, to determine their race; it proved that the herring from these places had the same racial character, which agreed with that of the summer-spawning herring of Iceland[2]; he concludes, there probably is only

[1] Paul Hansen l. c. 1937, p. 50.

[2] The data for the vertebrae have been published by Dr. Tåning in his paper: The Herring Stocks of the Faeroes, Iceland and Greenland. Rapp. et Proc.-Verbaux des Réunions, Vol. C, II, p. 33. Copenhague, 1936.

this one herring race at Greenland[1]. A spring-spawning herring is not found there.

On the east coast of Greenland the herring was first observed in 1932. PAUL HANSEN[2] reports, that in that year herring shoals were seen in the neighbourhood of Cape Walløe and at Griffenfeldts Island and that a large herring had been forwarded to him taken in the Angmagssalik district.

On his expedition to East Greenland in 1933 Captain THOR IVERSEN met with herring at several places in the Denmark Strait in July, along the deep-sea ridge from Greenland to the north-west coast of Iceland. Dr. RUNNSTRÖM[3] has examined specimens of these herring and found, that in regard to number of vertebrae and age-composition they are very similar to the large herring of North Iceland, thus the same conclusion as arrived at by PAUL HANSEN in the case of the herring of West Greenland.

The Salmon (*Salmo salar*) was previously only known and only in small numbers at two places, namely, Kapisigdlit in Godthaab Fjord and Amerdlok Fjord near Holsteinsborg. But towards the end of the twenties the observation was made, that in the autumn (October and November) a migration of large salmon takes place in the Sukkertoppen district, though not very near the coast. Later, especially in 1935 and 1936, the salmon occurred in numbers from October and on into December at several places in

[1] Beretninger og Kundgørelser vedr. Grønlands Styrelse, 1934, Nr. 2, p. 241.

[2] Beretninger og Kundgørelser vedr. Grønlands Styrelse, 1933, Nr. 1, p. 42.

[3] SVEN RUNNSTRÖM: The Distribution of the Atlanto-Scandian Spring Herring. Cons. Int. p.l'Explor. de la Mer, Rapp. et Proc.-Verbaux des Réunions, Vol. 100, p. 27. Copenhague, 1936.

the Sukkertoppen district, at the dwelling place Ikerasak and in fjords round about as also at the outpost Napassok and the dwelling place Agpamiut. In the autumn of 1935 about 200 of this stately fish were caught at Ikerasak. In September 1936 a number of salmon were caught at Lichtenau, in 1935 in October several salmon were noticed at Fiskenæsset, and in September 1938 a salmon was caught in the Tasermiut Fjord.

The Piked Dogfish (*Squalus acanthias*), which earlier was a great rarity at Greenland, has been taken there in recent years (1931—36), one specimen in the Sukkertoppen district (1933), two in Holsteinsborg district (1931 and 1934) and in 1936 one at Claushavn in the Christianshaab district and one at Ikerasak in the Umanak Fjord—5 specimens in all. The sex of 4 specimens was determined and they were all females (one from Sukkertoppen contained 7 embryos and one from Holsteinsborg 4 embryos, all large). All these dogfish were taken in the summer and autumn months (June—September).

The previously known specimens, taken at long intervals, were from the Sukkertoppen and Holsteinsborg districts, like the three above-mentioned. Remarkable, therefore, is the 1936 occurrence of the two last-mentioned specimens right up in Disko Bay and Umanak Fjord.

The North Atlantic open-sea Deal-fish (*Trachypterus arcticus*), previously only noted once as having stranded at Greenland (Frederikshaab 1890), has in recent years (1923—34) been found stranded on the beach no fewer than 7 times in the southern part of West Greenland, 5 in the Julianehaab district and 2 in the Sukkertoppen district.

The Norway Haddock (*Sebastes marinus*). When I investigated the waters west of Greenland in the spring and

summer of the years 1908 and 1909, I was unable to find the fry of this fish and concluded, that the species does not spawn at West Greenland. The older pelagic young found there in the autumn I believed to have come with the marine currents from the Denmark Strait, where newly hatched fry occur in great quantities in the spring[1].

When I investigated the same waters later with the ss. "Dana" in 1925, the conditions had greatly changed. During the period 7. June to 9. July we obtained the small pelagic fry (7—17 mm.) of this species in the ring-net on no fewer than 19 stations in the Davis Strait, but nowhere north of the submarine ridge which extends right across the Strait from the level of Holsteinsborg to Cumberland in America[2]. Later investigations came to the same result.

Thus, in contrast to the earlier period, the species had now been spawning in the Davis Strait[3].

The Capelan (*Mallotus villosus*) or "Angmagssak", as it is called in Greenland, was the object of special attention during my investigations of 1908 and 1909, as it plays such an important part in the food supply of the native population. At that time it came into the fjords in enormous shoals to spawn along the beach, right from the southernmost part of the land up to Disko Bay and the middle of Vaigat. It did not normally enter the North-east Bay, but

[1] Ad. S. Jensen: *Sebastes marinus*. Vidensk. Meddel. fra Dansk naturhist. Foren., Bd. 74, p. 90. København. 1922.

[2] Ad. S. Jensen: Investigations of the "Dana" in West Greenland Waters, 1925. Conseil Internat. pour l'Exploration de la Mer, Rapp. et Procès-Verb. des Réunions, Vol. XXXIX, p. 97. Copenhague, 1926.

[3] To avoid any possible misunderstanding I wish to note expressly, that on the voyage over to Greenland in 1908 I caught a number of the small fry of the Norway Haddock in the northern parts of the Atlantic, south of the Denmark Strait, with the same apparatus as used in the Davis Strait with negative result.

might occur, though not every year, in small shoals in the southern part of the Upernavik district, whereas it was unknown in the northern part of the district. A change has now set in. The capelan now penetrates into the North-east Bay in quantities and regarding this M. P. Porsild, Director of the Danish Arctic Station in Greenland, has given me the following information. At Igdlorssuit on Ubekendte Eiland the first capelan appeared in 1927; at Ikerasak they first appeared in 1933, but only a few, in 1934 there were very large shoals, in 1935 there was abundance everywhere on the island and its surroundings; many were also taken at Uvkusigssat. In 1929 Porsild did not find any capelan at the peninsula Svartenhuk, whereas in 1935 he found many on both south and east coasts. In 1936 Paul Hansen took an enormous quantity of capelan in July in the neighbourhood of Sermiarssuit; they occurred along a considerable stretch of the coast. In near Ikerasak he caught a couple of thousand of capelan of the I-Group. In 1935 the capelan appeared for the first time and in quantities at Krauls Havn in the northern part of Upernavik district. Further, the manager Lund-Drosvad has informed me, that in that year the capelan reached right up to Igdlulik at 74° 25′ N. L., in such large quantities that they could be scooped up with a baler. In 1935 a specimen was even taken at Thule (76° 30′ N. L.) and another at the same place in 1936; both specimens—adult males—were forwarded to me by the settlement manager at Thule Hans Nielsen, who reported at the same time, that none of the natives knew this fish. In the Thule district these capelan were caught on 22. September and 13. August respectively, whereas the fish arrive in Umanak Fjord in the beginning of July and, further south, at the end of May and in June.

That the capelan may even spawn in Umanak Bay and North-east Bay is shown by the fact, that the Godthaab Expedition of 1928 captured the fry of the year with the 2 m. ring-net at the following places:

70°44′ N.,	52°16′ W.;	35 m.	wire;	4/9;	28	spec.	13—27 mm.
—	—	250 -	—	—	6	—	13—23 -
71°16′ N.,	54°17′ W.;	80 -	—	3/9;	18	—	11—20 -

In East Greenland the capelan is a well-known fish at Angmagssalik where it is eagerly sought after by the population; in fact, the district is called after this fish, the name Angmagssalik meaning that the "Angmagssak" occurs there. North of this place the capelan has not been observed; E. Bay, who knew this fish well at Angmagssalik, states expressly that it was not found at Hekla Havn or anywhere else in or north of Scoresby Sound during the East Greenland Expedition of 1891—92[1]. Things have changed in recent years. From his visit to Scoresby Sound the zoologist Alwin Pedersen brought home 6 specimens (4 ♂, 2 ♀), taken in that Sound on 19. August—3. September 1927.

Regarding the Fjord-Cod or the "Uvak" (*Gadus ogac*) at West Greenland almost the same may be said as for the capelan. At many places in South Greenland, where the fjord-cod formerly appeared in abundance it has now almost vanished. At the same time it has spread further north; in 1932 it was taken for the first time as far north as at the dwelling place Ituvsalik 74° N. L., according to written communication from the settlement manager Lembcke Otto in Upernavik. And at various places in North Greenland, where it was previously few in numbers, it is now found in great quantities, for example, in Umanak Bay.

[1] Meddel. om Grønland. 19. Hefte, p. 57. København, 1896.

I am also able to mention, that a marine mammal has extended its range from southern waters to West Greenland, namely, the Ca'ing Whale (*Globiceps melas*) so well known at the Faeroes. Since 1926 it has become a more frequent visitor in summer than in earlier years, when it had only been noted a few times on the south-west coast[1]. Thus, in the middle of September 1926 about 200 were killed at Sukkertoppen. On 24. October 1928 a shoal of 185 was surrounded and caught in the Godthaab Fjord, and almost at the same time a small shoal was seen at Sukkertoppen. At the end of September 1931 120 specimens were caught at Kangamiut, at Itivdlek 150 and at Sarfanguak 50. In August 1935 about 40 ca'ing whales were killed on the Kvane Fjord in the neighbourhood of Frederikshaab. On 5. October 1935 a shoal was chased into the Ulke Bay at Holsteinsborg and about 300 whales in all were killed. In 1935 some shoals of this whale even reached up to Disko Bay at the end of September and the inhabitants of the southern district of Christianshaab pursued them with success, for example, 35 were killed at Akugdlet. At the end of September 1936 several hundreds were seen in Hamborg Sound, some of them being shot at Agpamiut. It has happened only of recent years—something never known before—that the ca'ing whale has passed the winter at Greenland; in the winter of 1931—32, for example, when a shoal remained in the neighbourhood of the outpost Utorkarmiut in the Godthaab south district.

In the nature of the case, as the foregoing pages indicate, changes in the marine fauna of a land like Greenland

[1] Winge gives several examples of the relative scarcity of the ca'ing whale at West Greenland in the 19th century, with exception of 1853. Cf. Herluf Winge: Grønlands Pattedyr, pp. 504—505. Meddel. om Grønland, XXI. København, 1902.

can on the whole only become known for such animals as are of economic importance and the subject of practical-scientific investigations. There is no purely scientific zoological station in Greenland and we cannot expect to be able to show corresponding changes among the invertebrate animals. Yet we have one example of an invertebrate coming within recent years into the Greenland waters from more southerly seas, namely, the jelly-fish *Halopsis ocellata*. This large craspedote medusa, belonging to the Fam. *Mitrocomidae* among the *Leptomedusae*, was first noted as occuring at Greenland by Dr. KRAMP in 1932[1]. "In 1928 I found several specimens off the west coast of Greenland as far north as the Disko Bay, about 69° N." In 1926 and 1927 Mag. PAUL HANSEN obtained this medusa in the plankton nets in the Sukkertoppen district, especially in the southern parts round Napassok. He also observed it in the boat harbour at Ivigtut in 1927, in Karssorssat harbour in 1929; in 1937 he saw many specimens at Julianehaab, where it was then the most common jelly-fish, and it was very abundant in the harbour at Sydprøven.

Since 1926, therefore, this medusa has been found along the west coast, from Sydprøven (60°25′ N.) to Disko Bay (69° N.).

It is quite inconceivable, that this fair-sized medusa (it measures 60—70 mm. in diameter) could have been overlooked, if it had occurred at West Greenland formerly. It is not given as Greenlandic in any earlier paper where its distribution is noted and we may conclude, that it has only come to West Greenland in recent years. On the east coast

[1] P. L. KRAMP: A Revision of the Medusae belonging to the Family Mitrocomidae. — Vidensk. Meddel. Dansk Naturh. Foren. Bd. 92 (p. 355). København, 1932.

of North America *Halopsis ocellata* is distributed from Grand Manan to Cape Cod; further, it occurs in the waters of north-western Europe, from the south-west of Ireland to the south coast of Iceland and western Norway up to Tromsø[1]. In relation to West Greenland, therefore, this species has a southern distribution and its occurrence there in recent years may be viewed in the light of changed climatic conditions.

Another invertebrate has in recent years become common in South-west Greenland, namely, the Common Starfish (*Asterias rubens*). This echinoderm is well-known from the lusitanian and boreal waters of Europe—in the north it reaches to the White Sea and Iceland—and was first taken, in 1895, by the Ingolf Expedition in the innermost part of Ameragdla, an inner branch of the Ameralik Fjord south of Godthaab. In 1926 Paul Hansen saw it right up in Angmagssivik in the Sukkertoppen district, and in 1927 he found it in large numbers in shallow water in Kapisigdlit, an inner arm covered with Zostera of the Godthaab Fjord. In recent years Paul Hansen has also found, that *Asterias rubens* is now very common from Julianehaab up to Holsteinsborg. Dr. Th. Mortensen, who has determined his material, gives as new localities: Sydprøven, Julianehaab harbour, Ikertok Fjord and off Holsteinsborg harbour[2]. As several naturalists had made large collections in South-west Greenland at earlier periods, it is difficult to believe, that they could have overlooked this large and obvious starfish.

As *Asterias rubens* earlier, in contrast to the present, was a great rarity and only found in a single locality, I am

[1] P. L. Kramp: Craspedote Medusen. 3. Teil. Leptomedusen. Nordisches Plankton, Bd. XII, p. 567. Kiel u. Leipzig, 1933.

[2] Th. Mortensen: Echinoderms (The Godthaab Expedition 1928). Meddel. om Grønland, Bd. 79, Nr. 2, p. 23. København, 1932.

most inclined to believe, that it is not really an immigrant, but a "relict", persisting since the warm period which prevailed in Greenland in postglacial times[1]. The species has survived the subsequent deterioration of the climate by retiring to the innermost branch of a fjord; and now that the conditions have become more favourable, it begins again in quite recent years to spread along the south-west coast of Greenland with great rapidity, almost like the results of an explosion.

In preceding pages it has been noted for West Greenland, how some forms of animal life (*Gadus aeglefinus, Brosmius brosme, Molva vulgaris, Halopsis ocellata*), unknown earlier there, have appeared on this coast in recent times; and how other forms (*Gadus callarias, G. virens, Clupea harengus, Salmo salar, Squalus acanthias, Trachypterus arcticus, Globiceps melas, Asterias rubens*), which previously had been comparatively rare and local in occurrence, have extended their distribution along the coast and at the same time in some cases (*Gadus callarias, Clupea harengus*) have enormously increased in numbers; further, some (*Hippoglossus vulgaris, Mallotus villosus*), which were previously common but now distributed further north than before; and lastly, one species (*Sebastes marinus*) which has only spawned at West Greenland in recent years.

On the other hand, we have the experience at the same time, that arctic forms have kept away from, or shortened

[1] Ad. S. Jensen: On Greenland's fossil Mollusc-Fauna from the quaternary time. Meddel. om Grønland, Bd. 29, p. 301, 1905. København, 1909. — Ad. S. Jensen & Poul Harder: Postglacial Changes of Climate in Arctic Regions as revealed by Investigations on Marine Deposits. Postglaziale Klimaveränderungen, p. 399. Stockholm, 1910.

their stay in South-west Greenland. The White whale, also called Whitefish (*Delphinapterus leucas*), which lives far to the north in summer but migrates south in the autumn to pass the winter along the west coast about the Polar Circle, has not arrived at Sukkertoppen in recent years before the middle of November and has again turned northwards already in January, according to information sent me by Mr. N. L. Nielsen, who has directed the white-fish fishery for the government during the past 25 years. In earlier years it came down to Sukkertoppen in the middle of October and did not depart before April and early May; in recent times indeed the white whale has almost disappeared here in the south in winter, but has been observed to prolong its stay in the north. And the capelan (*Mallotus villosus*), which used to come each year in enormous numbers close to the coast to spawn, from Disko Bay right down to the south point of the land, has for some years failed to appear at several of the earlier fishing places in the waters of South Greenland. On the other hand, the capelan has extended its area and even spawned further north than before (cf. pp. 19—21). And the fjord-cod (*Gadus ogac*) has become more numerous in North Greenland, but rarer in south-western Greenland. It should be noted further, that the arctic flatfish, the Greenland halibut (*Reinhardtius hippoglossoides*) has occurred during recent years in much smaller quantities than previously in the fjords of the most south-western Greenland (cf. pp. 30—31).

Review of the hydrographic conditions with some examples of the change in fish-fauna with the temperature of the sea.

If we should ask now, what can be the cause of these deep-going changes in the animal life at Greenland, the

answer must be, that we should seek for it in changed physical conditions, such as marine currents, ice conditions, temperature and conditions affecting productivity and so on.

We may consider first of all the marine currents, which must be discussed rather more closely, for the sake of other areas to be mentioned later.

Along the east coast of Greenland there runs a cold, ice-filled current from north to south. It comes from the Polar Sea and is in fact the principal outlet of this Sea to the south. The Polar Sea receives water first and foremost from the Norwegian Sea (Gulf Stream), but the large rivers, which open into it from the neighbouring continents, also bring water which sooner or later must find its way to the south. Owing to the deflecting force of the earth's rotation the descending Polar Current is restricted mainly to the East Greenland shelf, whilst out over the deeper parts of the Greenland Sea—the sea west of Spitzbergen and Jan Mayen—there is warmer water of Atlantic origin at corresponding depths. At one place, however, in the neighbourhood of 70° N.L., the Polar Current gives off a branch towards the south-east over towards North and East Iceland, but the great mass of it continues southward as far as the south point of Greenland, where it changes direction and runs northwards along the west coast of Greenland; here it gradually loses its force.

This ice-bearing current provides the reason, why the whole of East Greenland, and to some extent also South-west Greenland, is so unfavourably situated by comparison with West Norway, though it lies in the same latitude. It is only on the west coast, that a branch of the Gulf Stream brings some mitigation.

Whilst the Polar Current comes from the north, from

the Polar Sea, the Gulf Stream (more correctly called the "North Atlantic Current") comes from the south-west, from the Atlantic, passing up through the Faeroe-Shetland Channel and on to West Norway (Norwegian Atlantic Current), where the mild climate is due to this warm current combined with prevailing south-westerly winds. To the north of Norway the Gulf Stream sends branches into the Polar Sea, eastwards to the Barents Sea (North Cape Current) and northwards to the west coast of Spitzbergen (Spitzbergen Atlantic Current), where it keeps the water free of pack-ice in summer[1].

An arm of this Spitzbergen Atlantic Current flows northward into the Polar Basin[2]. Another flows westward into the Greenland Sea, where it comes to lie under and outside the East Greenland Polar Current; this relatively warm and salt stream, which was first detected just here and its Atlantic origin rightly indicated by Ryder, can be followed down along the coast of East Greenland as far as the submarine ridge, which extends from Iceland to Greenland

[1] Knipowitsch has drawn a schematic chart of the finger-like prolongations of the Gulf Stream in the European Polar Sea; it shows in broad lines, where the branches come to the surface and where they continue below the cold covering layers. Cf. N. Knipowitsch: Ichthyologische Untersuchungen im Eismeer, II, chart p. 38 with explanation pp. 39—40. Mem. de l'Acad. Imp. des Sciences, Cl. Phys.-Math. Vol. XXII, No. 4. St. Pétersbourg. 1908.

[2] This warm Atlantic water under the cold Polar water has been found by the recently concluded Russian North Polar investigations right up under the North Pole itself and the investigators were able to follow it down to off North-east Greenland at 75° N. North of 86° N. L. the surface layer with negative temperatures was 250 m. thick, the layer below with positive temperatures was 500 m. thick with maximum of 0.77° C.; further south, between 86° and 85° the cold layer was 200 m. thick, the warm layer 750 m. and its temperature 0.88° to 1° C.; still further south the Atlantic layer became more extensive and the temperature rose to 1.72° C.. P. Shirshov & E. Fedorov: Scientific Work of the Drifting North Polar Station. Nature, Vol. 141, p. 629. London, 1938.

across the northern part of the Denmark Strait; where the current goes thereafter is a matter of doubt, but in any case it does not affect Greenland any more. It is replaced by a very warm and mighty branch of the Gulf Stream, which has separated from the main stream in the Atlantic and flows northward towards the south coast of Iceland and further on along the west coast. This branch is called the Irminger Current after its discoverer, the Danish Admiral IRMINGER.

West of Iceland the Irminger Current divides into two arms, detected by MARTIN KNUDSEN on the Ingolf Expedition 1895—96, an east-going arm along the north coast of Iceland and a western, which continues in a southerly direction accompanying the East Greenland Polar Current. Some of this west-going water of the Irminger Current probably returns from East Greenland towards the south-east and east, in the great circulatory system between Iceland and Greenland[1], but a powerful part goes on round Cape Farewell like the Polar Current and is then turned northward by the earth's rotation along the west coast of Greenland. The major part of the water which flows north along the west coast of Greenland, thus consists in part of the East Greenland Polar Current and in part of the Irminger Current; they are however already well mixed along the east coast. As the currents gradually spread up along the west coast, they decrease in strength and importance, tending little by little out towards the west in the Davis Strait. Yet the warm current can still be traced at some depth (ca. 500 m.) through Baffin Bay.

[1] Dr. Å. VEDEL TÅNING informs me, that current bottles liberated from the "Dana" in 1931—34 on the west side of the Denmark Strait, stranded in large numbers on the west and south-west coasts of Iceland.

In the southern waters of Greenland there is therefore a conflict going on between the cold water of the Polar Current and the warm from the Atlantic Current; and according as to which obtains the upper hand, it is influencing the fauna, as the various examples mentioned in the foregoing have shown.

As a concrete example of the extent to which the fish fauna may change according to the temperature of the sea, I may mention the following:

The temperature of the water in Agdluitsok (Lichtenau Fjord) south of Julianehaab was measured in 1909 by Dr. phil. J. N. Nielsen and in 1934 by Cand. mag. Paul Hansen; comparing the results we have the following:

Agdluitsok Fjord

22. 8. 1909		16. 8. 1934
m.	°C.	°C.
0	3.85	5.20
10	1.45	3.65
25	0.52	2.52
50	0.62	1.36
75	0.44	..
100	0.07	1.09
125	0.38	..
200	0.61	1.50
275	..	2.19
300	0.59	..
390	0.58	..

We see that the column of water was throughout warmer in 1934 than in 1909. The fish fauna had also quite a different appearance; during the "cold period" of the fjord it was an arctic species, the Greenland halibut (*Reinhardtius hippoglossoides*), which dominated; in August 1909 I had long-lines laid out in the fjord and 808 Greenland halibut were caught on 4860 hooks, thus one Greenland

halibut on every 6th hook; on the other hand, not a single cod (*Gadus callarias*) was taken, neither on these lines nor in attempts with hand lines. In the "warm period" of Agdluitsok, on the other hand, it was the boreal *Gadus callarias* which dominated; in 1934 533,679 kg. of this fish were caught, whereas the arctic *Reinhardtius hippoglossoides* had become very scarce, as was shown by the experimental fishings of the biologist PAUL HANSEN in 1929, 1930 and 1932 at the same places where I had set out the lines in 1909[1].

An example from middle Greenland may also be given, namely, Godthaab Fjord.

In the cod-poor years (cf. p. 7) the following temperatures were measured by Dr. phil. J. N. NIELSEN.

Mouth of Godthaab Fjord

20. 6. 1908

m.	°C
0	0.75
25	0.60
50	0.62
100	0.58
200	0.61
385	0.54

Off Sardlok inside Godthaab Fjord

15. 6. 1908

m.	°C
0	1.90
25	0.82
50	0.64
100	0.24
200	0.29
300	0.56
400	0.85

[1] It should be noted, however, that the decline of the Greenland halibut might be due, though hardly to any great extent, to the fact, that quantities are taken out in the Davis Strait. As I have shown, this halibut does not spawn in the fjords and bays of West Greenland where the Greenlanders fish for them, but out in the deeper parts of the Davis Strait, south of the submarine ridge which extends about 67° N. L. from Greenland to America (AD. S. JENSEN: The Greenland Halibut (*Reinhardtius hippoglossoides*), its Development and Migrations. Kgl. Danske Vidensk. Selsk. Skrifter; Naturv. og Math. Afd., 9. Række, VI, 4. København, 1935). In recent years the waters out here have been fished by large fleets after the halibut (*Hippo-*

In the rich cod period (cf. p. 7) Cand. mag. Paul M. Hansen obtained the following temperatures:

Mouth of Godthaab Fjord		Off Sardlok inside Godthaab Fjord			
11. 6. 1935		8. 6. 1936		26. 6. 1937	
m.	°C.	m.	°C.	m.	°C
0	4.35	0	3.10	0	3.30
10	3.62	10	2.81	10	2.76
25	2.65	25	2.22	25	2.69
50	2.00	50	2.00	50	2.47
100	1.31	100	1.68	100	2.36
200	1.14	200	1.26	200	2.04
300	1.10	300	1.11	300	1.98

We see here again, that the water is considerably warmer in the rich cod years than in the poor period.

These are examples from the most southerly and central parts of Greenland; another may be added from the most northern occurrence of the cod. As mentioned previously, in 1931 the cod reached up to the Umanak district at 71° N. L. and in such large numbers, that a beginning was made of an export industry, and this has repeated itself each year since then. In this connection it is of interest, therefore, that Dr. Fritz Loewe[1], who carried out hydrographical investigations in the Umanak Bay in 1932, measured there temperatures of about 2° C. in the core of the warmer water-masses (400 m.), which surpasses by 0.6° to 0.7° the values found by the "Godthaab" (1928), whilst the salinity was 0.1 ‰ higher, certainly a small amount but clearly significant owing to its frequent recur-

glossus vulgaris), and during this fishery many Greenland halibut are taken on the hooks. (cf. Årsberetning vedkommende Norges Fiskerier 1935, No. IV, p. 112 and 1936, Nr. V, p. 132. Bergen, 1937).

[1] Fritz Loewe: Hydrographische Untersuchungen in Fjorden Westgrönlands. Arctica, No. 3, 1935, p. 69. Leningrad.

rence. By comparison with the measurements of the "Tjalfe" also (1908), the corresponding temperatures measured by him were 0.7° higher. It is reasonable to conclude, says Dr. LOEWE, that a connection exists between the appearance of the cod up here in Umanak Bay and the rising temperature, which has risen above the lower temperature limit (minimum) for the more abundant occurrence of the cod[1], a view which I quite agree with.

With regard to the conditions on the banks lying off the Southwest Greenland coasts, we find there in summer along their outer side a conflict prevailing between the cold water of the Polar Current and the warm Atlantic undercurrent; taken in conjunction with the heating of the surface water in summer, this conflict determines whether the watermasses over the banks in any year will be warm or not. In a work not yet published but already in manuscript, which he has kindly placed at my disposal, Mag. sci. A. KIILERICH has discussed the conditions on the banks very fully[2]. In these investigations KIILERICH found, among other things, that the Polar Current makes its strongest onslaught in the neighbourhood of the banks (Fyllas Bank) about the first of July, in some years very strongly, in others so feebly that it has but little influence. And KIILERICH comes to the conclusion, that the warm years, which began in the second half of the decade 1920—30, have continued up to the present without noteworthy interruption.

[1] Dr. LOEWE refers here to the investigations of AD. S. JENSEN & PAUL M. HANSEN, and of BEAUGÉ.

[2] A preliminary paper has been published: A. KIILERICH: Svingninger i de hydrografiske Forhold ved de vestgrønlandske Fiskebanker. Nordiska (19. Skandinaviska) Naturforskarmötet i Helsingfors den 11.—16. Augusti 1936, p. 317. Helsingfors, 1936.

This is in good agreement with the statistics which show, that it was from and including the year 1926 that the Faeroese and foreigners, especially Norwegian, French, English and Portuguese, have made good catches of cod on the West Greenland banks. — It will also be seen from the graphic representation in fig. 1 (p. 7), that it was from and including the year 1926, that the really big increase occurred in the returns of the cod fishery of the Greenlanders.

Air temperature.

It is not only in the sea-water that temperatures have risen, the air has also become warmer. At Upernavik, Jakobshavn, Godthaab and Ivigtut the winters have become milder in recent years than formerly. As a concrete example of this, we may consider the conditions at Jakobshavn.

In a paper on the coastal climate of Greenland the Danish State Meteorologist Helge Petersen has drawn up a table showing the variations of temperature in Jakobshavn (69° 13′ N. L.) during the period 1876 to 1932[1]. On the basis of this table Dr. Scherhag of the Weather Bureau (Reichsamt für Wetterdienst), Berlin, has compiled the temperature variations for the true winter months (January to March and November to December) during the last 50 years, and arranged them in 10-year periods so as to determine the deviations from the normal during the period 1876—1925[2]. It will be seen from the table 1, that the amelioration in the winter conditions at Jakobshavn has made steady progress from 1883 to 1922 and has advanced by almost 2° C.. Then

[1] Helge Petersen: Klima der Küsten von Grönland. Handbuch der Klimatologie von W. Köppen und R. Geiger, Bd. II, Teil K, Tabelle 25, pp. 62—63. Berlin, 1935.

[2] R. Scherhag: Die Erwärmung der Arktis. Journal du Conseil, Vol. XII (p. 263). Copenhague, 1937.

the temperature rises further with a jump and the winters at Jakobshavn during the period 1923—1932 have been more than 5° C. warmer than those registered 50 years ago.

Table 1. Deviations of temperature from the mean for 1876—1925 at Jakobshavn in winter (from SCHERHAG)

	1883—1892	1893—1902	1903—1912	1913—1922	1923—1932
°C	— 1.1	— 0.7	— 0.2	0.6	4.0

The summers have also become warmer, though the rise in summer has been considerably less than in winter. As will

Table 2. Deviations of the annual temperature from the normal (from SCHERHAG)

Year	Jakobshavn	Angmagssalik	Jan Mayen	Bear Island	Spitzbergen
1923...	1.7	0.5	0.7	2.3	2.9
1924...	1.06	— 0.3	1.4	1.9	2.5
1925...	0.9	0.3	1.3	2.2	1.9
1926...	2.2	1.7	1.1	0.6	0.8
1927...	2.0	1.6	0.6	0.8	0.3
1928...	4.0	2.3	1.1	1.4	0.7
1929...	4.0	2.5	1.5	0.6	— 0.4
1930...	2.8	1.0	2.0	2.8	2.5
1931...	2.3	0.5	1.1	2.6	2.9
1932...	3.1	2.4	1.1	1.3	1.3
1933...			1.9	3.3	3.0
Mean .	2.5	1.3	1.3	1.8	1.7

be seen from the table 2 the annual average temperature for the period 1923—1932 was 2.5° C. above the normal.[1]

[1] R. SCHERHAG: Eine bemerkenswerte Klimaänderung über Nordeuropa. Annalen der Hydrographie und Meteorologie, 64. Jahrg., Heft III (p. 97 and Table 4). Berlin, 1936. — Cf. also F. LOEWE: A Period of warm winters in Western Greenland and the Temperature See-Saw between Western Greenland and Central Europe. Quart. Journ. Royal Meteorological Society, Vol. LXIII, No. 271. London, 1937.

The east coast of Greenland has also enjoyed a milder climate in recent years. At Angmagssalik the annual average temperature lay 1.3° C. above the normal in the period 1923—32 (cf. table 2); and all winters from 1921 to 1934 have been warmer than the mean for 1895 to 1925[1].

It may be added, that according to Loewe the mean temperature in Upernavik was—5.6° C. in 1926 to 1931 against—8.6° C. in the 50-year period; hence the difference amounted to the large dimension of 3° C.. During the period 1926 to 1931 Godthaab had on an average a positive deviation from the mean of 1.9° C.[2].

Ice conditions.

It was to be expected, that the favourable temperature conditions in the air and sea would affect the ice conditions at Greenland, and there is evidence that this has actually been the case.

In a paper on the ice conditions in the Davis Strait the Danish State Meteorologist, Commander Speerschneider has described his investigations[3] and come to the conclusion, that apart from certain irregularities the period 1820—1860 was poor in ice, the period 1860—1900 was rich in ice and then again a period poor in ice from 1900—1930—the year in which the work was concluded—or pro tem. a period of 30 years while the two former periods each lasted forty years. From 1910 to 1930 the "Storis" (pack-ice) did not reach so far westward as before 1910. In the years 1910—1929 the pack-ice in the Davis Strait did not

[1] Helge Petersen: l. c. Tab. 25. p. 64. — Loewe, l.c. p. 365.

[2] Fritz Loewe, l.c. 1935, pp. 69—70.

[3] C. H. I. Speerschneider: The state of the ice in Davis Strait 1820—1930. Publications from the Danish Meteorological Institute. Meddelelser Nr. 8, København, 1931.

reach beyond 55° W. L., whereas in the years 1890—1909 it not rarely reached 56°—57° W. L., once even to 58°—59° W. L. In the period 1880—1899 the pack-ice reached rather far northward, after 1910 not so far north in the Strait as before 1910. In half of the years before 1910 the ice in the Strait reached to Godthaab (64° 10′ N.) or further northward, while after 1910 Fiskenaesset (63° 05′ N.) seems to be the normal northward limit of the pack-ice.

Along the east coast of Greenland the favourable ice years culminated in 1931, 1932 and 1933, according to the Danish Nautical-Meteorological Annuals. In August 1932 the coast from Cape Farewell to Scoresby Sound was almost free of polar ice. During all the 34 years in which the Danish Meteorological Institute has gathered information concerning the ice, the conditions for navigation have hardly in any other year been so favourable as during the summer of 1933, when in August the sea was free of ice over the whole distance from Cape Farewell to Scoresby Sound; during this month a vessel worked her way up to Lat. 79°, a feat that had never before been accomplished here. In 1934 and especially in 1935 the ice conditions seem to have become worse, but in 1936 in August the Denmark Strait and Greenland Sea up to about 75° N. were free of ice except for some small patches of pack-ice, which constituted no hindrance to navigation.

Iceland.

As mentioned in the foregoing, the south and west coasts of Iceland are washed by the Atlantic Current, whereas the north and east coasts come under the influence of the Polar Current. The considerable difference in the temperature of the sea resulting from this has a great influence on the

fauna, which in general has an arctic character to the north and east of Iceland, boreal to the west and south.

In recent years, however, numerous cases have occurred which indicate, that considerable changes have taken place in the animal life, especially the fish. Our information comes from the Icelandic Government's fisheries consultant, Dr. phil. Bjarni Sæmundsson, who has called attention to these changes, and I attach special importance to his judgment, for he has been untiring in his study of the animal life at Iceland and in the waters round about for the past 40 years and knows the conditions there better than any one. From his paper I take the following[1].

Cod (*Gadus callarias*). The great fishery based on the spawning cod takes place usually along the warmer coasts of Iceland (south and south-west) in the early spring (March-May), whilst this period is mostly a dead period for the fishery on the opposite, colder north coasts. But in most years since 1924, large shoals of mature cod have been met with at North Iceland in the spawning time, resulting in large catches there; and many of these cod were ripe with fully developed eggs and milt.

Here I may add some information received from Dr. Vedel Tåning. During the investigations with the "Dana" in the years 1924—38 the eggs and tiny fry of the cod were constantly obtained both on the north and east coasts, though in fairly small quantities. The conditions have thus changed since the beginning of the century, when neither cod eggs nor fry were found on North or East Iceland, according to Schmidt's classic investigations[2].

[1] Bjarni Sæmundsson: Probable influence of changes in temperature on the marine fauna of Iceland. Rapp. et Procès-Verb. du Conseil Internat. pour l'Explor. de la Mer, Vol. LXXXVI, 1. Copenhague, 1934.

[2] Johs. Schmidt: Fiskeriundersøgelser ved Island og Færøerne i

As to the Capelan (*Mallotus villosus*) B. SÆMUNDSSON writes, that it formerly used to visit the south and south-west coasts, to spawn almost every year in March-May; on the more northern and eastern coasts it spawned later, in May-July. But in the period from 1928 to 1935 very few capelan have appeared on the south and south-west coasts; at the same time they have appeared in great numbers on the north coast earlier in the year than previously[1].

The Herring (*Clupea harengus*) spawns under usual conditions on the south and south-west coasts, like the cod; but in 1926 and 1929 BJARNI SÆMUNDSSON was able to prove that considerable spawning had occurred on the east and north-west coasts, where previously such a spawning had been unknown. Later (1935) Mag. sci. FRIÐRIKSSON has also found summer-spawning at various places on North Iceland[2].

This change noted in the occurrence of the three common fishes mentioned, has according to B. SÆMUNDSSON also been noticed in the case of some of the rarer species. The Witch (*Pleuronectes cynoglossus*) and the Turbot (*Rhombus maximus*), which earlier only occurred on the south and west coasts, have spread in this period respectively to the north and east coast. Further, some of the southern species, which can only be regarded as stragglers in Icelandic waters, have on the one hand appeared in greater numbers than before, and on the other have found their way to the previously unvisited north and east coasts; for example, the Basking

Sommeren 1903. Skrifter udg. af Kommissionen for Havundersøgelser, No. 1 (pp. 59, 72 & 77). København, 1904.

[1] BJARNI SÆMUNDSSON: Fiskirannsóknir. Andvari, 62. Ár, p. 35. Reykjavík, 1937.

[2] ÁRNI FRIÐRIKSSON; Síld hrygnir við Norðurland. Aegir, XXVIII Ár, p. 206. Reykjavík, 1935.

Shark (*Selache maxima*), Tunny (*Orcynus thynnus*), Mackerel (*Scomber scombrus*), Skipper (*Scombresox saurus*), Sunfish (*Orthagoriscus mola*) and the Sudid *Paralepis Krøyeri*.

In later papers Dr. B. Sæmundsson was able to add three species of southern fish, which were previously not known from Iceland, but which have wandered up there in recent years, namely, the Six-gilled Shark (*Notidanus griseus*), Swordfish (*Xiphias gladius*) and the Horse Mackerel (*Caranx trachurus*)[1]. The subject is further discussed in a still later paper[2], which gives new information regarding the frequent occurrence of fish of southern origin at Iceland.

Bjarni Sæmundsson also mentions three species of common North-European Gulls (*Larus ridibundus, L. fuscus* and *L. argentatus*), which were previously rare but have become fairly abundant in recent years. He also notes, that certain Invertebrates, the large Sea-urchin (*Echinus esculentus*) and the well-known Polychaete, the Sea-mouse (*Aphrodite aculeata*), earlier known only from the south and south-west coasts, have now spread to the north coast. In a later paper[3] he adds the Stone-crab (*Lithodes maja*); previously this was only known from the south and west coasts, but in later years it has spread to the north coast. Further, the giant Polychaete *Nereis virens*, unknown earlier at Iceland was found in 1934 at Reykjavik and in the Dyra Fjord[4].

[1] Cf. Skýrsla um hið Islenzka Náttúrufraeðisfélag Félagsárin 1933 & 1934, p. 41; 1935 & 1936, p. 33. Reykjavik, 1935 & 1937. Nátturufraeðigurinn, VII Árg. p. 120. Reykjavik, 1937.

[2] Bjarni Sæmundsson: Zoologiske Meddelelser fra Island, XVII. Vidensk. Meddel. Dansk Naturh. Foren., Bd. 102, pp. 183—212. 1938—39.

[3] Bjarni Sæmundsson: Icelandic Malacostraca in the Museum of Reykjavik. Societas Scientiarum Islandica, XX. Reykjavik, 1937.

[4] Skýrsla um hið Islenzka Náttúrufraeðisfélag Félagsárin 1935 & 1936, p. 34. Reykjavik, 1937.

The cause of these changes in the animal life at Iceland is found by BJARNI SÆMUNDSSON in the increased temperature of the sea and air during the last 10—15 years. In the first place the polar ice or drift ice which often comes down on the north-west, north and east coasts, has been practically absent, except in the year 1929 when a lot of ice was about in July and August. At the same time the winters have been extraordinarily mild, especially in February and March, when the mean temperature was some 4° to 7° C. above normal. The high winter temperatures at Iceland in these years and the great scarcity of ice in these northern waters were due first and foremost to the higher temperature in the sea round Iceland, combined with the prevailing southerly winds during this period. The surface temperature has risen 0.5°—4° C. above normal, being higher on the east and north-east coasts, lower on the south and west coasts. The increase must be considered due to a stronger Irminger Current than usual, for this high temperature was not restricted to the surface, but penetrated down into the deeper layers (200—400 m.). Roughly speaking the temperature of the sea on the north and east coasts was very nearly the same as in the corresponding depths off the south and south-west coasts, where the water is usually warmer than on the other coasts of Iceland.

In connection with BJARNI SÆMUNDSSON's observation of the occurrence of spawning herring on the east coast, Dr. Å. VEDEL TÅNING makes the following remark on the summer-spawning herring at Iceland. The fact that a considerable spawning has taken place at south-east Iceland during the present period (1924—34), whereas anything of the kind was not known in the earlier period (1903—09),

may also be taken as a sign of a change produced by the present warmer period in the waters of Iceland[1].

An interesting feature mentioned by Dr. Runnström (l. c. pp. 25—26) may also be referred to in this connection. He found that the number of vertebrae in the herring of Iceland at present (1931—33) is lower than that found by A. C. Johansen in his racial investigations on the herring for the years 1900—1924; this result is in full agreement with the well-known phenomenon, that higher temperatures lower the average numbers of vertebrae.

Dr. Tåning[2] writes further, that larvae of the Capelan (*Mallotus villosus*) in recent years have occurred on the whole in much smaller numbers at most of the Icelandic coasts than in the beginning of the century. This agrees with the diminution in quantities of this fish and changes in its biology at Iceland within recent years, as described by Bjarni Sæmundsson.

The hydrographer Helge Thomsen has recently published a paper on the variations of the surface temperature at Selvogsbanki, Iceland, during the years 1895—1936[3], and summarises the results of his investigations as follows: "It is very striking that the period 1895—1912 contains three abnormal years only, while cold and warm years otherwise change; the period 1913—1925 on the other hand seems cold, 6 years definitely cold even, while the period 1926—1936 has been warm, 6 of the years definitely warm". This

[1] Å. Vedel Tåning: Distribution of postlarval stages of herring in Icelandic Waters 1924—34. Cons. Intern. p. l'Explor. de la Mer, Rapp. et Procès-Verbaux des Réunions, Vol. XCIX, VI, p. 17. Copenhague, 1936.

[2] Ibid. 1936.

[3] Rapports et Procès-Verbaux des Réunions, Vol. CV, X, p. 51. Copenhague, 1937.

falls in line with BJARNI SÆMUNDSSON's statement, that the surface temperature of the sea has risen since 1926[1].

Jan Mayen.

Captain IVERSEN has published an interesting report on the investigations he was able to carry out near and on this island[2].

From his account of the fishing experiments it appears, that in 1930 and 1931 both Cod (*Gadus callarias*) and Herring (*Clupea harengus*) were found at Jan Mayen, in no small quantities, though insufficient for a commercial fishery; this was also found to be the case in 1929, likewise from the Norwegian side.

Captain IVERSEN notes at the same time, that in 1900 during the fisheries investigations at Jan Mayen from the "Michael Sars" under the direction of JOHAN HJORT, no food-fishes were found though all sorts of fishing apparatus were used.

These changes in the occurrence of cod and herring at Jan Mayen, according to IVERSEN, must be ascribed to the great variations in the temperature of the water in this area, which lies on the borders of the drift ice; the comparison below shows what a different picture is shown by the hydrographical data for the years 1900 and 1930:

Jan Mayen

	8. 8. 1900	8. 8. 1930
m.	°C.	°C.
0	4.20	7.07
10	..	6.87
20	2.21	..
25	..	6.73

[1] Cf. agreement with South-west Greenland (p. 34, above).

[2] THOR IVERSEN: Sydøstgrønland, Jan Mayen. Fiskeridirektoratets Skrifter, Serie Havundersøgelser, Vol. V, Nr. 1, pp. 101—171. Bergen, 1936.

m	8. 8. 1900 °C.	8. 8. 1930 °C.
50	0.89	4.44
70	0.80	..
75	..	1.57
100	0.41	1.37
145	..	0.40

Further information given by Capt. Iversen includes, that in the period 1924—33 there was extremely little ice round Jan Mayen, in contrast to 1882—83 (the year when the Austrian Expedition stayed the winter there) which belonged to a period with much ice, severe winter and cold summer, by comparison with the years 1924—33. In the summer months (June—September) the average temperature was 4.7° C. in 1924—33 against 2.6° C. in 1882—83; for the whole year the mean temperature was 0.1°C. in 1924—33 against —2.3° C. in 1882—83 (cf. pp. 146—147, Fig. 109 and Tab. III in Iversen).

In this same paper (p. 116) Captain Iversen brings forward evidence to show, that the cod at Jan Mayen comes from Iceland. Both in 1930 and 1931 he found hooks of Icelandic origin in cod taken at Jan Mayen; and from marking experiments made in 1930 he found, that cod migrate from Jan Mayen to Iceland. The migration to Jan Mayen is explained as a wandering after food, whilst the return to Iceland is for the purpose of spawning.

Barents Sea, Murman Coast, White Sea, Novaya Zemlya, Kara Sea.

For these areas we have reports from a number of Russian observers, that the temperature of the sea has risen during recent years as the result of a strong inflow of warm,

Atlantic water; in consequence, great changes have come about in the fauna, both plankton and benthos, a number of new forms of boreal character having penetrated into the areas from the warmer waters lying to the west (North Norway).

Among the investigators who have occupied themselves with the conditions discussed here, Professor KNIPOWITSCH should be first mentioned and we may attach special importance to his statements, for his investigations and studies over many years have given him an intimate knowledge of the hydrological and biological conditions in the European Arctic Ocean, both as they were at the beginning of the present century and also later.

KNIPOWITSCH has shown[1], that a warmer period has prevailed in the south-western part of the Barents Sea from the year 1921 than in the beginning of the century. At a series of hydrographic stations ($69^1/_2°$—$72^1/_2°$ N. L.) along the Kola meridian (33° 30′ E.) the temperature at depths of 0—200 m. was 1.06° to 3.48° C. higher at the end of May 1921 than at the same date in the years 1900 and 1901 ; the mean difference was 1. 9° C.. After the year 1921 there were certainly considerable fluctuations in the temperature of the sea water, but on the whole we must regard this period as relatively warm.

It was possible to show, that simultaneously with the rise in temperature of the sea considerable changes occurred in the composition and distribution of the fauna. Food-fishes of Atlantic origin were caught in large quantities farther to the east and north-east than in earlier years; thus, in the

[1] N. M. KNIPOWITSCH: Hydrologie und Fischerei. Explorations des Mers d'U. R. S. S., Fasc. II, 1930, p. 33. — Idem: Rasche Veränderungen hydrologischer und biologischer Verhältnisse im Barents-Meer. Bulletin de la Commission pour l'Etude du Quaternaire, 1931, No. 3, p. 19. Leningrad.

autumn of 1921 the Cod (*Gadus callarias*) was found in large numbers in the south-eastern part of the Barents Sea, considerably farther east than previously[1]. Further, in the plankton of the Kola Fjord Knipowitsch found the colony-forming Radiolarian *Collozoum* several times in May 1921 already, although this form had not previously been taken east of Finmark. In the following years a number of animal forms were found on the Murman Coast (*Gibbula tumida*[2], *Acera bullata*) which were previously unknown there, and

[1] During the "cold" period something happened, which was diametrically opposite to what occurred in the following "warm" period. In the years 1902 and 1903 arctic animals, such as the Greenland Seal (*Phoca groenlandica*), the Ringed Seal (*Phoca foetida*) and the White Whale (*Delphinapterus leucas*), whose mass-wanderings are otherwise restricted to purely arctic regions, came down in enormous schools to the northern coasts of Norway and farther also along the west coast. Previously the fixed ice-boundary had advanced farther west and south than any one could remember; even in May 1902 it extended as a continuous wall from Spitzbergen and Bear Island down towards the Murman Coast, not far from Varanger Fjord, presumably owing to the prevailing northerly and easterly winds over Spitzbergen and the Barents Sea. The temperature of the sea and air was noted as low. The special meteorological and hydrographical conditions in conjunction with the advance of the ice were taken to be the cause of the mass-incursion of arctic animals to Norway. At the same time the great cod fisheries in the northern districts of Norway were a failure and the fishermen believed, that the arrival of the seals had driven the cod from the coast. It might be questioned, however, whether it was not the changed natural conditions, which enabled the arctic seals and whales to thrive on the northern coasts of Norway, which had the opposite influence on the boreal cod and forced them to go elsewhere. — Detailed reports on these remarkable occurrences are given by Alf Wollebaek: Über die Biologie der Seehunde und die Seehundjagd im Europäischen Eismeer, hauptsächlich nach norwegischen Quellen, pp. 20—24. Rapports et Procès-Verbaux, Vol. VIII. Copenhague, 1907. And by R. Collett: Norges Pattedyr, pp. 391—392, 399—404 and 660—664. Kristiania, 1911—1912.

[2] Interestingly enough shells of this gastropod were found earlier on the Murman Coast in marine deposits of interglacial age, the molluscan fauna in which shows indications of having lived in a warm period of distinctly oceanic character.

for which the eastern boundary had been Finmark; other species, rare before, had now become common (e. g. *Cardium edule* and *Echinus esculentus*).

In a paper published at the same time DERJUGIN deals with the same theme[1] and comes to similar results. In addition to the animal forms mentioned by KNIPOWITSCH as penetrating to the Murman Coast from western areas, DERJUGIN notes *Galvina* and *Cuthona* among the nudibranchs and the Hermit Crab (*Eupagurus bernhardus*) among the crustaceans. Referring to investigations carried out by professional specialists, DERJUGIN also mentions a number of plankton organisms, belonging to both the zoo- and phytoplankton, which were of more western, Atlantic origin but since 1921 had been observed in the warm currents of the open sea.

TANASIJCUK[2] mentions from the Kola Fjord a number of boreal species, which had not previously been known from this fjord, but were found in the years 1921—28: *Primnoa resedaeformis, Poraniomorpha hispida, Amphipholis murmanica*[3], *Lamellidoris bilamellata, Acera bullata, Munida rugosa*[4], *Eupagurus bernhardus, Goniada maculata* and *Nereis virens*. All the forms mentioned—writes TANASIJCUK—are so conspicuous, that we can hardly believe they could have been

[1] K. M. DERJUGIN: Hydrologie und Biologie. Explorations des Mers d'U. R. S. S., Fasc. II, 1930, p. 44.

[2] N. TANASIJCUK: Über den Einfluss des Nordkapstromes auf die Fauna des Kola-Fjords. Travaux de la Station Biologique de Murman, Vol. III, No. 1, p. 24. Murmansk, 1929.

[3] TANASIJCUK considers this species, first described as new in 1929, as a boreal species for the reason that it belongs to the family *Amphiuridae*, which lives mainly in warmer waters.

[4] Is presumably the same as *Munida Sarsii;* cf. AUGUST BRINKMANN: Die nordischen Munidaarten und ihre Rhizocephalen, p. 9 et seq. Bergens Museums Skrifter Nr. 18. Bergen, 1936.

overlooked by previous observers, especially as they are all (with exception of *P. hispida*, *M. rugosa* and *N. virens*) found in fairly large numbers at several places. Further, some boreal species (*Echinus esculentus*, *Cardium edule*), previously rare, have now spread and become common. Tanasijcuk explains this phenomenon in the following manner: It is evident, that the fauna of the fjord is changing with an enrichment of the boreal elements and that this enrichment has been produced by the special character of the hydrological conditions in the Barents Sea during the years 1921—27. Comparing the hydrological graphs from the seasonal cruises along the Kola meridian (33° 30′ E. L.) in the years 1900—1906 with those of the years 1921—27, we find that the temperature of the waters of the Barents Sea was lower in the first than in the second period. This was the period of the so-called depression in the tension of the North Cape Current. The waters were characterized not only by the lower temperature, but also by the reduction in the quantity of warm water which flowed into the Barents Sea from the Atlantic Ocean. In addition, the largest branch of the North Cape Current (Murman Current) was more distant from the Murman coast than in the years 1921—27. These later years we may call years of increase in the tension of the North Cape Current. In two tables (pp. 10—11) Tanasijcuk gives a summary of the differences in the bottom temperatures in the periods 1900—1906 and 1921—26, partly at the stations in question and partly in the Murman Current itself; in the second period the mean temperature in the Murman Current was for May 0.98°, for August 1.05°, for the whole period 1.01° above the corresponding temperatures in the period 1900—1906.

In connection with these observations TANASIJCUK states, that SV. RUNNSTRÖM has proved experimentally, that the embryonic development only proceeds normally within very restricted temperature limits; change of temperature to 1—2° higher or lower than certain critical points brings about the anormal development of the fertilised eggs. TANASIJCUK is of the opinion then, that hydrological factors would approximate more to the optimum for boreal species in the years when the tension of the North Cape Current had increased. Hence we should have either a mass-occurence of such boreal species or a considerably larger number of their representatives or both together.

Regarding the Murman Coast it is reported by RASS[1], that his investigation of the fishes taken in the Kola Fjord in the summer of 1926 revealed but few specimens of the arctic species *Leptagonus decagonus* (2 spec.) and *Gymnacanthus tricuspis* (1 spec.), whilst they were taken earlier in larger quantities; and, on the other hand, they obtained such species as *Zeugopterus* (*Scophthalmus*) *norvegicus* and *Chirolophus galerita*, the usual distribution of which only extends from the British Isles to Northern Norway. These observations, writes RASS, may indicate an increase of the more seldom, boreal forms and a reduction of the arctic forms in the Kola Fjord, which probably is connected with the greater heating of the water-masses in the Kola Fjord in 1925—26.

According to BERG[2] (pp. 6—7), in the autumn of 1931

[1] THEODOR RASS: Übersicht der Fische, welche von der Biologischen Station an der Murman-Küste während des Sommers 1926 gesammelt wurden. Travaux de la Station Biologique de Murman, Vol. III, 7, p. 26. Murmansk, 1929. — Cf. also ibid., Vol. II, p. 76. Murmansk, 1926.

[2] LEO S. BERG: Rezente Klimaschwankungen und ihr Einfluss auf die geographische Verbreitung der Seefische. Zoogeographica, Bd. 3, Heft 1. Jena, 1935.

considerable numbers of the Haddock (*Gadus aeglefinus*) were found in the White Sea, where none previously had been noticed at any time. Further, in the same year large quantities of the Coalfish (*Gadus virens*) also appeared in the White Sea and this had not been known since the middle of last century.

Awerinzew has also stated, in 1935[1], that during the last 20 years some warm-water forms had appeared in the White Sea and Barents Sea, which had not been observed there earlier. In 1924—26 he himself had observed the Mackerel (*Scomber scombrus*) in catches from the White Sea, and on the Murman Coast shoals of mackerel had been encountered, yielding catches of 300—400 kg.; twenty to thirty years ago nothing was known of a mass-occurrence of herring in the gulfs of the Murman Coast, such as one may observe nowadays. Awerinzew adds, that according to his earlier experiences in the years 1904—06 one could not rely upon catching large quantities of the cod and other food-fishes with the otter-trawl in the waters round Bear Island, whereas it is common knowledge that the area between Spitzbergen and Bear Island is now teeming with these fishes (cf. similar reports from the Norwegian side, pp. 52—53).

Berg[2] quotes statements from various Russian investigators which dwell upon the fact, that cod have been found in recent years, partly in enormous quantities, on the coasts of Novaya Zemlya. The cod was undoubtedly observed earlier and right back into the last century on the coasts of Novaya Zemlya, but we may believe, that in recent years

[1] S. Awerinzew in Journ. du Conseil Internat. pour l'Exploration de la Mer, Vol. X, No. 1, pp. 73—74. Copenhague, 1935.

[2] l. c. pp. 2—3.

especially its numbers have increased. At the island of Kolgujew, which lies to the south of Novaya Zemlya, an expedition sent out by the Arctic Institute in 1932 found, according to JESSIPOW[1], a fairly large number of Herring (*Clupea harengus*), a fish previously unknown there. Later, AGAPOV and TOPORKOV have reported[2], that they discovered for the first time the following boreal species during their 1936 investigations along the west coast of Novaya Zemlya: Herring (*Clupea harengus*), Mackerel (*Scomber scombrus*) and Coalfish (*Gadus virens*). And they add: "The appearance of these fishes, aliens to the Arctic, is caused by the same factor as the appearance of the cod and some other fishes in the Kara Sea, namely the general warm spell of the past few years".

Here these authors refer to the information given by LEO S. BERG (l.c. p.2), that PROBATOV in the year 1932 caught ripe Herring (*Clupea harengus*) as well as Cod (*Gadus callarias*) in the Kara Bay; further, Salmon (*Salmo salar*) in the River Kara, which flows into the southern end of the Kara Sea;—all fishes that had not previously been recorded so far to the east.

Spitzbergen, Bear Island, Franz Joseph Land and Northern Land.

At Spitzbergen, the northern limit for the cod, the Norwegian sealers found by chance in 1873 that cod were present there in large quantities. Instigated by this discovery, 3 fishing vessels sailed up there in 1874 and brought

[1] Arctica, No. 2, 1934, p. 168. Leningrad.

[2] I. D. AGAPOV and G. N. TOPORKOV: Some data concerning the Fishes of Novaya Zemlya. Problems of the Arctic. 2. Leningrad, 1937, p. 108 (Arctic Institute of the USSR.).

home 37,500 cod. From then onwards there was a general participation from Norway in this fishery on the west coast of Spitzbergen, up to and including the year 1882, and the catches landed each year at Tromsø and Hammerfest alone amounted to between 147,000 and 595,000 cod. But in 1883 all the vessels returned without a catch; the total was 3 cod—the fishing period at Spitzbergen was at an end. A detailed account of this short-lasting cod fishery at Spitzbergen has been given by the fisheries adviser Iversen[1].

Later attempts at a Spitzbergen cod fishery in 1898 and 1901 from German and Norwegian sides gave a negative result. It was not until in 1923 and 1924 that the investigations were renewed, by the Norwegians, and the cod were again found, though but few. In 1925 both cod and haddock were plentiful, and good catches of both were made in trial fishings in 1926, 1930, 1931 and 1932[2]. In consequence of these investigations a commercial fishery was started on the banks west of Spitzbergen in 1934; in 1935 about 200 vessels with 1500 men took part and the catch amounted to about 4,500 tons, by far the most part consisting of cod and haddock, with a value of ca. 1.2 million kroner, according to Orvin. Several shoals of herring were also observed[3]. In 1936 again the cod fishing was carried on by Norwegian line-fishermen on the west coast of Spitzbergen from the beginning of July. The Faeroese took part for the first time in this fishery and returned with

[1] Thor Iversen: Torskefiske ved Spitsbergen i gamle Dage. Norsk Fiskeritidende, 42. Aargang. Bergen, 1923.

[2] Thor Iversen: Some Observations on Cod in Northern Waters. Report on Norwegian Fishery and Marine Investigations, Vol. IV, No. 8, pp. 6—8. Bergen, 1934. — Årsberetning vedk. Norges Fiskerier 1937, Nr. 4, pp. 87—89. Bergen, 1937.

[3] K. Anders Orvin, in Norsk Geografisk Tidsskrift, Bind V, p. 472. Oslo, 1935.

very good catches, taken on hand-lines. The trawlers also made a good fishery[1].

Bear Island, south from Spitzbergen, belongs like the latter group of islands to the boundary zone where warm Atlantic and cold Polar waters contend for the mastery. Like Spitzbergen Bear Island had also its cod period during last century, but for a long period of years (1898—1914) trial fishings only gave unfavourable results. In 1925 however good catches of cod were obtained and since then the cod fishery has been carried on each year at Bear Island, not only by Norwegian but also by foreign vessels[2].

According to IVERSEN the spawning of the cod in these northern waters seems to be so insignificant, that it cannot form the foundation for the great masses of every age-group, which now occur there. The cod stock frequenting Bear Island and Spitzbergen (and also the Barents Sea) is probably mainly connected with the spawning grounds off the coast of Norway (Lofoten). Marking experiments carried out by the Norwegians and Russians seem also to verify this[3].

So far as I am aware, no scientific publication has yet appeared dealing with the very extensive hydrographical material, which has been collected in recent years by the Norwegian investigations. From a paper not yet published Prof. AHLMANN states, however, that MOSBY's investigations

[1] Årsberetning vedk. Norges Fiskerier 1936, Nr. V, pp. 127—28. Bergen, 1937.

[2] THOR IVERSEN, l. c. 1934, pp. 4—5.

[3] JOHAN HJORT: Fluctuations in the year-classes of important food-fishes. Journal du Conseil Internat. pour l'Explor. de la Mer, Vol. I, No. 1, 1926 (p. 8).—E. MESSIATZEVA: Chief results of the Fishery Research in the Barents Sea in 1930 by GOIN. Rapports et Procès-Verbaux des Réunions, Vol. LXXXI (p. 147). Copenhague, 1932.—THOR IVERSEN l. c. 1934 (p. 11.)

during the Swedish-Norwegian Arctic Expedition in 1931 showed higher temperatures and salinities than any observation from former years. North of Spitzbergen he found that both temperature and salinity had obviously decreased from 1910 to 1912; from 1912 to 1922, in 1923 and in 1931 there was on the whole an increase[1].

We have the information that the temperature of the air has risen, from B. J. Birkeland, O. V. Johansson and R. Scherhag. From Scherhag, who has also cited the two first-mentioned authors, we take the following brief extracts[2].

At Spitzbergen and Bear Island the annual temperature during the period 1923—33 was on an average 1.7° C. and 1.8° C. above the normal (cf. Table 2, p. 35 in the present paper).

The accompanying table 3, from Scherhag, shows the mean temperatures at Spitzbergen in the winter months (January to March and November to December), arranged in 5-yearly groups for the period 1911—1935:

Table 3. Mean winter temperature at Spitzbergen (from Scherhag).

	1911—1915	1916—1920	1921—1925	1926—1930	1931—1935
°C.	— 17.6	— 17.6	— 12.5	— 13.9	— 8.6

We see that in the third 5-year period an amelioration has set in of 5° C.; and in the period 1931—1935 the mean temperature of the winter has been even 9° C. higher than in the period 1911—1920.

"Such a change of temperature as we have experienced

[1] The Geographical Review, Vol. XXVIII, p. 436. New York, 1938.

[2] R. Scherhag: Eine bemerkenswerte Klimaänderung über Nordeuropa. Annalen der Hydrographie und Maritimen Meteorologie, 64. Jahrg., p. 96. Berlin, 1936.—Idem, l.c. 1937, p. 263.

at Spitzbergen must be counted among the greatest known climatic changes!" comments SCHERHAG (l. c. 1937, p. 265).

At Franz Joseph Land the mean temperature of the air, as determined by the observations of the Meteorological Station established there in 1929, has been 3.6° C., for the three winter months even 7° C., higher than the temperatures measured on various earlier expeditions, according to Prof. WIESE[1].

With regard to the ice conditions, basing his conclusions on the publications of the Danish Meteorological Institute dealing with the ice conditions in the Arctic seas 1898—1934, Dr. SCHERHAG[2] has calculated, that in the period 1909—1918 the late-summer ice-limit in the waters east of Spitzbergen lay south of the average position in the period 1898—1922, whereas since then the ice-limit in 1919-1934 has not at all advanced beyond the average position in a southerly direction. How the position of the ice-limit is most closely connected with the air temperature at Spitzbergen, appears clearly from a comparison of both values for the separate years, as represented in SCHERHAG's publication of 1936[3].

The Russians also report, that it has been observed on their numerous expeditions in the Polar Sea, that the ice-edge has withdrawn far to the north in the course of the last 15 years. As a striking example of the retreat of the polar ice we are given the following facts. In 1935 the ice-breaker "Sadko" was able to cover the distance from Cape Jelanija (north point of Novaya Zemlya) to the northern end of Ssevernaya Zemlya (Northern Land) and farther north in open water up to 82° 41′,—the northernmost point ever reached by

[1] V. J. WIESE: Cause of the rise of temperature in the Arctic. Sovjetic Arctica, January 1937, p. 59 (in Russian).

[2] l. c. 1937, pp. 266—267.

[3] l. c. 1936, Tafel 12, Fig. 1.

a ship in the Arctic Ocean (i. e. under its own power; other ships reached farther north drifting with the ice). Alongside this voyage for comparison we have the information, that the powerful icebreaker "Jermak" sought in vain to reach Cape Jelanija in 1901[1]. Further, we are reminded, that in the summer 1932 the ship "Knipowitsch" was able for the first time in the whole history of Arctic voyaging to sail round Franz Joseph Land[2].

In connection with these interesting observations we may also remember, that the waters east of North-East Land were perfectly free of ice in the summer of 1930, and a Norwegian scientific expedition—with the sealing vessel "Bratvaag" and under the direction of the geologist Dr. Gunner Horn—was able to land on the dazzling white Giles Land (White Island, Kvitøya of the Norwegians), covered with ice and snow, on the way to Franz Joseph Land, and there found the remains of the ill-fated Andrée Expedition, previously searched for in vain, with the bodies of the three members of the Expedition who in October 1897, 33 years before, had succumbed to exhaustion and the cold[3]. The discovery of their last camp in the summer of 1930 was due on the one hand just to the unusual ice-conditions permitting a landing on Giles' White Island, which as a rule is surrounded by drift-ice and is considered to be one of the most inaccessible spots in the whole Svalbard Archipelago (l. c. p. 187), and on the other to the favourable climatic conditions, as the snow and ice melting seem to have been greater than for many years (l. c. p. 212).

[1] Wiese l. c. p. 60.

[2] N. N. Zubov: The Circumnavigation of Franz Josef Land. The Geographical Review, Vol. XXIII, p. 394. New York, 1933.

[3] Andrées Polarfærd 1897, af S. A. Andrée, Nils Strindberg og Knut Frænkel. Published by Svenska Sällskapet för Antropologi och Geografi. København, 1930.

On variations in the temperature of the Gulf Stream and its offshoots.

Various cases have been noted in the foregoing of temperature variations in widely separated areas of the ocean. And the view has been put forward, that the rise of temperature is due to the influence of the branch of the Atlantic Current (Gulf Stream), which penetrates into the respective areas.

What do we find now in the case of the Gulf Stream itself? Can we detect corresponding fluctuations in its temperature and volume, before it reaches the Arctic?

Dr. SCHERHAG[1] states in this connection, that according to a comparative study made by G. SLOCUM of an extensive material the surface temperature was on an average 0.42° C. warmer during the period 1926 to 1933 than in the period 1912 to 1918 in all the areas where the Gulf Stream takes its origin (Yucatan Channel, east, north-west, north and south-west parts of the Gulf of Mexico, Caribbean Sea and Florida Channel). A rise of the water temperature by 0.4° C. within a period of 15 years and over such an extended area must be regarded as very considerable, Dr. SCHERHAG explains.

We may also refer to Professor HELLAND-HANSEN, whose exact measurements of the physical conditions of the Gulf Stream, in a section from the entrance to the Sogne Fjord towards the north-west, has shown that abnormal quantities of warm Atlantic water may sometimes flow into the Norwegian Sea[2].

In May of the years 1901—1905 temperatures above 8° C. were only rarely found at a depth of 50 m. or more, and

[1] R. SCHERHAG l. c. 1937, p. 268.

[2] BJØRN HELLAND-HANSEN: The Sognefjord Section. James Johnstone Memorial Volume, p. 257. Liverpool, 1934.

none as high as 9°C.; the investigations of later years demonstrate the presence of considerable quantities of water of more than 8°C.; in May 1929, temperatures even higher than 9°C. were observed below 50 m. in many places. In May of the years 1901—1905, 1925 and 1927 the salinity was 35.30—35.35 ‰ at depths of 50 m. and deeper, but in 1929 the salinity was >35.4 the maximum being 35.45, that is to say, water of a more distinctive Atlantic character than usual in the month of May had entered the Norwegian Sea; in August the maximum salinity was 35.43 ‰ in 1928, 35.40 ‰ in 1932 against 35.37 ‰ in the earlier years. From these data Helland-Hansen draws the following conclusion: "It might seem probable that comparatively "strong" Atlantic water flowed into the Norwegian Sea in the summer of 1928 and continued so to flow for many months or even years afterwards". Further, Helland-Hansen found that quite considerable variations occurred in the volume of Atlantic water flowing northwards through the Norwegian Sea; according to his calculations the volume was about 20 per cent. greater in May 1929 than in 1927. At the same time the average temperature of this water was much higher in 1929 than in 1927 and, consequently, the quantity of heat, brought from the Atlantic to the Norwegian Sea much greater.

In a paper just published[1] Professor H. Ahlmann writes, that Helland-Hansen has informed him, that new investigations in the Sogne Fjord section were undertaken in May—June 1935 and 1936. The observations indicate, that at the entrance to the Norwegian Sea the temperature of the Atlantic Current was invariably higher in these years than it was at the beginning of the century.

[1] The Geographical Review, Vol. XXVIII, p. 436. New York, 1938.

Further, Professor SVERDRUP[1] has called attention to the fact, that great variations are found in the character of the Atlantic intermediate layer to the north of Spitzbergen[2]. He shows this by comparing the mean values of temperature and salinity at 200, 300 and 400 m., as observed by Norwegian expeditions at three neighbouring stations in August for the years 1912, 1922 and 1931:

	1912	1922	1931
Mean temperature 200—400 m. . .	1.7°	3.70°	3.18° C.
Mean salinity 200—400 m.	34.90‰	35.05‰	35.10‰

In this connection also a paper by Dr. JAKHELLN deserves close attention.

Dr. JAKHELLN, who has worked up the hydrographical material from the Norwegian expeditions to North-East Greenland (ca. 72° – 75° N. L.) in the summers of 1930, 1931 and 1932[3], has found that in the years 1931 and 1932 the Atlantic water off East Greenland was of an exceptional character. The salinity in the core of the Atlantic water was up to 35‰, and the temperature above 2.10° C.. The maximum temperatures previously found (the "Belgica" Expedition in 1905 and the "Danmark" Expedition of 1906—1908) are all under 1.5° C.. and the maximum salinity under 34.95‰—with two exceptions (34.96 and 34.97). And Dr. JAKHELLN places this phenomenon in connection with Professor HELLAND-HANSEN's evidence of the fluctuation in

[1] H. U. SVERDRUP: Oceanography. Scientific Results of the "Nautilus" Expedition, 1931, II, p. 37. Papers in physical Oceanography and Meteorology, Vol. II, No. 1, Cambridge, Mass., 1933.

[2] Cf. also the statement of Prof. AHLMANN quoted p. 53 in the present paper on the result of the Swedish-Norwegian investigations in 1931.

[3] ANTON JAKHELLN: Oceanographic Investigations in East Greenland Waters in the Summers of 1930—1932. Norges Svalbard- og Ishavs-Undersøgelser, Nr. 67. Oslo, 1936.

the Norwegian Atlantic Current. "These extraordinary conditions are obviously the same as those first pointed out by Helland-Hansen off the Norwegian coast in the autumn of 1928, and also found by H. Mosby in August 1931 in the Atlantic water in the Polar Sea north-east of Spitzbergen, as mentioned by Helland-Hansen. It looks as if this extraordinary water appeared for the first time in 1931 off East Greenland, and that the water had a still more extraordinary character in the late part of the summer of 1932".

It appears from this last sentence, however, that Dr. Jakhelln has overlooked the observations of Commander Riis-Carstensen, who found on the "Godthaab" Expedition to East Greenland in 1930 a maximum temperature in the warm current of 2.15° C. with an accompanying salinity of 34.98 ‰[1].

Reduction in thickness of the layer of Polar water in the Arctic Sea north of Eurasia.

To throw further light on the changes in the hydrographical condition of the Arctic Sea north of Eurasia, we may refer to a paper by Professor Schokalsky[2]. This is so much the more worthy of attention in that it gives us some impression of the intensive work carried out by Russian scientists in their investigation of these arctic waters. For example, during the last 12 years the Moscow Oceanographical Institute has sent out over a hundred expeditions, each lasting about two months, to study the physical and

[1] Bulletin Hydrographique pour l'Année 1930, p. 97. Copenhague, 1931.

[2] Jules Schokalsky: Recent Russian researches in the Arctic Sea and in the mountains of Central Asia. The Scottish Geographical Magazine, Vol. 52, No. 2. Edinburgh, 1936.

biological oceanography of the Barents Sea and even of the Greenland Sea.

With regard to the present subject, Professor SCHOKALSKY takes as starting point the memorable voyage of the "Fram" (1893—1896), when NANSEN discovered that the upper layer of the Arctic Ocean from 200 to 250 metres in thickness was less saline than the deeper water, and that it had a temperature of $-1.0°$ to $-1.9°$ C., while the deeper layer, from 600 to 700 metres thick, was of oceanic salinity (over 35.00‰) and had a temperature of 1.2° C..—Five years later (1901) S. O. MAKAZOV on the ice-breaker "Ermak" found, between Franz Joseph Land and Novaya Zemlya, that the zero temperature occurred at a depth of about 200 metres and that, below this, the temperature rose to 1.1° C.. This confirmed NANSEN's observations.

But, during the oceanographical investigations carried out by numerous scientific expeditions (Russian with the vessels "Elding", "Krassin", "Sedov", "Persée", "Lomohasov", "Knipowitsch" and "Sadko", and an American with the "Nautilus") during recent years (1927, 1928, 1929, 1931, 1932, 1934 and 1935) in the waters between Novaya Zemlya and Franz Joseph Land, north of Spitzbergen, between Franz Joseph Land and the Northern Land Archipelago, and a little north-west of Northern Land right up to 82° 42′ N., —in all these waters it has been found, that the cold and less saline surface layer was only ca. 70—125 metres thick, and that beneath this lay a thick saline layer of Atlantic water with a temperature of 0.6° to 2.6° C.. In 1929 the "Sedov" and the "Persée", at almost the same place as MAKAZOV chose for his observations in 1901, found the isotherm of zero at the depth of 125 metres instead of

200 metres. Again, in 1931, the "Persée" found this isotherm at 75 metres in the same vicinity.

"These records"—writes Prof. Schokalsky—"and others not cited here, together provide incontestable evidence of a progressive warming of the Arctic Ocean. The branch of the North Atlantic Current which enters it by way of the edge of the continental shelf round Spitzbergen has evidently been increasing in volume, and has introduced a body of warm water so great, that the surface layer of cold water, which was 200 metres thick in Nansen's time, has now been reduced to less than 100 metres in thickness".

Changes in glaciers and tundras.

The amelioration of the climate in arctic and subarctic regions which has occurred in recent years, has also affected the land-ice, both glaciers and ground-ice.

Among the scientists who have been engaged in late years with the study of glaciological conditions we must mention H. Ahlmann[1], Professor of Geography at the Stockholm Högskola, who has contributed greatly to our knowledge of the nature and life of glaciers. After studying a glacier in Jotunheim in Norway for 5 years Professor Ahlmann in 1931 investigated the glaciers on North-East Land, an island in the group embraced under the name of Svalbard. Wherever he went on this North-East Land he was able to detect, that the glaciers were much declining; to a

[1] Hans W: Son Ahlmann: Scientific Results of the Swedish-Norwegian Arctic Expedition in the Summer of 1931. VIII, Glaciology (especially pp. 180—186). Geografiska Annaler, Bd. XV. Stockholm, 1933.—Idem: Investigations into the Life of Glaciers. Arctica, Vol. III, p. 33. Leningrad, 1935.—Idem: The Fourteenth of July Glacier. Geografiska Annaler, Bd. 17. Stockholm, 1935.—Idem: Tre Nordiska Forskningsexpeditioner. Nordisk Tidsskrift, pp. 121—138. Stockholm, 1936.

great extent he would characterize them as dying. And he explains later, that the English Oxford Expedition, which continued his investigations in 1935—36, found the glaciers of North-East Land in even worse condition than he had calculated.

In 1934 Professor AHLMANN examined the 14th of July Glacier on West Spitzbergen. The study of the increase or decrease of glaciers was brought by him into new and rational lines; above all he sought by exact methods (digging, boring, measuring etc.) to throw light on the aspect of the glaciers' condition, which he calls their economy: how much snow is added yearly as income to the glacier and how much water it is deprived of yearly as expense. He found, that the income of the 14th of July glacier during the winter of 1933—34, in the form of snow converted into water, amounted to 78 million cubic metres, whilst the loss during the summer of 1934 by melting and evaporation was equal to 113 million cubic metres of water. The "negative balance in the budget" is so great, that it would bring about a catastrophe to the glacier if such conditions prevailed for a number of years. The result of this investigation was thus a confirmation of the conditions discovered during the previous expedition, namely, that the glaciers on Spitzbergen are now in a period of degeneration.

As a result of his own and other observers' investigations of the condition of the Spitzbergen glaciers Professor AHLMANN states, that for the last couple of decades so great a majority of the glaciers have been in a recessive stage, that we might speak of a present, general retrogression of glaciation in Spitzbergen.

In 1936 Professor AHLMANN undertook an expedition to Iceland to study the Vatnajøkull; from his combined

methods of investigation he found, that this like the Spitzbergen glaciers was in recession for the time being.

This great recession of the glaciers in recent years is due, according to Professor Ahlmann, to the transfer of heat from milder areas, probably from the warm areas of the sea.

On East Greenland also it has been found that the glaciers are receding. On the 7th Thule Expedition, under the leadership of Dr. Knud Rasmussen, glaciological investigations were carried out by the geologist Mag. sci. Keld Milthers. These investigations have not yet been published, but Milthers permits me to refer to his preliminary report to the second-in-command of the expedition, Captain C. C. A. Gabel-Jørgensen, who forwarded it to the Committee of the Expedition, of which I am a member and thus came to be acquainted with its contents.

Immediately on his arrival at Angmagssalik (13. 7. 1933) Milthers made a journey of reconnaissance round the district and selected 11 glaciers in all for his investigations. On this journey he found not a single glacier showing forward progress; a proposed comparison between ice-edges of glaciers advancing and those retreating could therefore not be made. Chief importance was then attached to the photogrammetric registration of the changes at the edge of the glaciers and for this purpose a phototheodolite was used.

The success was attained of registering 4 suitable glaciers in this way and a chart of the present state of 5 glaciers in all can be made, whilst photographs were taken of altogether 11 so-called "dead" glaciers.

All the glaciers bore evidence of a retrograde movement of the edge during a long series of years and it was typical of them all, that a vacated terminal moraine lay in front of the edge for a distance of about 100 m.

In connection with this we may also draw attention to the statement of Prof. AHLMANN: It is generally known that the Greenland glaciers are diminishing[1].

In the east also, on the island group of Franz Joseph Land in the Siberian Arctic Sea, the glaciers are in retreat. The results of the Russian investigations here have been summed up as follows: "The glaciation of the archipelago is at present in a regressive phase of its development, to which point the existence of glacier remnants and the border-remains, at some distance from the border of the glacier"[2].

Lastly, we have reports, that the boundary of the ground-ice in the northern districts of the Sovjet Union is moving northwards[3].

In 1837 A. SCHRENK visited the town of Mesen (65° 50′ N.) whilst on an expedition through the north-eastern part of European Russia and he reported, on the base of his own observations and statements of the inhabitants, that the ground remains permanently frozen from a depth of 2 m.

In 1933 a Commission appointed by the Russian Scientific Academy sent out a special expedition to investigate the limits of the ever-frozen soil. This expedition found, that ground-ice no longer persists at Mesen and its immediate neighbourhood; the ground-ice was first met with 40 km. north of the town, at the village of Sjomsha on the west coast of the Kanin Peninsula. Nor is there any ground-ice now found in the regions about Cape Bubnov and Cape Olchovsky.

[1] l. c. 1935, p. 206.

[2] T. N. SPIZHARSKY: On the Glaciation of Franz-Joseph Land. Transactions of the Arctic Institute, Vol. XLI, Geology, p. 37. Leningrad, 1936.

[3] cf. among others LEO S. BERG, l. c., pp. 11—12.

The belief is, that the shifting of the edge of the ground-ice northwards is connected with the increasing warmth of recent years in these northern regions.

Importance of air currents on the rise of temperature.

It may be recalled, that Dr. B. Sæmundsson sought for the cause of the high winter temperatures in Iceland and of the great scarcity of ice in these northern waters, in the higher temperature of the sea round Iceland in conjunction with the prevalent southerly winds during this period.

In his glacier studies Professor Ahlmann points out, that there is a connection between glacier recession and atmospheric activity. Regarding Vatnajøkull on Iceland Ahlmann writes, that the melting depends in high degree on the quantities of heat carried in over the glacier by the southerly winds. And regarding the Spitzbergen glaciers he writes, that their great recession in recent years is due to the transfer of heat from milder regions, probably from the warm areas of the sea, which means that an increased exchange or greater work of transport has taken place in the atmosphere. In this connection he brings into consideration Professor Helland-Hansen's investigations into the variations of the heat content of the Gulf Stream (cf. present paper pp. 57—58).

By including the atmospheric circulation in the discussion of these conditions Dr. B. Sæmundsson and Professor Ahlmann have touched upon one side of the problem, which has recently been brought strongly into the foreground by the meteorologists (Scherhag, Wiese). It is emphasized, that the cause of the rise in temperature and recession of the sea-ice in the Arctic must first and foremost be sought for in the great current movements of the air.

In 1929 already A. WAGNER showed, that the atmospheric circulation was greater in the decade 1911—1920 than in 1886—1895. Then SCHERHAG in his paper of 1936 indicated, that this increase had continued on a greater scale in 1921—1930[1]. The cause of this increased circulation is, that during this period a strengthening of the subtropical high-pressure belt has taken place simultaneously with a deepening of the barometric minima on the polar front. This would of necessity lead to the result, that large quantities of warm Atlantic air would stream in over Europe and over the Arctic regions and there effect a climatic change towards more oceanic conditions. It is just since 1920 that such a rise of air temperature has been detected in the Arctic regions, especially in the winter.

Although the cause of the rise of temperature in the higher latitudes must be found first and foremost in the atmospheric circulation, this may agree quite well with the fact, demonstrated by SLOCUM, that a rise of 0.4° C. has occurred in the areas of origin of the Gulf Stream itself, since indeed there is a close connection and interchange between these two influences.

WIESE[2] gives the same explanation as SCHERHAG of the rise in temperature both in the air and sea, maintaining that the common cause must be sought for in an increase of the atmospheric circulation; this leads on the one hand to warm masses of air being brought up into the higher latitudes, eastwards up to the New Siberian Islands at least, and on the other to an increase in the activity of

[1] R. SCHERHAG: Die Zunahme der atmosphärischen Zirkulation in den letzten 25 Jahren. Annalen der Hydrographie und maritimen Meteorologie, 64. Jahrg., pp. 397—407, Tafel 58—63. Berlin, 1936.

[2] V. J. Wiese l. c.—Idem: Ice prognoses. Problems of the Arctic, No. 1, p. 80. Leningrad, 1937.

the marine currents, with consequently a stronger inflow of Atlantic water into the Norwegian Sea and the Polar Sea. Whilst these warm masses of water are certainly able to react on the atmospheric circulation and increase this further by deepening the barometric minima round Iceland and South Greenland, they cannot have any direct influence on the air temperature in the high-arctic regions, since here they are at a depth of 100 to 200 m. below the greatly cooled upper layer. Further, he points out, that the more prevalent south-westerly winds of recent years have forced the southern limit of the ice in the Barents Sea farther north.

Concluding remarks.

It is of course not in every single case, where southern animal forms as described in the foregoing have been discovered in the arctic and subarctic regions during the warm period, that we can see clear evidence of a heat increase in the sea. Naturally enough, since in recent years increasing interest has been shown in northern seas; consequently, far more observations are made than in earlier years, so that possibly one or other animal form now discovered for the first time may wrongly be regarded as a new immigrant.

Nevertheless, in the abundance of the discoveries we are justified in seeing clear signs of the rise of temperature. Further, some of the southern species—especially those of importance economically or commercially—have occurred in northern waters in such quantities, that they could not have been overlooked earlier, if in similar numbers.

That the temperature conditions have great influence on the distribution of the heat-variable (poikilothermic) marine animals—and it is these especially that come into

consideration here—has become manifest from abundant observations in nature and examination of museum materials brought home by expeditions.

Experimentally also endeavours have been made, to throw light on the influence of the temperature. Of these only a few need be mentioned here, which seem to be of special interest for the present investigation.

Dr. RUNNSTRÖM has been able to determine experimentally in the case of a number of invertebrate animals, that the eggs only develop in a normal way within certain limits of temperature; for arctic-boreal species these lie between —1° and 11° C.; in boreal species between 4° and 16° C.; in Mediterranean-boreal species between 8° and 23° C.. On the other hand, the hatched-out larva or the young, as also adults, are much less sensitive to the temperature than the earliest developmental stages[1].—A rise of temperature in northern seas must consequently have the effect, that the lower limit for the normal embryonic development of boreal species can be reached or passed and, consequently, the existence of the species in all stages is assured. Should unfavourable temperature conditions again recur, and the eggs perish just after fertilisation, the continuance of the species in the region is threatened, unless the pelagic larvae, which are remarkable for their greater eurythermy, are conveyed by the currents from the original spawning places in neighbouring boreal regions[2].

[1] SVEN RUNNSTRÖM: Über die Thermophatie der Fortpflanzung und Entwicklung mariner Tiere in Beziehung zu ihrer geographischen Verbreitung. Bergens Museums Årbok 1927. Naturvidensk. Række, Nr. 1, 2. Bergen, 1928.—Idem: Weitere Studien über die Temperaturanpassung der Fortpflanzung und Entwicklung mariner Tiere. Ibid. 1929, Nr. 3, 10. Bergen, 1930.

[2] For animals highly able to move about, that is certain fishes, it might perhaps be added: or unless constant immigration of adult individuals on their food wanderings takes place.

Many investigators have studied the influence of the temperature on the development of the eggs of fishes and the results all show, that low temperatures delay the development. In the case of the Cod *(Gadus callarias)* Dannevig obtained the following results[1]:

Temp. in °C.....	—1°	3°	4°	5°	6°	8°	10°	12°	14°
Time of incubation in days (24 hours)	42	23	20½	17½	15½	12¾	10½	9⅔	8½

This is a striking example again of the great influence exercised by the temperature on the development of the eggs.

It is quite evident, that the relatively high temperatures in the water at places in West Greenland have in recent years been favourable to the development of the cod eggs, even as the cod have adapted their spawning to the special conditions and changed their time of spawning to the months of May and June, when the water has reached a temperature suitable for hatching (4° to 6° C. is considered the most favourable temperature). For the sake of comparison it may be mentioned, that the main spawning time on the south coast of Iceland falls in March and April for *Gadus callarias*; the surface temperature is then above 5°

[1] 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., Edinburgh, 1895.— In some earlier experiments carried out by R. E. Earll the hatching lasted longer at negative temperatures, namely 50 days at —0.6° C. (U. S. Commission of Fish and Fisheries, Part VI, Rep. of the Commissioner for 1878, p. 724. Washington, 1880). A. C. Johansen and A. Krogh found in their experiments, that the lower limit for the full development of the cod eggs lies at about 0° to —1.0° C., but that cod eggs were not hatched at temperatures as high as 12° and 14° C. (Publ. de Circonstance, No. 68, p. 19. Copenhague, 1914).

already, a temperature only reached later in the year at West Greenland[1].

One circumstance would be of great interest to have determined experimentally, namely, whether a fish like the Cod (*Gadus callarias*), which, as we have frequently learnt, has undertaken extensive wanderings during the warm period into the arctic seas lying beyond their usual area of distribution — whether such a fish can really appreciate such small differences of temperature as come in question here.

Such an investigation has recently been undertaken by Dr. BULL at the Dove Marine Laboratory[2]. Through experiments planned on a large scale, with well-thought out technique as well as with great patience he has shown, that fishes are able to appreciate and react to even very small changes in temperature. Dr. BULL summarises the result of his investigations as follows: "By the use of a "conditioned response" technique it has been shown that teleostean fishes respond "purposively" to an increase in temperature of the water surrounding them of between 0.03° and 0.10° C.". The experiments concerned 19 species of marine fishes (Teleosts) including *Gadus callarias*.

At my request Dr. phil. Å VEDEL TÅNING and Mag. scient. A. KIILERICH have kindly read through my manuscript crit-

[1] On 15. June 1925 the following temperatures were measured in Kapisigdlit Fjord, one of the innermost ramifications of the extensive fjord complex known as the Godthaab Fjord: 0 m. . . 9.25° C.; 5 m. . . 7.34° C.; 10 m. . . 6.49° C.; 13 m. . . 5.77° C.; 20 m. . . 4.28° C.; 25 m. . . 2.06° C.. Half an hour's haul with a 2 m. stramine net at the surface yielded 2712 cod eggs in all stages of development. Cf. AD. S. JENSEN l. c. 1926, pp. 87 and 89.

[2] HERBERT O. BULL: Studies on Conditioned Responses in Fishes. Part VII. Temperature Perception in Teleosts. Journal of the Marine Biological Association of the United Kingdom, N. S., Vol. XXI, No. 1, p. 1. Plymouth, 1936.

ically and I am indebted to them for both corrections and supplementary notes; my heartiest thanks are due to them. To Dr. H. M. Kyle, who has undertaken the translation of this work into English, I wish also to express my best thanks.

Summary.

The region dealt with in this paper embraces the arctic and subarctic from Greenland in the west to Eurasia in the east.

From the review undertaken it appears, that many southern (boreal) species of animals, including mammals, birds, fish and invertebrates, have in recent years been able to extend their area of distribution farther north, whilst on the other hand the southern limit for certain northern (arctic) species has retreated northwards. Further, a number of southern (boreal) species, which formerly only occurred here and there and in small numbers, have now become common and occur in large quantities.

The cause of these zoogeographical changes is sought in the fact, which has occurred contemporaneously, that the temperature of the sea and air has risen in the regions in question. Along with this rise of temperature there has also been a retreat of the ice-boundary in arctic seas, whilst on land the glaciers and tundras have diminished and retreated.

Postscript.

Within quite recent years changes have again occurred in the hydrographical conditions at South-west Greenland. In 1937 and especially in 1938 unusually low temperatures have been found everywhere in the waters of the fjords and on the coasts, according to the investigations undertaken

by Cand. mag. PAUL HANSEN for the Greenland Administration.

We could hardly expect this cooling of the water-layers to produce already any outstanding change in the biological conditions after such a relatively short period. Nevertheless, we can note certain changes in the animal life, which may be briefly referred to here.

At South-west Greenland the arctic Fjord Cod (*Gadus ogac*) has again become common. The arctic Deep-sea Halibut (*Reinhardtius hippoglossoides*) again penetrates in increasing numbers to the earlier rich fishing grounds in the Julianehaab district; in the Lichtenau Fjord the stock was on the increase in 1937 and in the summer of 1938 the number of standard-size fish had shown such an increase, that a commercial fishery could be restarted.

From the Sukkertoppen district it is reported, that quantities of Cod (*Gadus callarias*) and other species, such as the Lumpsucker (*Cyclopterus lumpus*), Sea-cat (*Anarrhichas*) and Norway Haddock (*Sebastes marinus*), came up dead to the surface in the winter and spring of 1937—38; it is quite fifty years since such a phenomenon has been known. And from Amerdlok Fjord in the Holsteinsborg district it is stated, that in 1938 from the end of May and through the whole of June a large number of dead or dying large cod came up in the trawl, when fishing for the deep-water prawns, which has never happened before in the years this fishery has been carried on. In both cases it has evidently been the great cold in the water, which has killed or deadened the fish. In Amerdlok Fjord the temperature was negative in the last half of June 1938 at depths of 150—500 m., lying between $-0.26°$ and $-0.93°$ C.; for comparison it may be mentioned, that at the same period in 1936 at corres-

ponding depths the temperatures were about 2° C. higher.

Again it is reported from various fishing stations, that the weight of liver taken from the cod caught in 1938 was less than in the previous year (ca. 40 % less in similar quantities of fish) and the oil contents of the liver were small. This points to a badly nourished condition, presumably owing to the cold in the water affecting the fish, making them less desirous of food. Experiments, recently carried out by R. A. Mc. Kenzie, have shown, that in the matter of feeding the water temperature is a greater controlling factor than anything else: and at low temperatures it has been found that the cod practically cease feeding at 0° C. or lower[1]. How the liver of the cod is affected in circumstances like these, is a question I put to the zoophysiologist, Dr. phil. P. Brandt Rehberg, who has very kindly answered in the following manner: "The liver in fishes may, *inter alia*, be regarded as a storing organ, its weight being influenced to a great extent by the nourishment of the fish and we should expect that failing supply of food will make itself felt in the weight of this organ before in that of any other".

Further, it is reported from Amerdlok Fjord, where as mentioned trawling for the deep-water prawn (*Pandalus borealis*) is carried on for the cannery in Holsteinsborg, that the spawning of this species has been greatly delayed; normally it should begin in the middle of July, but in 1938 the first egg-carrying prawns were not seen before the middle of August, presumably because the sea-water even at the great depths where the prawns live (ca. 200—500 m.), remained ice-cold far on into the summer (cf. above).

[1] Atlantic Biological Station, St. Andrews, New Brunswick, Canada, Note Nr. 47. 1936.

It may be added, that *Halopsis ocellata* (cf. pp. 23—24) an immigrant medusa from warmer seas to West Greenland, was not observed in the summer of 1938, though a sharp look-out was kept for it.

At the present moment we cannot tell, whether these quite recent changes in the temperature of the sea-water in the fjords of South-west Greenland and along its coasts indicate a returning tendency of the "cold" period; or whether it is just a brief fluctuation, which may soon be replaced by a new onslaught of the "warm" period.

As mentioned on p. 53, Prof. AHLMANN had access to a paper by Dr. MOSBY, then not yet in print, and from it cited some remarks regarding the changes in sea temperatures at Spitzbergen; I have quoted these at the place mentioned. Dr. MOSBY's work has appeared since the present manuscript was sent to be printed and I must content myself therefore, with referring readers to this important work[1] for information regarding the hydrographical conditions in the area of Svalbard.

[1] HÅKON MOSBY: Svalbard Waters. Geofysiske Publikasjoner, Vol. XII, No. 4. Oslo, 1938.

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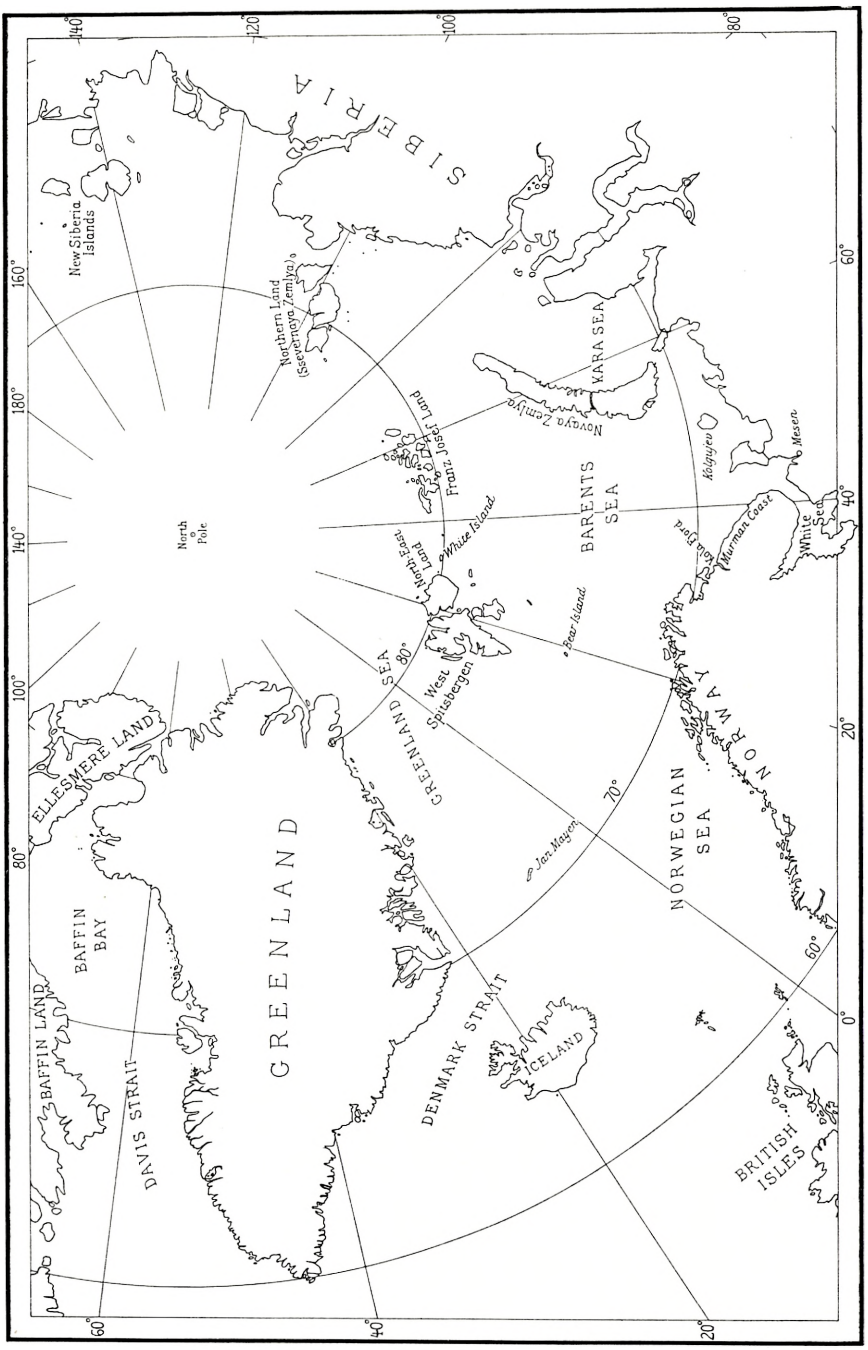
Indleveret til Selskabet den 12. Januar 1939.
Færdig fra Trykkeriet den 26. April 1939.

Chart of Greenland, showing position of most of the places mentioned in text. The sign ⊙ indicates settlements after which the districts are named.



Survey Chart of the whole region dealt with.

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