INVESTIGATIONS UPON IMMUNISATION AGAINST METASTASIS FORMATION IN EXPERIMENTAL CANCER

BY

JOHANNES FIBIGER AND POUL MØLLER

WITH 5 PLATES

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THE classical paper published by C. O. JENSEN in 1903 contained the first observations showing that mice which have once or repeatedly appeared resistant to the transmission of cancer by transplantation of tumor tissue from cancerous mice, may also be found refractory to subsequent graftings.

A series of later investigations (APOLANT, BASHFORD, BORREL, BRIDRÉ, CLOWES, CLUNET, EHRLICH, HAALAND, LEWIN, MURRAY, SCHÖNE and others) confirmed these observations, and demonstrated that such a resistance to transplantation of malignant tumors does not merely depend upon natural immunity but upon artificially induced immunity as well. The acquired immunity is produced by inoculation of living tissue originating from animals belonging to the same species as those into whom implantation is made, and it is possible to obtain immunity no matter whether living tumor tissue revealing only receding growth or no proliferative energy at all after transplantation, or living normal tissue (blood, spleen, placenta, mouse-embryo tissue, mouse-embryo skin etc.) is used for inoculation.

The resistance developing after absorption of the inoculated tissue is not, however, so specific that it will protect only against a subsequent transplantation of tumor

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tissue, the structure of which corresponds closely or exactly to that of the inoculated and absorbed tissue. Although the resistance will generally be most marked toward the transplantation of such tumors, it will, as a rule, yield protection also against the inoculation of tumor tissue belonging to other and different morphological types and, in fact, so often that the term: pluri-immunity, nay even pan-immunity has been used (Ehrlich).

The existence of such a far-reaching or universal immunity has not, however, been generally acknowledged. That, on the other hand, the inoculation of carcinomatous tissue may produce resistance to the transplantation of sarcoma, and inversely the implantation of sarcomatous tissue may prevent growth of transplanted carcinoma, was demonstrated by earlier investigations, and also confirmed by later experiments (Caspari[1]), in which it was asserted that, in conformity to Ehrlich's results, no essential difference exists between the immunizing power of these two types of tumors nor between their faculty of producing a mutually immunizing effect.

The immunizing power of normal tissue is analogous to that of tumor tissue. Inoculation of placentar tissue is thus able (Higuchi)[2] — as is the inoculation of blood (Lewin[3]) — to produce resistance to the transplantation of both carcinoma and sarcoma. That embryo skin, too, protects against both these forms of tumor was shown by Tsurumi[4], although the immunizing power of the skin may fail in the case of some sarcomatous tumors (Itami)[5].

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[1] Zeitschrift für Krebsforschung XXI. 1924.
[2] 5th Scientific Report of the Imperial Cancer Research Fund 1912.
[3] Zeitschrift für Krebsforschung VI. 1908.
[4] The Journal of Pathology and Bacteriology Vol. XX. 1915.
[5] The Journal of Cancer Research. Vol. IV. 1919.

The normal tissues are of varied efficacy, as also recently emphasized by ITAMI[1]. Some of them are inactive.

The hope of basing upon the experimental results immunizing methods that might be utilized in preventing and combating human cancer, could not be maintained, since experiments showed that immunity to the transplantation of tumor tissue is far from being identical with immunity to spontaneous tumor development. Thus, as generally known, it was demonstrated that mice, which were made resistant to transplantation by unsuccessful inoculation of tumor tissue, might nevertheless develop primary spontaneous cancerous tumors later on.

Extensive investigations on the immunizing effect of normal tissue have further evidenced that the implantation into mice of mouse-embryo skin, which among all homologous normal tissues is the most potent immunizing factor (BASHFORD, HIGUCHI, TSURUMI), is not able to inhibit the growth of spontaneous tumors, nor to prevent either local recurrence after removal of the tumor by excision, or the development of new primary tumors.

These observations most of which are due to investigations made by HAALAND[2] in the laboratories of the Imperial Cancer Research Fund in London, have thus furnished decisive arguments against the assumption that immunisation by means of the said methods might be able to exert any influence upon the development, growth, and recurrence of primary spontaneous tumors.

Nor has it been possible to trace any inhibiting effect of the immunizing treatment upon metastasis formation, all experiments having shown that metastases will occur

[1] The Journal of Cancer Research. Vol. X. 1926.
[2] 4th Scientific Report of the Imp. Cancer Research Fund. 1911.

with about the same frequency in cancerous mice, whether or not the mice have been subjected to immunisation.

But this result of the investigations cannot be considered fully convincing. The experiments, the great majority of which are also due to Haaland, were finished about the year 1911, viz. at a time when it was only possible to transplant fully developed cancerous tumors into mice, the artificial production of primary cancerous tumors in normal mice being still beyond the scope of possibility. Therefore only mice suffering from spontaneously developed cancerous tumors of unknown etiology and of quite obscure date of earliest beginning could be used for the experiments. Thus, the result of these researches must necessarily be less conclusive owing to the fact that the age of the metastases found at the death of the mice must also — and to a still greater extent — escape exact estimation. And, accordingly, it is impossible to preclude that metastatic nodules may have been present and perhaps even fully developed in the experimental animals already before treatment was instituted.

It was chiefly this difficulty of deciding the moment at which the metastatic nodules established themselves which made Haaland[1] extend his investigations to include also the effect of immunizing treatment upon autoplasts. The successful transplantation of a primary tumor into the same mouse in which it has primarily developed may, according to Haaland[2], Murray and Woglom[3], be regarded as an artificial metastasis formation, the earliest beginning of which is of course fixed by the moment of the autoplasty itself.

[1] loc. cit.

[2] loc. cit.

[3] 7th Scientific Report of the Imp. Cancer Research Fund. 1921.

As experiments by Haaland[1] have demonstrated that autoplasty will give a positive result both in treated and in non-treated animals in nearly all cases, and that the immunizing treatment is thus not capable of preventing the success of autoplasty, this fact too, was quoted in favour of the view that immunisation exerts no influence upon natural metastasis formation.

We should not, however, be warranted in looking upon autoplasty and spontaneous metastasis formation as quite analogous processes arising and taking their course under equal conditions.

Apart from the different way in which they come into existence, there seems to be a striking contrast between the success and the rapid growth of the autoplasty on one side, and the less frequent occurrence and slower development within the same time of the natural metastases on the other side.

Autoplasty is nearly always successful (in 95 per cent (Bashford)[2], or in 85 per cent (Murray)[3] of the experiments), and the autoplastic graft will in the vast majority of experiments develop into a palpable tumor already 2—3 weeks after inoculation.

As an illustration of the frequency of metastasis formation in spontaneous tumors may, on the other hand, be quoted that Apolant[4] found macroscopic metastases in the different organs only in 6 out of 221 mice bearing spontaneous tumors, while Murray[5] observed metastases

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[1] loc. cit.

[2] Proceedings of the Royal Soc. of Med. March 1910.

[3] Personal communication to Johannes Fibiger.

[4] Arbeiten aus dem Kgl. Institut für experimentelle Therapie zu Frankfurt a. M. 1906.

[5] 3rd Scientific Report of the Imperial Cancer Research Fund 1908.

in the lungs of 27 out of 68 spontaneously affected tumor mice, 16 of which were however only subjected to macroscopical examination. Gierke[1] found macroscopic metastases in the lungs of 8 out of 35 mice presenting hemorrhagic mammary tumors, whereas Haaland[2] observed such metastases in 103 (38 per cent) out of 275 mice bearing spontaneous tumors (most of which were no doubt mammary carcinomata). In experiments carried out in the Anatomo-Pathological Institute of the University of Copenhagen Fridtjof Bang[3] found macroscopic metastases in lungs and lymphatic glands in 33 out of 113 i. e. in 29.2 per cent of mice affected with fully developed experimental tar cancer.

And let it be remembered that in all these different investigations the cancerous mice, or the majority of them, and also those without metastases, have no doubt been suffering from their primary tumors during a period exceeding the few weeks sufficient for autoplasts to develop into palpable and relatively large nodules.

Furthermore, Haaland's experiments demonstrate that the development of natural metastases is far from being an exceedingly frequent or constant finding in the same tumor-bearing mice in which autoplasty has turned out successful. Although metastasis formation must seem a priori to be capable of arising in these animals not only from the spontaneous primary tumor itself, but in addition from the inoculated autoplast, even microscopic examination did not reveal the presence of metastases in a great number of the autotransplanted mice.

Altogether, according to various investigations, the diffi-

[1] 3rd Scientific Report of the Imperial Cancer Research Fund 1908.
[2] 4th Scientific Report of the Imperial Cancer Research Fund 1911.
[3] Bidrag til Studiet af Kræftsygdommens Klinik og Patogenese. Thesis for a doctor's degree. Copenhagen 1924.

culties opposing natural metastasis formation in mice bearing spontaneous primary tumors thus seem to be greater than those opposing the growth of autoplasts in such mice. But this difference is perhaps in great measure or entirely due to the difference between the origin of autoplasts and that of metastases. By autoplasty a block of cells in abundant number are introduced simultaneously and violently into the tissue, whereas the metastases arise from a few cells only, deposited little by little in the organs through the lymph or blood circulation.

For this reason alone, a natural or acquired immunity might perhaps exert a greater influence upon the metastatic cells than upon the autoplasts, and an immunizing treatment would thus be supposed to be able to prevent more easily the formation of metastatic deposits than the development of autoplasts.

Experiments recently performed (ITAMI)[1] seem to confirm the probability of this supposition, since they show that autoplasts are able to grow when inoculated into an immunized mouse as a block graft, but not when introduced as a suspension into the circulation. Consequently, the success of autotransplantation of block grafts into immunized mice does not justify the conclusion that immunisation will leave spontaneous metastasis formation unaffected too.

From these various considerations it may be deduced that, although it has been shown that immunizing treatment by the implantation of normal tissue produces no effect upon the development and growth of primary cancerous tumors, experiments have not yet furnished any decisive proof whether or not such immunizing methods

[1] The Journal of Cancer Research. Vol. X. 1926.

might be able to retard or to prevent natural metastasis formation.

In a minor series of experiments J. Fibiger and J. Maisin[1] did not succeed in demonstrating any difference as to the development of experimentally produced tar tumors or their metastases in mice which prior to the tarring had been found resistant to transplantation of artificially produced tar tumors, or had been treated by inoculation of the tissue of mouse-spleen. These experiments, however, are too few in number to admit of decisive conclusions.

Thus, altogether, a revision of the question seems amply justified; extensive experiments must be carried out upon a great number of mice bearing experimentally produced primary tumors, the age of which can be estimated more exactly, and upon which treatment must be instituted, before metastasis formation has begun or has manifested itself through distinct nodules.

Experimental Investigations.

In order to institute such researches we then, at the beginning of 1924, entered upon a series of experiments with the aim of elucidating the question whether or not injections of living mouse-embryo skin would produce any effect upon metastasis formation in experimental tar cancer. Such immunizing treatment against tumors of this origin would a priori seem to promise a result, not only because of the strong immunizing power of the embryo skin per se, but also because of this tissue being com-

[1] First Meeting of the Leeuwenhoekvereeniging. Amsterdam 1922.

posed of the same cellular elements as those from which arise the cutaneous tar tumors, and inducing especially a perfect protection against squamous-celled carcinomata of the mouse (BASHFORD)[1].

Method and Technique.

At the beginning our investigations comprised altogether 305 white mice, out of which, however, 12 destined for treatment with embryo skin died so early after the beginning of the tarring that no injection could be made upon them, the total number of mice available for the investigations being thus reduced to 293. The mice were of varying age and weight, the minor half weighing about 20 grammes and more, while the remainder were younger and smaller animals. All mice were nourished in the same way, were kept under equal conditions, isolated each of them, and were painted with the same coal tar on alternate days during the same period, application being made in the interscapular region on an area of about 1 square centimeter of the dorsal skin. The tarring was continued for 4 months (120 days), but a rather considerable number of the mice died before this period had elapsed.

Two analyses carried out in the Institute of Chemistry of the University of Copenhagen (Director Professor Dr. BIILMANN) by Dr. phil. HAKON LUND gave as a mean result that the tar employed contained arsenic in very small quantities (0.0033 per cent).

137 mice kept as test animals were only tarred, while upon 156 mice, in addition to the tarring, injections were made of an emulsion of living mouse-embryo skin.

[1] The Immunity Reaction to Cancer. Proceedings of the Royal Society of Medicine. March 1910.

This emulsion was procured in the following way: female mice in the last stage of pregnancy were killed by ether and decapitation, after which the embryos were removed without delay from the uterus under aseptic conditions, and skinned. The skin, from which any trace of adhering fluid and blood was absorbed by means of a piece of sterilized filtering paper, was weighed, and minced in a sterile saline solution (0.9 per cent).

As generally known according to most investigators, dead homologous tissue will produce no immunity, and other researches furthermore indicate that sometimes it may possess, as does heterologous tissue too, a power of inducing hypersensibility to cancerous growth. Therefore, in order to make sure that the treatment was made with living cells, the emulsion was injected immediately after its preparation. Injections were made by means of a sterile syringe subcutaneously into the abdominal wall of the mice, the dosage being at each injection constantly 2.5 centigrammes of as far as possible fluidless skin. Absorption took place within about 3 weeks, but by microscopical examination we found, in accordance with Da Fano's[1] results, fragments of the inoculated skin persisting for a longer time. In one mouse a small epithelial cyst was found 38 days after the inoculation. In no case, however, could pronounced proliferation or teratoma-like growth of the inoculated skin be traced.

As it turned out impossible to procure the number of pregnant mice necessary for the treatment of all 156 mice exactly on the same days, we were forced to regulate the days of injection according to the number of pregnant mice ready for use at some selected day, and partly for this reason, partly because some animals died before treatment was completed, all the mice have not received the same number of inoculations. Injections were

[1] 4th Scientific Report of the Imperial Cancer Research Fund 1911.

given at earliest on the 19th day, at latest on the 137th day after the beginning of the tarring (17 days after its cessation). Owing to outer circumstances treatment could not be further continued, as it had been planned. Most unfortunately one emulsion, injected into altogether 15 mice, must have been infected, since injection was followed in these animals by localized transitory suppuration. As a matter of course these injections, being non-effective, are not included in the total of injections made upon these 15 mice. The number of injections made into each mouse varied from 1—7. Details are given below.

The mice seemed to bear the injections very well, shock-similar symptoms were not observed.

All mice of both groups were left alive until they died spontaneously, after which systematic examination was made of the skin, especially the tarred area, and of all lymphatic glands and internal organs (see below).

Results.

In communicating the results of our experiments, we shall first deal with the cutaneous primary tar tumors observed in all treated and non-treated mice, turning thereupon to the metastasis formation for which these primary tumors were responsible in both groups.

Primary cutaneous tumors in all treated and in all non-treated mice.

The macroscopic appearance of the tarred area did not differ at all from that observed by most investigators upon the skin of tarred mice.

The intensity of the changes visible to the naked eye and palpable, was on the whole in all treated and non-treated mice proportional to the period during which the

animals had been tarred and had survived the tarring. As
the most pronounced changes in the longest lived mice
extensive cancerous infiltration of the skin was found,
associated generally with enormous cutaneous horns. A
gigantic case of this type is shown in Plate I, Fig. 1. In
other mice, also surviving for a relatively long time, less
pronounced changes of the same type occurred, in some
cases accompanied by ulcer formation. Mice surviving only
for a shorter time presented more or less marked hyper-
plastic thickening and papillomatous growth, whereas mice
of the shortest survival only showed cutaneous thickening
and alopecy, or this last phenomenon alone.

On comparing the pathological changes found in the
treated mice with those found in the tests, we are un-
able to point out any essential difference what-
ever between the macroscopic appearance and
structure of the cutaneous changes observed in
the mice of the two groups.

If only the slightest changes were visible, all the tarred
area of the dorsal skin was subjected to a thorough micro-
scopic examination, and in all cases this was done
by means of serial sections, a technical method which of
course was applied in all mice to demonstrate or preclude
the presence of invasive growth. The determination of the
non-carcinomatous nature of papillomata has thus always
been based upon a systematic examination of serial sec-
tions of the whole tarred area and all the papilloma.

Papillomatous proliferation and papillomata
without any atypical or invasive cancerous down-
growth whatever were found altogether in 16
(treated and non-treated), mice, whereas in 229 mice
true cancerous tumors developed.

In planning and performing these investigations we have not intended to subject to any quite exhaustive examination the much discussed question whether or not experimental tar tumors are only pure carcinomata, or may be carcino-sarcomata or sarcomata as well, an examination that would require special and extensive researches which up to the present we have not been able to perform.

Therefore, we shall restrict ourselves to giving here only a summary report on the structure of the tumors observed and of the classification which, in our opinion, it would be justified to make of them, after examining them by means of a few of the usual methods of staining[1].

In 29 out of the 229 cancerous tumors observed altogether in treated and non-treated mice the epithelial elements were situated in abundant bundles of closely packed, rather uniform spindle-shaped cells, differing distinctly not only from usual spindle-shaped epithelial cells but also from the cells of which the stroma of tar cancers is generally composed. In some areas the spindle-shaped cells predominated completely, so as to make the tumor tissue appear here as a pure spindle-celled sarcoma, while in other places an admixture of epithelial cells and strands of epithelium produced the well-known picture of a carcino-sarcoma (see Plate II figg. 4—5). Furthermore, in 2 tumors the typical structure of a pure spindle-celled sarcoma was predominant everywhere (see Plate II figg. 6 and 7). Such carcino-sarcomatous or sarcomatous structures were not only observed in the primary tumors, but in a great number of cases also in their pulmonary and glandular metastases which would then contain spindle-

[1] Hematoxyline-Eosine. Hematoxyline-van GIESON-HANSEN.

shaped sarcomatous elements alone or mixed with epithelial cells (see Plate III figg. 8, 10, 11 and Plate IV figg. 12—13).

Attempts were made in 7 cases to transplant tumors of a carcino-sarcomatous, more or less sarcomatous type, but in 4 cases without success. In 3 cases, however, the subcutaneous transplantation of metastatic nodules from axillary lymphatic glands gave positive results. In 2 of these mice the transplanted tissue was apparently purely sarcomatous, in one mouse it also contained epithelial elements. In all 3 cases in which transplantations were continued in 4, 5 and 14 generations respectively, the tumors developing in the transplanted mice presented a pure spindle-celled sarcomatous structure (s. Plate IV fig. 15).

Hoping later on to have the opportunity of treating thoroughly the question of the nature of tar tumors composed of spindle-shaped cellular elements, we shall not enter here upon any discussion of this problem, but only point out that, recognizing the great difficulties opposing any exact classification of such tumors, we feel inclined to consider 29 out of the 229 primary cancerous tumors observed altogether, as carcino-sarcomata, and 2 as pure sarcomata.

The remaining 198 tumors must without doubt be classified as typical flat-celled keratinizing pure carcinomata. In 185 out of these tumors the diagnosis carcinoma was ascertained by the demonstration of indubitable invasive downgrowth into the cutaneous muscular layers, combined in a great number of cases with carcinomatous invasion of lymphatics, blood vessels or nerves, with or without metastasis formation.

In the remaining 13 tumors considered by us as indubitable carcinomata, no invasive growth into the mus-

cular layers could be definitely traced. The tumors of these 13 mice, 8 of which were test animals and 5 treated mice, presented a histological structure corresponding fully to that of the well-known usual type of epithelioma malignum, the atypical epithelial proliferation completely penetrating the connective tissue and reaching down to the cutaneous muscular layers without invading them. In 2 of these mice belonging to the test animals and surviving for 124 and 171 days respectively, the diagnosis carcinoma was confirmed by the finding of carcinomatous metastases, in the former mouse in the one lung, in the latter both in lungs and axillary lymphatic glands.

Table I shows the total number of mice and all cases of tar cancer among them, grouped according to the time during which the mice survived the first tarring.

All figures indicating treated mice are printed in blacker types.

As will be seen from the table, the 229 cases of cancer observed altogether, developed in 127 out of 156 treated mice, and in 102 out of 137 test mice, i. e. in 81 and 74.4 per cent respectively.

This percentage difference which per se is too small to establish any real difference between the frequency of cancer within the two groups, must, however, be further diminished by the fact that the 12 mice mentioned above, destined for treatment, died so early after the first tarring, that no injection was made upon them. Therefore, we might be justified when calculating the frequency of cancer in the treated mice, in using as a basis the number 168 instead of 156, an increase which will practically make the difference disappear.

At any rate the figures do not admit of the as-

sumption of any indubitable difference between the frequency of primary cutaneous tar cancers observed in treated and in non-treated mice. And this result is further confirmed by a comparison of the treated mice with the test mice after grouping them according to their time of survival. It will be seen from the table that all mice, treated and non-treated, surviving the first tarring for a period of 2 months (0—60 days) presented only slight cutaneous changes, and none of them papilloma nor cancer. In mice surviving for 2—3 months (61—90 days) besides the papillomata, only one case of cancer developed in each of the groups, the treated group comprising 13, the non-treated group 9 mice, whereas half of the 18 test- and 20 treated mice surviving for 3—4 months (91—120 days) were bearing cancerous tumors. So were 10 out of 11 test mice and 22 out of 28 treated mice within the group surviving for 4—5 months (121—150 days), and finally cancer developed without any exception in all the remaining mice (82 tests and 94 treated mice).

Thus, the percentage number of cancers in such treated and non-treated mice as passed through the whole tarring and survived the initial tarring for 4 months or more, was found to be 95 and 99 respectively, while within the groups of tests and treated mice surviving for 5 months or more it was found to be 100.

These results, like those mentioned above, demonstrate that, on the basis of our experiments, there can be no question at all of attributing to injections of living homologous embryo skin any faculty whatever of preventing or retarding the development of primary cutaneous tar cancer in mice.

Table I. All Cases of Cancer in treated and non-treated Mice.

All figures indicating treated mice are printed in blacker types.

Span of life after 1st tarring Days	Number of mice	Slight changes or none	Hyperplasia papilloma	Cancer invasion not observed	invasive	All cancerous mice	Percentage frequency of all cases of cancer
0— 60	17 / **1 (+ 12)**	17 / **1 (+ 12)**				0 / **0**	
61— 90	9 / **13**	7 / **8**	1 / **4**	1	/ **1**	1 / **1**	
91—120	18 / **20**	6 / **8**	3 / **2**	2 / **2**	7 / **8**	9 / **10**	27 / **33** ; 10 / **11** ; 37 / **33**

— tarring stopped —

Span of life after 1st tarring Days	Number of mice	Slight changes or none	Hyperplasia papilloma	Cancer invasion not observed	invasive	All cancerous mice	Percentage frequency of all cases of cancer
121—150	11 / **28**	/ **1**	1 / **5**	4 / **2**	6 / **20**	10 / **22**	
151—180	21 / **19**			1 / **1**	20 / **18**	21 / **19**	
181—210	28 / **35**				28 / **35**	28 / **35**	
211—240	24 / **24**				24 / **24**	24 / **24**	
241—270	6 / **11**				6 / **11**	6 / **11**	93 / **122** ; 92 / **116** ; c. 99 / **95** ; 100 / **100**
271—300	2 / **1**				2 / **1**	2 / **1**	
301—362	1 / **3**				1 / **3**	1 / **3**	
578	**1**				**1**	**1**	
total	137 / **156**	30 / **18**	5 / **11**	8 / **5**	94 / **122**	102 / **127**	74.4 / **81**

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Metastasis formation in all treated and in all non-treated mice.

As already stated cancerous tumors developed in 102 out of 137 non-treated test mice, and in 127 out of 156 treated mice.

Upon these 127 treated mice the injections of the emulsion of mouse-embryo skin were made at the following periods[1] after the tarring began.

| | | days after beginning of tarring | |
number of mice	number of injections	1st injection	last injection
5	7	19	137
34	6	19	128
62	5	25	127
23	4	25	101
2[2]	3	43	87
1[2]	2	45	68

Before comparing the metastasis formation in these treated mice with that of the test animals, we shall communicate the technique employed by us in investigating the frequency of metastasis formation in all mice.

After exact examination at the autopsy of all abdominal and thoracic organs and all lymphatic glands, microscopic examination was made in all cases where metastases, metastasis-like nodules or spots were visible to the naked eye[3]. Lungs and axillary

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[1] As mentioned above, the intervals between the injections could not be exactly the same throughout the experiments. As a rule injection was made every third week, so that no interval exceeded this space of time.

[2] mice dying 20—37 days before cessation of tarring.

[3] Besides metastases in the lungs and the axillary lymphatic glands, one mouse presented metastases in the heart and in the diaphragma, one mouse metastases in the spleen, and one mouse metastases in both inguinal glands.

lymphatic glands were cut totally into serial sections in all mice the skin of which presented pronounced changes, papillomatous hyperplasia, papilloma or cancerous tumors.

In the mouse both axillae will, as a rule, contain 2, very exceptionally 3 lymphatic glands, but in a certain number of animals one or both axillae may contain only one lymphatic gland visible to the naked eye[1]. To ascertain definitely, however, that no axillary lymphatic gland had escaped attention, we made microscopic examination of all the tissue of the axillae in all cases where we did not succeed in distinguishing at least 2 glands in each axilla. In 6 mice, 3 test and 3 treated mice, the cutaneous tumors extended so far into the axillary region that it was impossible for us to decide whether or not metastases were present in the axillary glands (see the table below).

In most cases in which large metastases were easily visible to the naked eye (see Plate I, fig. 2—3), the microscopic examination was suspended when the histological structure of the metastatic nodules was sufficiently recognized.

In all other mice every 10th section (à 6 μ) of the lungs, and every 6th section of the axillary lymphatic glands were examined microscopically, and if metastasis-like changes or small metastatic nodules were traced, examination was made of every 5th and every 3rd section respectively.

Thus the non-existence of metastases was not finally decided upon, before lungs and all axillary lymphatic glands had been subjected to this systematic examination of serial sections.

Strict measures have been taken to avoid that even the tiniest metastases escaped microscopic examination. During the process of staining, the preparations were examined preliminarily by one of the assistants of the Institute, being thereafter all of them subjected to a systematic microscopic examination by another assistant, who for a series of years has made examinations of this kind at the Institute, and finally the preparations were once more

[1] This was the case with 5 out of 50 normal non-tarred mice examined for this special purpose.

thoroughly examined and revised microscopically by one of the authors (Fibiger), in several cases by both of us.

In accordance with the primary cutaneous tumors the great majority of metastases were flatcelled, keratinizing carcinomata (see Plate III, fig. 9, Plate V, figg. 16—17), but as mentioned above, also carcino-sarcomatous and pure sarcomatous metastases were found (see Plate III, figg. 8, 10 and 11, Plate IV, figg. 12—13). The metastases rather often contained necrotic areas. We do not, however, feel entitled to state any pronounced difference in this respect between the metastases in non-treated and in treated mice. And only once or perhaps twice have we observed in the latter such tiny intravascular cellular groups as could be supposed to be composed of metastatic tumor cells in a stage of degeneration, necrosis and absorption. We wish, however, to point out that we must consider our investigations on this problem as only preliminary, and not as definitely finished or decisive.

Primary pulmonary adenomata of the wellknown usual type (see Plate V. figg. 18—19) as described by Tyzzer,[1] Jobling,[2] Murray,[3] Haaland[3] and others were found altogether in 9 mice (4 treated and 5 tests) out of the 245 mice of both groups, the lungs of which were subjected to microscopic examination. In one treated mouse out of these 9, the lung contained in addition a primary bronchial carcinoma. In none of these mice, no more than in a mouse (test) the lungs of which presented a typical strongly keratinizing primary bronchial carcinoma, could

[1] 5th Report of the Cancer Commission of Harvard University 1909.

[2] Monographs of the Rockefeller Institute for Medical Research. No. 1. 1910.

[3] 3rd and 4th Scientific Report of the Imperial Cancer Research Fund 1908 and 1911.

parasitic worms be traced in the lungs. In a few cases where slighter or stronger signs of pulmonary lymphomatosis were observed, no worms were found either.

Altogether the lungs of 23 mice contained worms (alive or dead and calcified), mostly very few in number, sometimes numerous, and in several cases situated in the vessels. In most cases the surrounding pulmonary tissue presented marked pneumonic changes which were also frequently found in mice not infested with worms.

Table II shows the total number of treated and non-treated mice bearing cancerous tumors, and all mice in which metastasis formation occurred, arranged according to the space of time in which these mice survived the first tarring.

As mentioned above the propagation of the primary tumors into the axillary regions prevented us from ascertaining definitely, whether or not metastases were present in the axillary glands of 3 tests and of 3 treated mice. Consequently there is a possibility that the number of mice in which metastases had developed must be raised by 3 in both groups, and therefore the number of metastases applying to these 6 mice is given in 2 figures in the table.

Also in Table II all figures indicating treated mice are printed in blacker types.

As will be seen from the table, metastasis formation was found in 97 — or perhaps, if the 6 mice just mentioned are included — in 103 out of altogether 229 cancerous treated and non-treated mice.

It will be noted from the table that among all the cancerous mice metastases were found in:[1]

[1] If against our opinion one will deny the right of maintaining the diagnosis cancer in the 6 test mice and in the 5 treated mice (s. p. 17)

59—62 out of 102 test mice i. e. 57.8—60 per cent, and in
38—41 „ „ 127 treated mice i. e. 29.9—32 per cent.

If in both groups we exclude all mice dying before
cessation of the tarring or just coincidently with this
(which is to say 120 days after its beginning), and in
which metastases were not found at all, the figures will be:

*In 59—62 out of 92 test mice metastases were found i. e.
64—68 per cent.*

**In 38—41 out of 116 treated mice metastases were found i. e.
32.7—35 per cent.**

*Altogether the percentage frequency of metastases was thus
found to be about half as high in treated cancerous mice as
in the tests,* and this result is not due to the circumstance
that the test mice might have more frequently survived the
tarring for longer periods than did the treated mice, and that
metastases would accordingly find better conditions of
evolution in the tests than in the treated mice. That such
a difference must be out of the question appears from
Chart I (page 26), according to which the percentage mor-
tality of cancerous test mice within the different periods
after beginning and cessation of the tarring, follows almost
the same curve as does the percentage mortality of the
treated cancerous mice.

61 out of the cancerous tests and 75 out of the treated
cancerous mice survived cessation of the tarring for more
than 2 months, and the percentage frequency of metasta-
ses among them was *77—80* and **45—48** respectively.

in which no invasive down growth into the muscular layers and no meta-
stases could be traced, the total number of cancerous test mice and
treated mice will be reduced to 96 and 122 respectively. The percentage
numbers of cancerous test mice and cancerous treated mice bearing meta-
stases then will be: *61—64.5* and **31—33.6** respectively.

Table II. Metastasis formation its Occurrence and Frequency in all treated and non-treated cancerous Mice.

All figures indicating treated mice are printed in blacker types.

Span of life after 1st tarring Days	Total number of cancerous mice	Number of mice presenting metastasis formation				Macroscopic metastasis-formation	Metastasis formation in all cancerous mice Percentage number	
		in the lungs	in the lymph. glands	in the lungs and in the lymph. glands	total			
0— 60								
61— 90	1 **1**							
91—120	9 **10**							
tarring stopped								
121—150	10 **22**	3	**1**	2	3 **3**		30 **13.6**	
151—180	21 **19**	3 **1**	2 (3) **(1)**	4	9 (10) **1 (2)**	6	42.8-47.6 **5.2-10**	
181—210	28 **35**	9 **4 (3)**	3 (4) **3**	9 **7 (8)**	21 (22) **14**	10 **7**	75-78.5 **40**	
211—240	24 **24**	7 (8) **6**	4 **4 (5)**	7 (6) **2**	18 **12 (13)**	14 **4**	75 **50-54**	64-68 **32.7-35**
241—270	6 **11**	**3**	(1)	5 **2**	5 (6) **5**	3 **3**		
271—300	2 **1**	1 **1**		1	2 **1**	1	88-100 **50-56**	
301—362	1 **3**	1	(1)	1	1 **1 (2)**	1		
578	**1**		**1**		**1**	**1**		
total	102 **127**	24 (25) **15 (14)**	9 (12) **9 (12)**	26 (25) **14 (15)**	59 (62) **38 (41)**	34 **16**	57.8-60 **29.9-32**	

But from the Table will be noted the far more pro-
nounced difference between the frequency of metastases in
the treated mice and that observed in the tests surviving
cessation of the tarring only for 1—2 months. While
12—13 out of 31 cancerous test mice of these
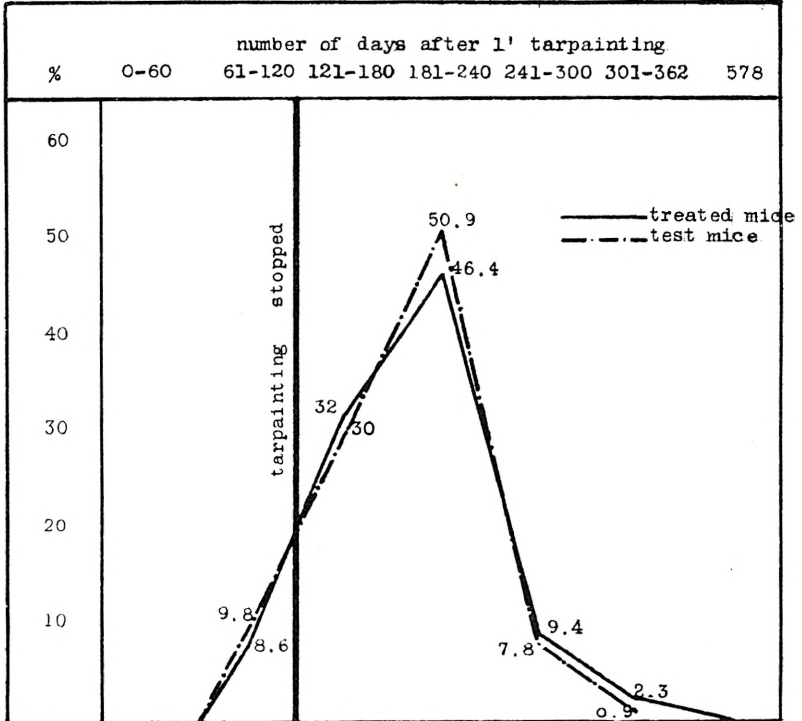
Chart I.

groups had developed metastases (about 38—42
per cent), only 4—5 out of 41 treated mice (9—12
per cent) presented metastases.

For further evidence of the difference between the fre-
quency of metastases in treated and in non-treated mice,
we refer the reader to the Chart II (page 27) giving a
graphical view of the difference.

But also the frequency of macroscopic metastasis formation is different in the two groups.

As mentioned above, FRIDTJOF BANG found macroscopic metastasis formation in the lungs and lymphatic glands in 29.2 per cent of mice affected with experimental tar-cancer. To this number correspond very well those found by us in these investigations, as 34 out of 102 cancerous test mice presented macroscopic metastases, viz. 33.3 per cent. On the contrary, only 16 out of our **127** treated cancerous mice, viz. **12.6** per cent, presented metastases visible to the naked eye.

It further appears from Table II, that metastases visible to the naked eye occurred in 34 out of 59—62 metastases-bearing test mice and in **16** out of **38—41** metastases-bearing treated mice.

Furthermore, coincident occurrence of metastases both in lungs and lymphatic axillary glands within the same animal was observed:

in 25—26 out of the 102 cancerous test mice and out of the

Legend (Chart II):
- □ non-cancerous mice
- ▨ cancerous non-treated test mice
- ▨ cancerous treated mice
- ■ cancerous mice with metastases

tarring suspended

Injections of skin emulsion

beginning of tarring

Survival after 1' tarring Days

0-60, 61-90, 91-120, 121-150, 151-180, 181-210, 211-240, 241-270, 271-300, 301-362, 572
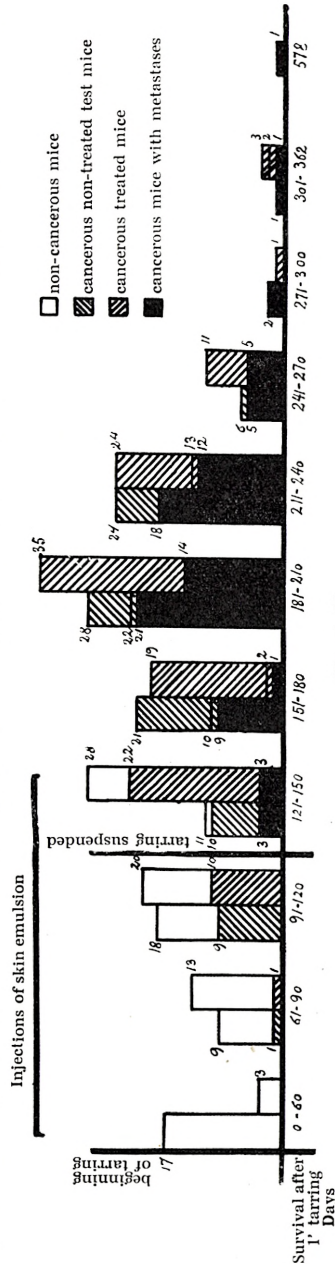
Chart II.

59—62 test mice bearing metastases, and in **14—15** out of the **127** cancerous treated mice and out of the **38—41** treated mice bearing metastases.

Thus, the experiments seem to indicate that also the size and dissemination of the metastases is probably smaller in the treated mice than in the tests. The numerical differences here are not, however, sufficient to admit of any convincing statistical proof.

But this cannot be maintained against the chief result that: Out of the total number of mice metastases occurred about half as frequently in the treated mice as in the tests.

This result emphasizes the efficacy of the treatment, and according to Professor Dr. jur. and polit. H. Wester-gaard who has kindly reviewed critically the numerical material no lawful objection can be made to this conclusion from a statistical point of view.

Thus we seem entitled to assume that the strikingly rare occurrence of metastases in the treated mice surviving cessation of the tarring for 1—2 months only must most probably be ascribed to the fact that these mice belong to a period that is least distant from the last immunizing treatments, a period in which the active power of the immunizing factor will accordingly be higher than in subsequent periods.

Summary.

The chief results of our investigations here recorded may be summarized as follows:

1) *treatment of cancerous mice by inoculation of homologous, living mouse-embryo skin has* — in accordance

with previous investigations — *appeared ineffective against the development and growth of primary cancerous tumors.*

2) On the other hand, in contradistinction from the current opinion *it must be regarded as proved that treatment with homologous, living mouse-embryo skin may bring about inhibition of the metastasis formation in cancerous mice.*

In which way this inhibition takes place must be made the subject of later researches. Our experiments do not contribute to the elucidation of this question, their chief aim being only to investigate whether experimental researches would offer any basis whatever for the assumption that immunizing tissue treatment is capable of preventing metastasis formation in cancer.

Possibly future investigations will show that treatment during longer periods or application of larger doses than those injected by us, or inoculation of other tissues (as e. g. blood) may give still better results.

We need not point out that for the present there is no way of judging whether or not it might prove possible by further researches to build up, on the basis of the above investigations, methods that might be utilized to prevent metastasis formation in human cancer. But great practical difficulties seem to oppose the establishing of such methods. According to most of the investigations made up to the present in this field, not only the attainment of perfect efficacy of immunizing tissue treatment, but also the possibility of avoiding hypersensibility to cancerous growth, must presumably neccessitate the appli-

cation of homologous and living, sterile cellular material, material which is difficult to procure.

We desire to acknowledge our indebtedness to the Carlsberg Fund and to the William Bendix and Mrs. Bendix Legacy for their support of these investigations.

We should like, also in this place, to beg Miss I. Breds-dorff and Miss M. Falck, assistants at the Anatomo-pathological Institute of the University of Copenhagen, to accept our thanks for their valuable assistance at our researches.

PLATES

PLATE I.

Fig. 1. Mouse treated with 6 injections of emulsion of embryo skin. Died 350 days after 1st tarpainting.
Enormous cutaneous Cancer (carcinosarcoma) and gigantic cutaneous horn. Natural size.

Fig. 2. Pulmonary metastases in non-treated mouse dying 220 days after 1st tarpainting.
Natural size.

Fig. 3. Axillary lymphatic glands with metastasis formation. Non-treated mice. Natural size.
a. from mouse dying 200 days after 1ste tarpainting.
b.   »   »   »   185   »   »   »   »
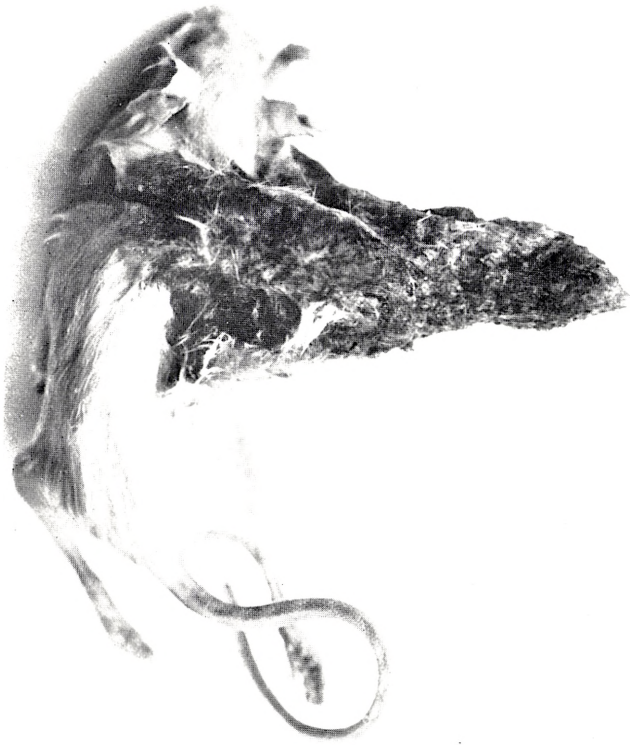
Fig. 1.
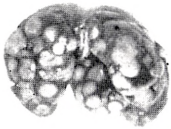
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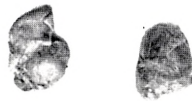
a.   b.

Fig. 3.

PLATE II.

Fig. 4. Primary cutaneous tar cancer. Carcinosarcoma. $\times$ 75.
Fig. 5. The same tumor $\times$ 190.
Fig. 6. Primary cutaneous tar cancer. Sarcoma. $\times$ 75.
Fig. 7. Primary cutaneous tar cancer. Sarcoma. $\times$ 190.
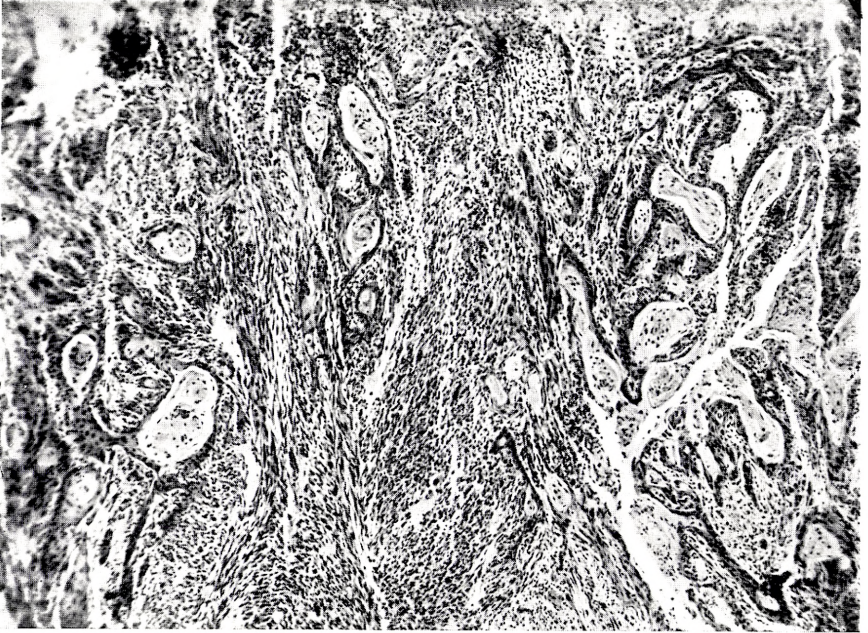
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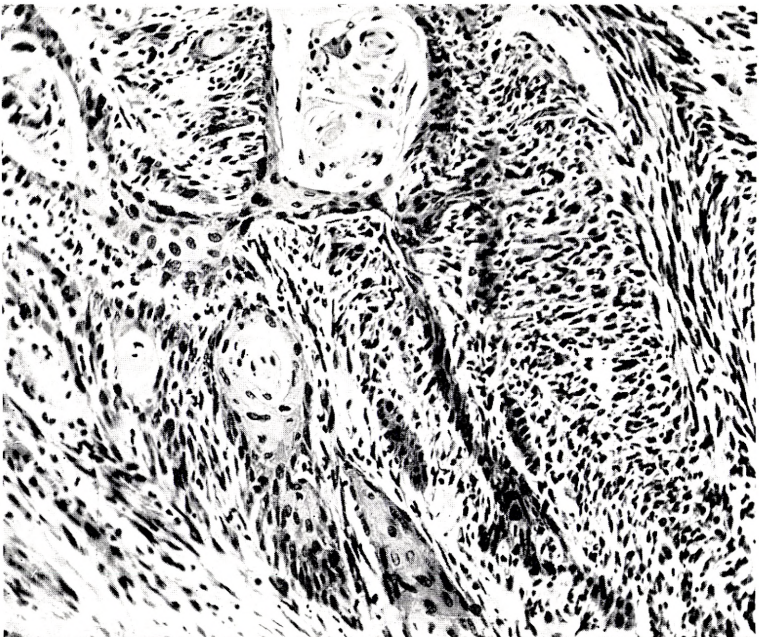
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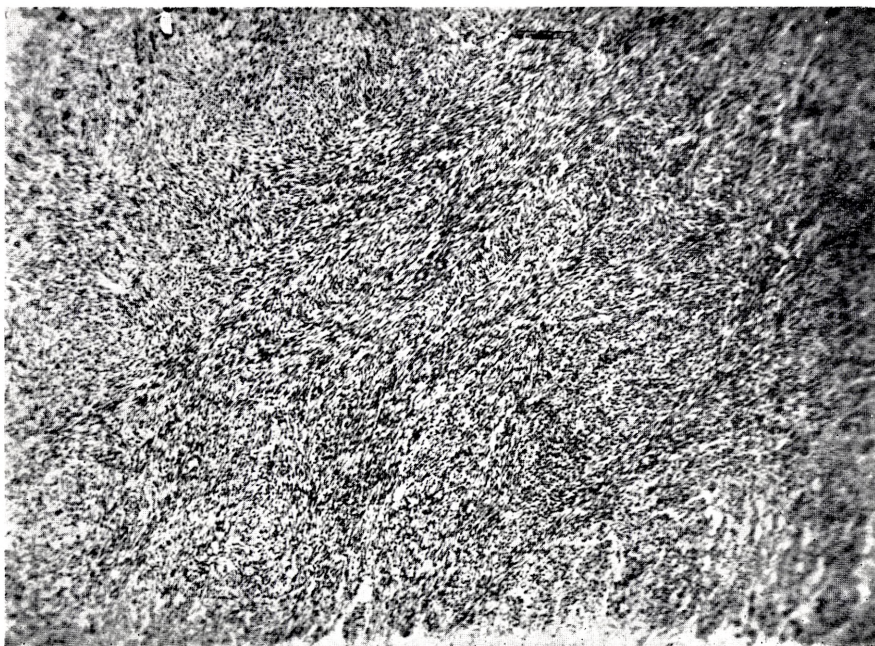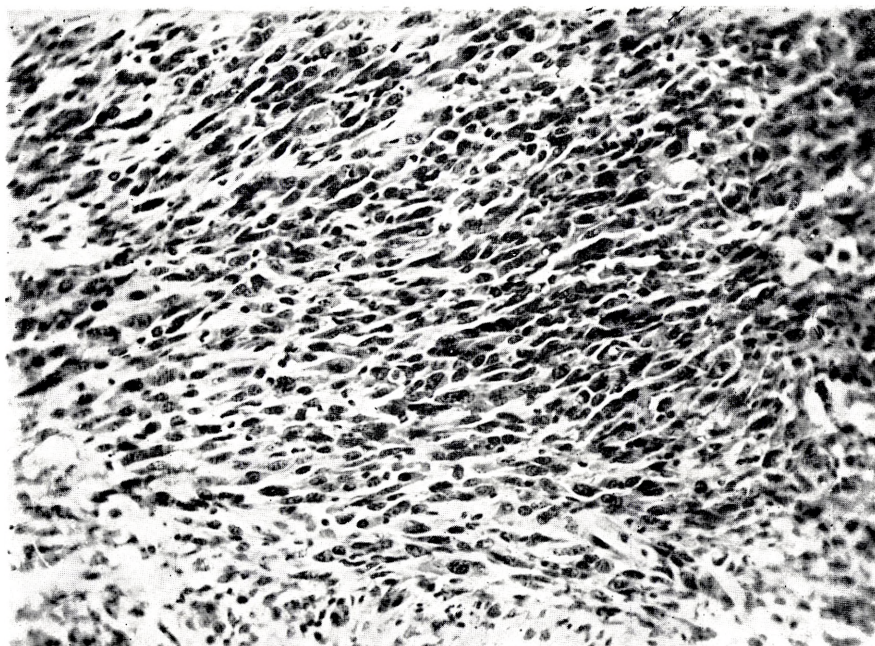
Plate II

Fig. 6.

Fig. 7.

PLATE III.
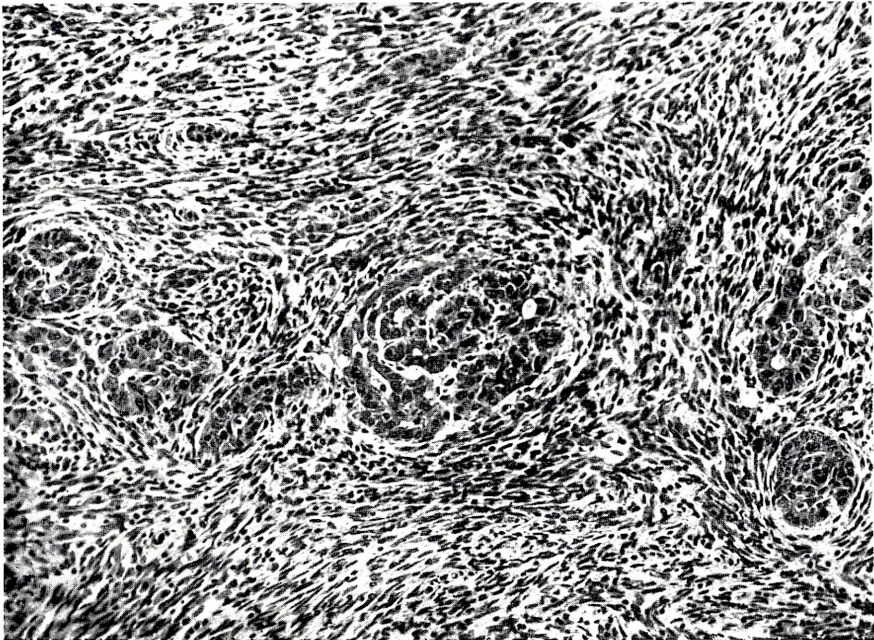
Fig. 8.
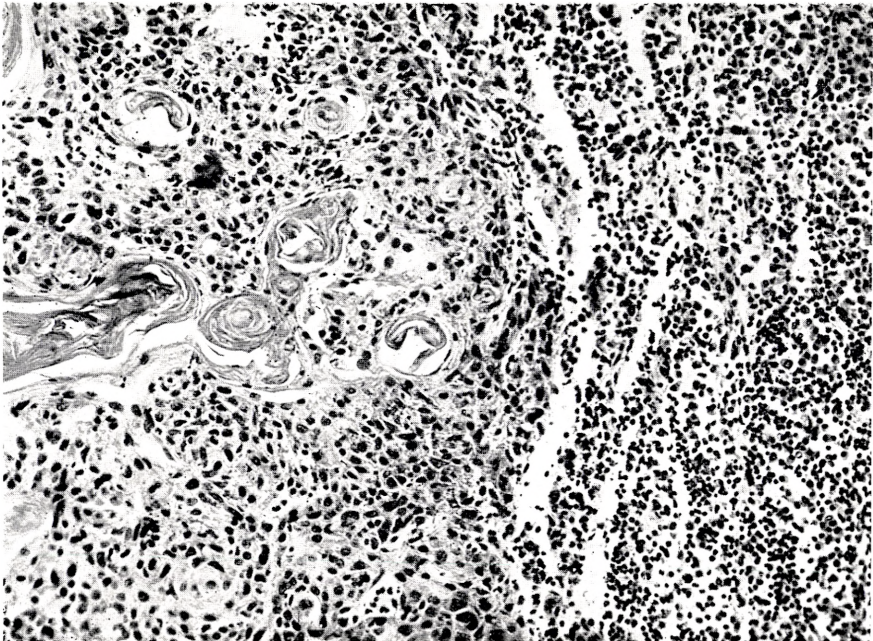
Fig. 9.
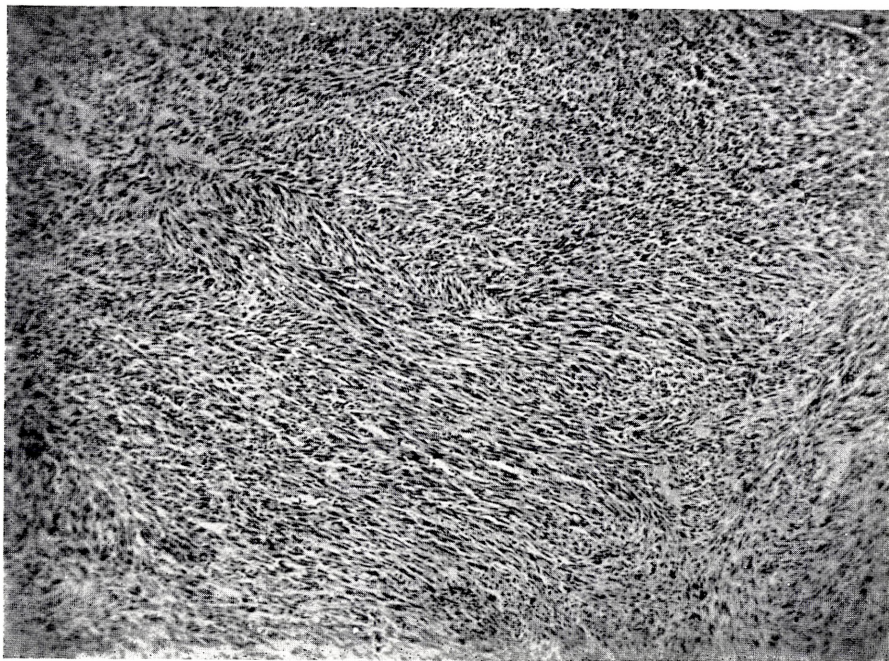
Plate III

Fig. 10.
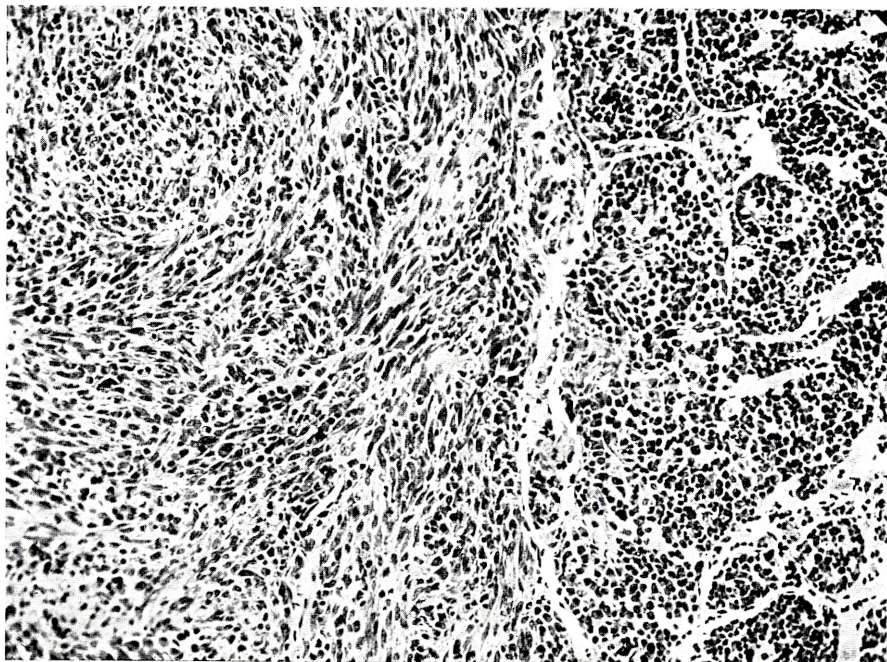
Fig. 11.

PLATE IV.

Fig. 12. Pulmonary metastasis. Pure sarcoma. $\times$ 170.

Fig. 13. Metastasis in the heart muscle. Pure sarcoma. $\times$ 100.

Fig. 14. Transplanted metastatic pure carcinomatous tar tumor. 12th generation. $\times$ 170.

Fig. 15. Transplanted metastatic sarcomatous tar tumor. Pure sarcoma. 14th generation. $\times$ 170.

Fig. 12.

Fig. 13.

Plate IV
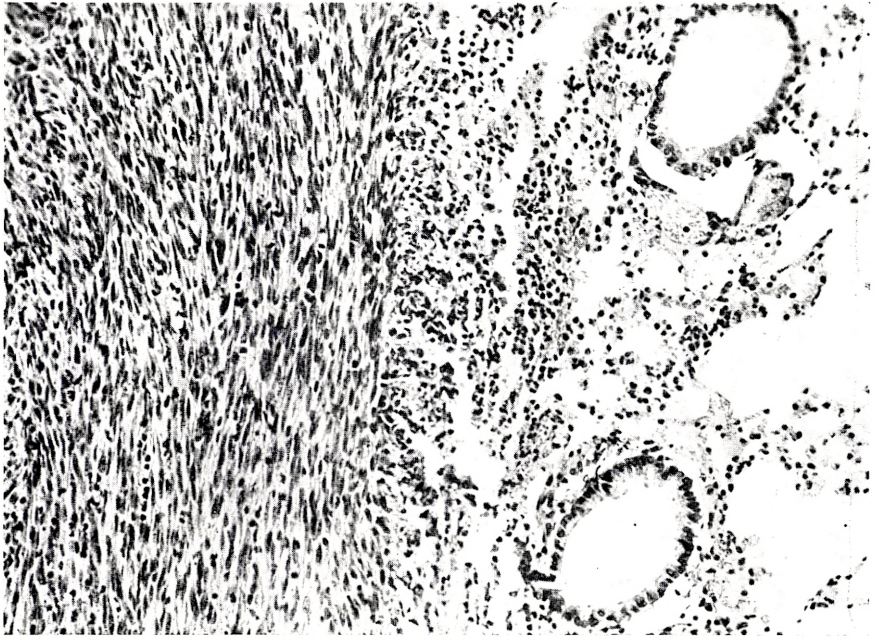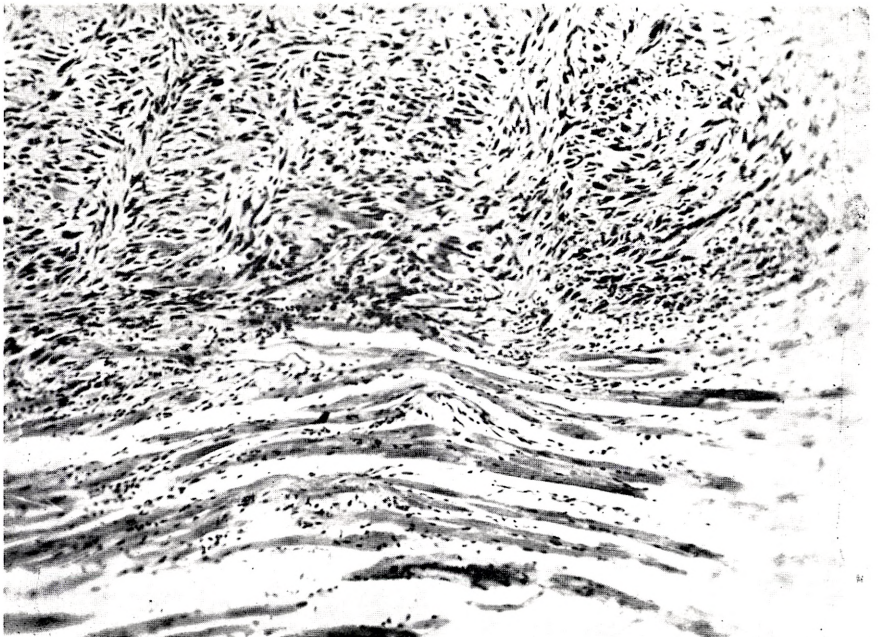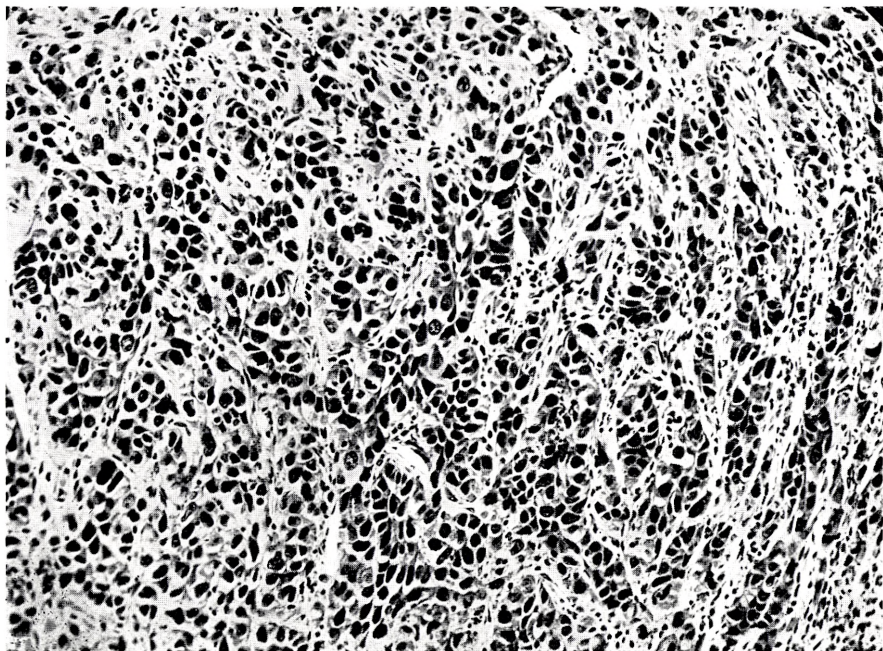
Fig. 14.

Fig. 15.

PLATE V.

Fig. 16.

Fig. 17.

Plate V
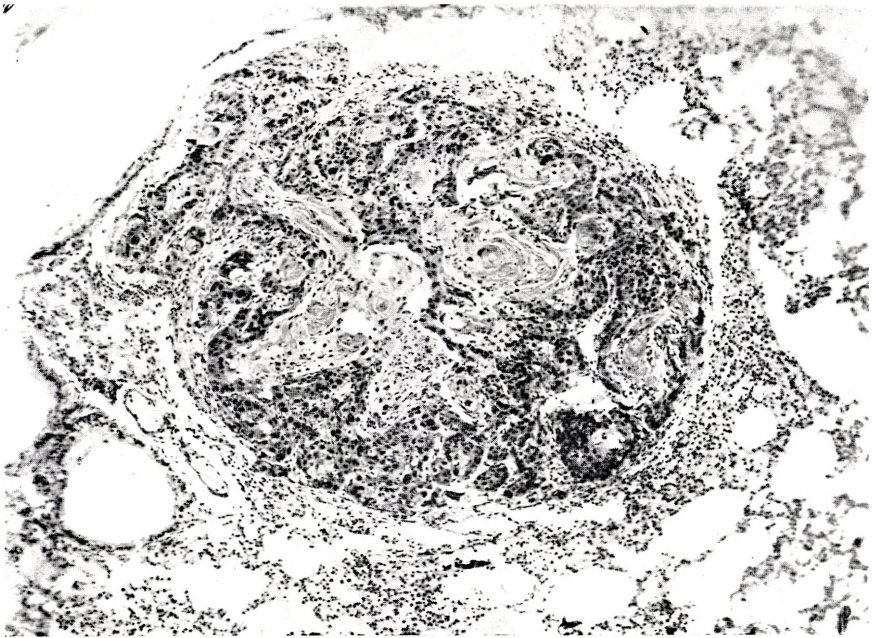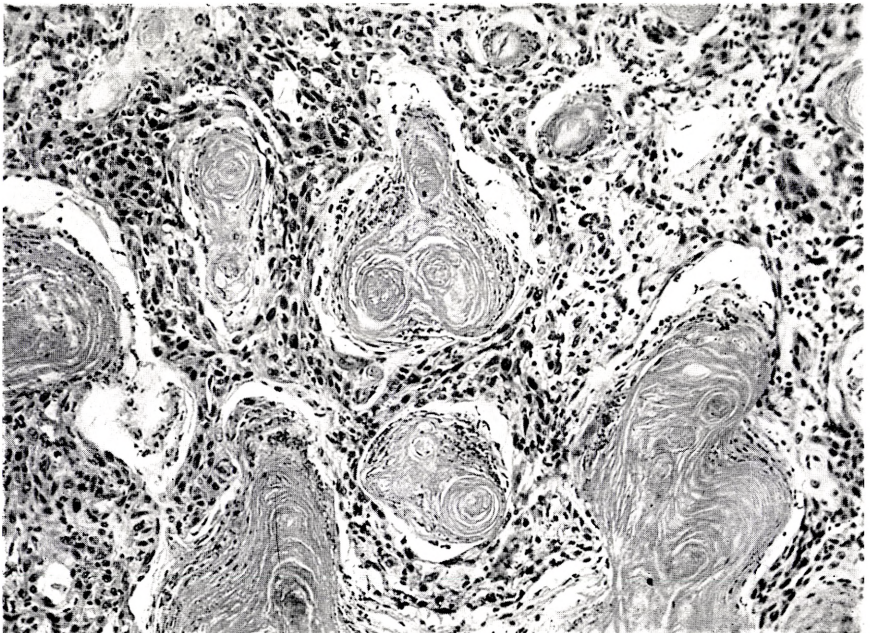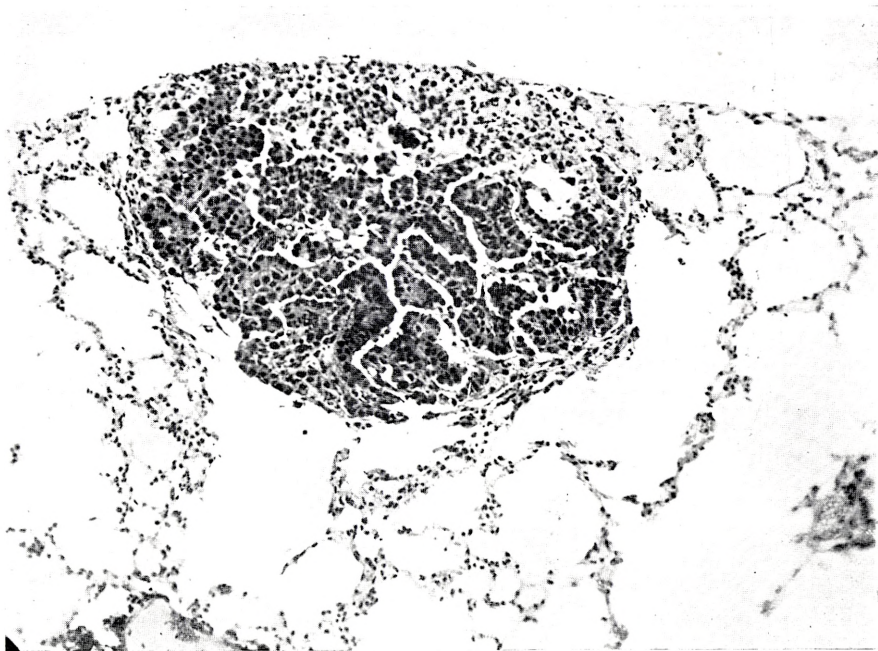
Fig. 18.
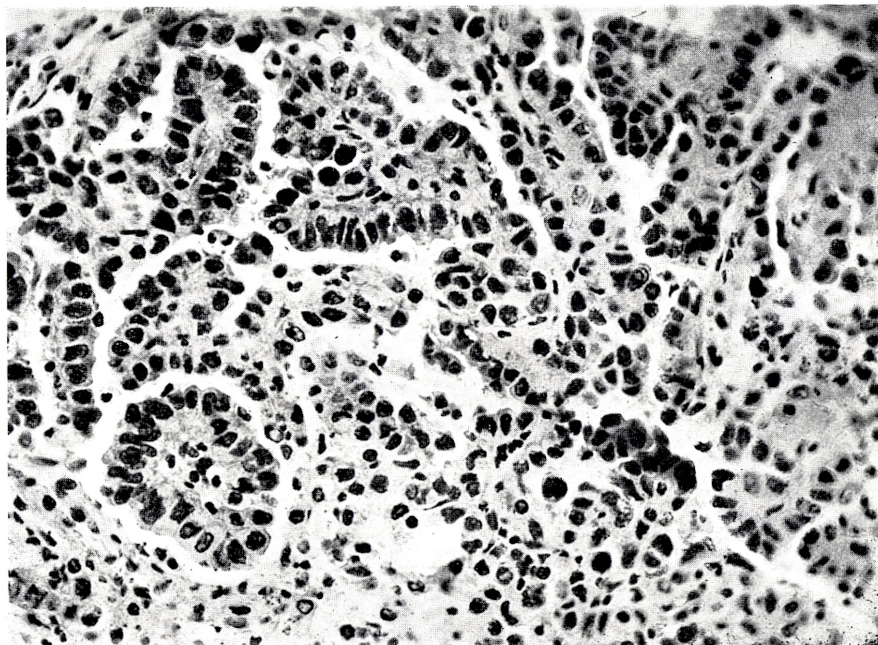
Fig. 19.

BIOLOGISKE MEDDELELSER

UDGIVNE AF

DET KGL. DANSKE VIDENSKABERNES SELSKAB

2. BIND (KR. 15,40):

3. BIND (KR. 19,95):