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EXPERIMENTAL PRODUCTION OF TAR CANCER IN WHITE MICE

BY

JOHANNES FIBIGER AND FRIDTJOF BANG

WITH SIX PLATES

KØBENHAVN
HOVEDKOMMISSIONÆR: ANDR. FRED. HØST & SØN, KGL. HOF-BOGHANDEL
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It is a long recognised fact that the skin, when exposed for a longer period to the action of substances due to incomplete combustion of coal, or to distillation of coal, tar or allied compounds, may become the seat of dermatosis, papillomatous and carcinomatous growth.

As will be generally known, the first observation in this domain was "the chimney sweeps' cancer" (PERCIVAL POTT 1775), caused by the influence of the soot; already in the early half of the nineteenth century literature contained a considerable number of reports on this cancer.

Later observations (VOLKMANN[1], TILLMANS[2], SCHUCHARDT[3], a. o.) have shown that cutaneous diseases and cancer of quite a similar type may occur among workers engaged in the manufacture of tar and crude paraffin; and finally it has been reported (LEGGE[4], LUSH[5], ROSS and CROPPER[6],

[1] Berl. klin. Wochenschrift. 1874.

[2] Deutsche Zeitschrift f. Chirurgie. 1880, cit. by Schuchardt.

[3] Volkmann's Sammlung klin. Vorträge, No. 257, 1885.

[4] Home Office. Manufacture of Patent Fuel. Special Report on Ulceration of the Skin and Epitheliomatous Cancer in the Manufacture of Patent Fuel, and of Grease. London 1912.

[5] Report to His Majesty's Secretary of State for the Home Department on the Draft Regulations proposed to be made for the Manufacture of Patent Fuel (Briquettes) with addition of Pitch. Presented to Parliament by Command of His Majesty. London 1911. Second Report 1913. Annual Report of the Chief Inspector of Factories and Workshops for the Year 1918, presented to Parliament by Command of His Majesty. London 1919.

[6] The Problem of the Gasworks Pitch Industries and Cancer. London 1913. H. C. Ross: Occupational Cancer. The Journal of Cancer Research. Vol. III. 1918.

Herman[1], Bayet and Slosse[2], Glibert[3], a. o.) that also workers employed in the briquette factories are liable to suffer from similar affections, which are ascribed to the action of the pitch contained in this sort of fuel. Also among workers at petroleum wells a series of cutaneous carcinomata has been observed (Ullmann[4], a. o.).

The skin affections of the chimney sweeps and those of the tar, paraffin and briquette workers present very great conformities in regard to localisation, development, symptoms and course of disease. In all these workers the affections will make their first appearance — most commonly and most strongly — on the skin of scrotum, and epitheliomatous growth will occur with relative frequency in this region too. The lesions, however, may affect other parts of the body (hands, neck, arms, face, lips).

Initial symptoms are: dermatitis, pigmentation, chronical thickening of the skin, keratosis and warty growths (soot-warts, pitchwarts), after which ulcers and carcinomata of the common malignant keratinising epitheliomatous type may develop.

The quite similar character of these cutaneous affections and their occurrence after the action of substances which may, to some extent, be ascribed to the same origin, most naturally has given rise to the opinion that in all these affections the same or closely allied chemical factors in the strongly composed substances: soot, tar, crude pa-

[1] Prophylaxie de la maladie du brai. Bull. de l'Académie Royale de Médecine de Belgique. IV Série. Tome XXIX. 1919.

[2] L'intoxication houillère arsenicale. Ibidem.

[3] Contribution à l'étude du cancer professionel. Ibidem.

[4] Ueber das Wesen und die Verbreitung einiger bei der Erdölgewinnung und Paraffinfabrikation entstehender Berufsdermatosen. Das oesterreichische Sanitätswesen. No. 18. 1912.

raffin and pitch, might in reality be made responsible for the irritation of the skin and the development of cancer. Creosote and carbolic acid have been pointed out as dangerous agents, and arsenic, anthracene and aniline as well, but we do not yet possess any real knowledge of the nature of the active substances, and up till now there has been no practicable way whatever of solving this question by means of experimental investigations, because it has hitherto proved impossible to produce cutaneous cancer in animals on exposing their skin either to such composed products as are considered etiological factors, or to various of the chemical compounds contained therein.

It was thus reported by HANAU[1] (1889) that a greater series of experiments, in which for months rats had been painted on the skin of scrotum with pitch or tar (with or without paraffin admixed) has given negative results. CAZIN's[2] investigations (1894) in which for 5 months a dog was brushed with tar and soot on an inflamed area of the skin, did not prove successful either, no more did the experiments of BROSCH[3] (1900) in which the skin of guinea-pigs (after previous bruising) was rubbed with xylol-paraffin for 2—3 months every 3—4 day.

Nor did STAHR[4] (1907) succeed in producing cutaneous cancer by means of injuring and treating the skin of rabbits, mice and rats with xylol-paraffin, soot or tar, — and on rubbing the skin of rabbits' ears with crude paraffin oils (ULLMANN[5] 1912), soot (HAGA[6] 1913), anilinous

[1] Fortschr. der Medizin. VII. 1889.
[2] Des origines et des modes de transmission du cancer, Paris 1894.
[3] Virchow's Archiv Bd. 162. 1900.
[4] Münch. med. Wochenschrift. 1907.
[5] l, c.
[6] Zeitschr. f. Krebsforschung. 1913. Vol. XII.

ointments (Haxthausen[1] 1916) only epithelial proliferation and no carcinomatous growth was produced. Also on the skin of rabbits' scrotum soot had no effect. (Haga)[2].

Finally a considerable number of investigations have been published most of which were impelled by the renowned experiments of Fischer[3] on the effect of injections with Scharlach R. oil. For subcutaneous injection on the rabbits' ears were used partly various crude paraffin oils (with or without admixture of soot), partly extracts of tobacco tar, coal tar, pitch and the like. Also in these experiments (Meyer[4], Benthin[5], Wacker and Schminke[6], Greischer[7], Ross and Cropper[8], Bayon[9]) the result — when most successful — was only proliferation and some downgrowth of the surface epithelium, but no development of carcinoma.

The first reports on experiments with positive results appeared from Japan in 1915, and were due to Yamagiwa and Ichikawa[10].

These investigations, commenced in 1913, were inspired (Yamagiwa and Ichikawa[10]) by the success of Fibiger's[11]

[1] Dermatologische Zeitschr. Bd. XXIII. 1916.

[2] Zeitschr. f. Krebsforschung. 1913. Vol. XII.

[3] Münch. med. Wochenschrift. 1906.

[4] Ziegler's Beiträge Bd. 46. 1909.

[5] Zeitschrift f. Krebsforschung. Bd. 10. 1911.

[6] Münch. med. Wochenschrift. 1911.

[7] Zeitschr. f. Krebsforschung Bd. 11. 1912.

[8] l. c.

[9] The Problem of the Gasworks Pitch Industries and Cancer. London 1913.

[10] Experimentelle Studie über die Pathogenese der Epithelialgeschwülste I, II and III. Mittheil. der med. Fakultät der Kaiserlichen Universität zu Tokyo. Bd. XV. 1915. Bd. XVII. 1917. Bd. XIX. 1918. The Journal of Cancer Research. Vol. III. 1918.

[11] Zeitschrift für Krebsforschung. XIII. 1913.

experimental production of cancer on transmission of the *Spiroptera neoplastica (Gongylonema neoplasticum).*

As experimental animals YAMAGIWA and ICHIKAWA used rabbits the ears of which were painted with coal tar every 2nd or 3rd day. The result of these experiments was published in a series of reports, the last of which appeared in 1918.

It will be seen from these reports that papillomatous new growth ("folliculoepitheliomata") may be produced on the rabbit's ear by application of coal tar for 30 to 100 days; that by the repeated application complete carcinoma was produced in 12 out of 196 rabbits, and furthermore that carcinoma was found in its earliest stage in 23[1] and in an early stage in 26 ears[1]. The carcinomatous change was discovered between the 55th and the 360th day, in most of the cases it was found after the 150th day.

The presence of metastasis was microscopically proved in the regional lymph nodes in three cases.

The hyperkeratotic papillomatous new growths continued to grow after the irritant had been discontinued and eventually developed into cutaneous horns. From such horns carcinoma developed in some cases.

Investigations of this kind have hitherto been published by no other author, whereas BULLOCK and ROHDENBURG[2] in 1918 — without having tested the Japanese experiments — expressed their doubt as to YAMAGIWA's and ICHIKAWA's success in producing real carcinoma.

In 1918 information was given by a third Japanese scientist HIDEJIRŌ TSUTSUI[3], who by painting the skin of

[1] The number of animals is not communicated.

[2] The Journal of Cancer Research. III. 1918.

[3] Ueber das künstlich erzeugte Cancroid bei der Maus. Gann. Japanische Zeitschrift für Krebsforschung. XII. 2. Juli. 1918.

mice repeatedly with tar had also succeeded in producing hyperkeratosis, papillomatous growth and carcinoma. Only an extremely brief report of these experiments is at hand, from which it appears that out of 67 mice which survived more than 100 days after the beginning of the painting, cutaneous carcinoma developed in 16, sarcoma in 1 mouse. Metastatic nodules were found in the lungs of 2 mice.

Also experiments of this kind are hitherto unpublished from other laboratories.

Finally YAMAGIWA and ICHIKAWA[1] in 1919 reported, that on repeated injection of mixtures of lanoline and tar, or extracts from tar in lanoline into the mammae of 47 rabbits initial stages of cancroids were produced in 3 cases, taking their rise from the large ducts. In a 4th rabbit cancroid developed as well, and in a 5th a myxofibrosarcoma mammae, which produced metastases and according to information in a letter from Prof. YAMAGIWA to FIBIGER proved transplantable.

In spite of BULLOCK and ROHDENBURG's criticism of YAMAGIWA's and ICHIKAWA's statements it has seemed to us to admit of no doubt whatever, that these latter authors have actually succeeded in producing experimentally tar carcinoma of the skin in rabbits, — and the examination of some microscopical preparations which were most kindly sent to FIBIGER by Prof. YAMAGIWA proved the successful result of the experiments.

It seems to us, however, that still more importance must be assigned to the investigations made by TSUTSUI on mice, and not only because, generally speaking, it is

[1] Experimentelle Studie über die Pathogenese der Epithelialgeschwülste. Mitt. der med. Fakultät der kaiserlichen Universität zu Tokyo. Bd. XXII. 1919.

an essential advantage to use for experimental purposes such animals as mice, that are easily procured and less expensive to breed, but especially because the great majority of previous experimental investigations on cancer — and particularly the transplantation experiments — have been performed on mice, whose relation to cancer, on the whole, must be considered better known than that of any other animal.

In continuation of investigations carried on by FIBIGER in the Anatomo-Pathological Institute of the University of Copenhagen with a view to the experimental production of cacinoma, we have then — on the base of the communications given by TSUTSUI — entered upon a series of experiments, the preliminary result of which we shall state in the following pages.

Our investigations comprise 2 series of experiments, the first of which (*I*), commenced June 30th 1919, includes *15*, the second (*II*), commenced October 31st 1919, *10* white mice. Ten mice weighed 17—19 grammes, 15 20—27 grammes. 14 were males, 11 females. To this may be added a third series (*III*) made by one of us (F. BANG) and comprising *20* mice, which will be dealt with later on.

All mice were painted every 2—3 day. For painting was used coal tar from the same supply. Application of the tar was cautiously made on the same spot of the skin of the back (between the scapulae).

In order to facilitate the action of the tar upon the skin of the painted area, in the first series the hairs were epilated before the beginning of the paintings, but this procedure was not repeated later on, as it soon turned out that

the hairs were removed by the mice themselves or fell off spontaneously.

The results of the two first experimental series will be accounted for together.

In *8* mice (which died 23—34 days after the beginning of the paintings) and in *1* mouse (which died 62 days after the first painting) the skin did not present any special pathological changes except losing of hairs and dubious or quite slight thickenings.

Nor did microscopical examination reveal any pronounced changes. In 4 of these mice (which died respectively 25, 29, 29 and 34 days after the beginning of the paintings) pathological changes could not be traced with any certainty. In 4 mice (which died respectively 23, 27, 33 and 62 days after the 1st painting) partly slight desquamation and thickening of epidermis, partly inflammatory processes were found in corium, which in 3 of these mice contained small foci of lymphocytes and leucocytes, in the 4th (which died 27 days after the 1st painting) small abscesses situated in the connective tissue and the hair follicles.

In the 9th mouse (which died 25 days after the beginning of the paintings) slight downgrowth of the surface epithelium was found. Pronounced or heterotopical proliferation had not developed either in this mouse or in any of the 8 others.

In *1* mouse (which died 89 days after the beginning of painting) similar slight changes were found, whereas in 2 mice (which survived the 1st painting for 121 and 123 days respectively) more advanced desquamation and inflammatory changes had developed. Pathological downgrowth of the epithelium was not, however, detected in any of these animals.

In the remaining 13 mice far more pronounced changes and development of growths — in some cases very violent — were produced. Out of these mice we shall first take *3* which survived the 1st painting for the shortest time (102, 105 and 184 days respectively) presenting less pronounced changes than did the remaining 10 longest lived mice. As the initial effect of the paintings in all mice were found slight changes of the above-mentioned type: losing of hairs, slight thickenings and roughness of the skin, in one of the mice (see below) combined with small fissures. These alterations continued, and after about 3 months (82—86 days) development of small warty or papillomatous nodules was found, in 2 mice (named I_2 and II_{18}) increasing uniformly until the death of the animals, while in the third mouse a nodule developed which fell off after about $4^1/_2$ months and was replaced by a new one about 40 days later. In order to illustrate the progress of the changes in these mice, the following particulars about the last-mentioned mouse are subjoined:

White male mouse named II_{24}, weight 25 grammes was painted from October 31st 1919. On November 18th 1919, 18 days after the 1st application of tar, small fissures were visible on the painted area; 14 days later, on December 1st, an extensive dermatitis with thickenings, fissures, covered all over by a crust had developed. On January 21st 1920, 82 days after the 1st painting, a strongly circumscribed nodule as large as the head of a pin was found on the painted spot in addition to the changes above. In the time following the nodule increased in size, and 28 days later had reached the height of 5 mm, its diameter being about 2 mm.

The growth again decreased in size. On March 15th (26 days later) is noted: The growth has nearly disappeared, but roughness is still to be felt in its place.

Then a new and papillomatous growth developed, the height and diameter of which on April 24th (40 days later on, 176 days after the 1st painting) measured about 2 mm. The mouse died on May 2nd 1920, 184 days after the 1st application of tar.

On microscopical examination the growth, cut in serial

sections, was found to be a papilloma of typical structure. The surrounding skin was somewhat thickened, owing partly to hyperplasia of the covering epithelium, partly to proliferation of the elements of the connective tissue of corium, which in and under the papilloma—and in the neighbouring areas as well—contained a considerable number of lymphocytes, leucocytes and mastcells. Heterotopical or invasive downgrowth of the epithelium was not observed.

In this mouse which died 184 days after the beginning of the paintings, inflammatory processes were found in addition to a small papillomatous growth, the structure of which corresponded entirely to that of ordinary papillomata of the skin.

Also the 2 mice mentioned above (which survived the 1st painting for 102 and 105 days respectively) presented typical papillomata (see Plate II figs. 10 and 11) and inflammatory changes. Neither heterotopical downgrowth nor signs of carcinomatous growth were detected in any of these 3 mice.

Quite different changes, however, were found in the other *10* mice which survived the 1st painting for 243—333 days. Not only inflammatory processes and papillomatous growths of far more violent nature had developed in all of them, but besides, pronounced new growth of malignant type (carcinomata and carcino-sarcomata).

The following extract from the journals may demonstrate the development of the pathological processes in these 10 mice. (the letters b. o. p. will indicate: beginning of painting).

Mouse No. 1. (named II_{25}). White female mouse, weight 15 gr. was painted from October 31st 1919. On the 82th day some thickening of epidermis was found besides losing of hairs in the painted area, but 4 weeks later the skin was again smooth. During the following month, however, a small warty nodule developed, having on the 144th day gained nearly miliary size. 32 days later, all in all, 3 small nodules were visible, the largest of which measured

about 2 mm in height and about 3 mm in basal diameter. These nodules kept unchanged until the 213th day after b.o.p. on May 31st 20, when infiltration was observed at the base of one of the papillomatous growths. 9 days later the infiltration was pronounced and painting was now discontinued. 243 days after the first painting (June 30th) a group of small papillomata had developed, and in the deeper layers an infiltration of the size of a pea was found. The mouse was then killed (weight 21 gr). The infiltration proved to be a whitish, partly solid, partly necrotic nodular mass containing horny tissue. Parts of the growth were used for transplantation (see below page 24). In the lymphatic glands and other organs no pathological changes.

On microscopical examination the non-transplanted remaining part of tumor was found to be a papillary, strongly keratinising carcinoma (type: *Epithelioma malignum*), infiltrating the connective tissue of corium and the underlying muscular layer (see Plate IV fig. 20). Axillary glands and lungs were cut in serial sections, presence of metastases not detectable.

Mouse No. 2. (named I_9). White male mouse, weight 25 gr painted from June 30th 1919. For the first 6 months the painted area did not present any special pathological changes except losing of hairs and roughness of the skin. Not until January 21st 1920, 205 days after b.o.p. a nodule was observed on the thickened skin, nearly as large as the head of a pin. The increase of the growth during the repeated paintings was very slight. On March 15th 20 a new, quite small papillomatous nodule of the same kind had developed. The mouse died on April 4th 20, 279 days after b.o.p., its weight being then 18 gr. Lymphatic glands and other organs normal.

Microscopical examination of the painted area showed besides proliferation of the fibrillar elements of the connective tissue, foci of lymphocytes and mast cells situated in corium, in the place of one of the tumors moreover, pronounced thickening with downgrowth of epidermis and keratinised retort—shaped cystic cavities,—whereas, corresponding to the other nodule, atypical downgrowth of the surface epithelium as irregular strands, containing epithelial pearls and horny globes, infiltrated the underlying connective tissue of corium (*Epithelioma malignum*, see Plate II fig. 12). The axillary lymphatic glands as well as the lungs were cut in serial sections. No metastases were found.

Mouse No. 3. (named II_{20}). White male mouse, weight 25 gr painted from October 31st 1919. As early as the 18th day after b.o.p.

not only losing of hairs, but also fissures were observed in the painted area of the skin. These fissures, however, soon disappeared, and about $2^3/_4$ months (82 days) after the 1st painting the skin appeared nearly normal and continued to be so for some time. On April 24th 1920 (176 days after b.o.p.) a papillomatous nodule of miliary size was found, which 5 weeks later had attained a height and diameter of about 4 mm. During the following month several small flat papillomatous nodules developed, which, however, again disappeared. 276 days after b.o.p. (August 2nd 20) an area of the skin measuring 8 mm by 9 mm was felt to be infiltrated, and on pressing this part a small amount of necrotic cellular masses could be squeezed out. The mouse died on August 7th 1920, 281 days after b.o.p. Post mortem examination showed the papillomatously changed surface of the skin to be covering a tumor-like thickening nearly as large as a pea and non-adherent to the muscles of the back. No metastases were found in the lymphatic glands nor in other organs.

On microscopical examination of the papillomatous growth its central part was found to be the seat of a strongly advanced necrosis, consisting of enormous layers of keratinising epithelial cells, the structure of which like that of the scarce elements of the connective tissue in most places was lost, owing to the very extensive necrosis. Also in the underlying part of corium necrosis had taken place in addition to strong inflammatory processes and purulent infiltration. On the margin of the papillomatous necrotic area the structure, however, was perfectly infact, and here a typical downgrowth of strands of flat-celled epithelium was seen, containing horny globes and epithelial pearls and invading the deeper layers of the connective tissue and the muscles *(Epithelioma malignum)*. Axillary lymphatic glands and lungs were cut in serial sections but no metastases were found.

Mouse No. 4. (named H_{17}). White male mouse, weight 25 gr painted from October 31st 1919. After the usual initial changes a distinct crust was found on January 21st 1920 on the painted area. 192 days after the b.o.p., moreover, infiltration of the skin, and 3 weeks later a small papillomatous nodule was observed. Some few days later this nodule, however, fell off, leaving a small ulcer with thickened margins. This ulcer gradually increased in size, a necrotic mass could be squeezed from out its borders, and 264 days after the b.o.p. it had reached nearly the size of a pea, being on its surface covered by a crust of secretion and tar. From its margin an infiltration extended 2—3 mm into the surrounding tissue.

12 days later the ulcer measured 14 mm by 17 mm. The paintings were now discontinued. 16 days later the ul-

cer measured 15 mm by 17 mm and was covered by a high conical-shaped crust of tar and cellular masses as hard as bone (see Plate I fig. 3). This crust being on the point of loosening consequently was removed, and the underlying layer, covered with pus, was washed with a normal saline solution. The mouse died on August 28th 1920, 302 days after the b.o.p. Weight 20 gr. The ulcer then measured 21 mm by 21 mm. No metastasis formation in the lymphatic glands and other organs.

Microscopical examination showed the ulcer to be due to necrosis of the surface of a typical non-keratinising flat-celled carcinoma with invasive downgrowth into the muscles of the back.

Axillary lymphatic glands and lungs were cut in serial sections. Metastases were not found.

Mouse No. 5. (named I_5). White female mouse, weight 20 gr painted from June 30th 1919. After the usual initial symptoms a small nodular growth developed, which 191 days after b.o.p. (on January 7th 1920) had gained the size of the head of a pin, presenting a month later the aspect of a cutaneous horn, the height of which measured 7 mm, the diameter being 4,5 mm. The paintings were discontinued on February 9th 1920, 224 days after their beginning. (The size of the cutaneous horn is seen from Plate I fig. 4).

In spite of suspended painting the horn increased in size, and nine days later had gained the height of 8 mm, a quite similar, but somewhat smaller nodule having at the same time developed next to it. In the following period small quickly growing papillomata constantly arose. On April 3rd 1920, 278 days after b.o.p. infiltration was found at the base of the papillomata, increasing very conspicuously during the following time in depth and extension, while the papillomata broke off and the painted area was covered with irregular horny masses presenting the appearance of cutaneous horns (see photograph from April 14th Plate I fig. 5). On April 20th the infiltration had reached the dimensions of 5 mm by 10 mm. The mouse was killed on May 10th 1920, 315 days after b.o.p. weight 20 gr.

Metastases were not found in lymphatic glands nor in other organs which were all perfectly healthy. In the painted area of the skin infiltration was found measuring 8 mm by 10 mm.

Microscopical examination showed a strongly keratinising papillary carcinoma (of the type: *Epithelioma malignum* see Plate II fig. 14), extending invasively into corium and muscular layers. The axillary lymphatic glands and the lungs were cut in serial sections. No metastases were found.

Transplantation experiments gave negative results.

Mouse No. 6. (named I_{15}). White female mouse, weight 24 gr was painted from June 30th 1919. Not until January 21st 1920 205 days after b.o.p. was found a quite unquestionable thickening of the skin on the painted area, where 3 weeks later (February 10th) a nodule as large as the head of a pin had developed. During the following time this nodule gradually increased in size, and on February 23rd had gained the height of about 4 mm (see photograph from this day, see Plate I fig. 1). On March 5th 1920, 249 days after b.o.p. a papillomatous growth was observed measuring 5 mm in height, and separated from this, an ulcer the diameter of which was about 3 mm. The paintings were now discontinued. On March 15th an infiltration of the surrounding skin was seen to enclose the ulcer like an elevated margin, the diameter of which was 9 mm. The growth constantly increased in size. On March 18th necrotic cellular masses could be squeezed from its base. On April 24th an area of 9 mm by 14 mm was found to be partly ulcerated, partly covered with horny masses (see photograph from April 14th, see Plate I fig. 2).

The mouse died on May 15th 1920, 320 days after b.o.p., weight 19 grammes.

Besides the local changes no pathological processes were found. No metastasis formation.

Microscopical examination of the painted area showed a typical carcinoma with invasive downgrowth (of the type: *Epithelioma malignum* with strongly pronounced keratinisation) (see Plate III fig. 15). The axillary lymphatic glands and the lungs were cut in serial sections. An enlarged axillary gland was, to a great extent, destroyed by carcinomatous tissue of quite the same structure as the mother tumor (see Plate III fig. 16).

Mouse No. 7. (named 1_{12}). White male mouse, weight 20 grammes, painted from June 30th 1919. Beyond the usual initial processes no particular changes were met with until the 204th day after b.o.p., when thickening of the skin was found in the painted area. 41 days later, a nodule nearly as large as the head of a pin had, moreover, developed, but did not apparently increase in size during the following month. On April 8th 1920 was noted: the papilloma-like nodule is covered by a crust of tar, fissures are found at its base. On April 24th: No infiltration is to be detected at the base of the growth. On May 10th: several small new papillomatous growths and some infiltration (315 days after the first painting).

The mouse was killed on May 26th 1920, 331 days after

b. o. p. Weight 22 grammes. Besides local changes no pathological findings. The axillary glands normal.

Microscopical examination of the painted area showed tumor to be a papillomatous carcinoma with strong keratinisation and heterotopic invasive downgrowth of strands of flat-celled epithelium admixed with horny globes and epithelial pearls (type: epithelioma malignum).

The axillary lymphatic glands and the lungs were cut in serial sections. Metastases were not detected.

Transplantation experiments gave negative results.

Mouse No. 8. (named II_{23}). White male mouse. Weight 20 grammes, painted from October 31st 1919. 18 days later the skin of the painted area appeared slightly thickened and remained so for the following 2 months. On January 21st 1920 it was covered by a crust. These changes, however, healed up, and on February 18th 1920 the skin was again quite smooth. 176 days after b. o. p. a papillomatous growth had developed, measuring 2 mm by 2 mm. It increased in size and on the 213th day had attained a height of nearly 6 mm, a smaller new nodule having developed next to it.

14 days later, initial infiltration was found at the base of the papilloma. The paintings were now discontinued on the 231st day after their beginning (June 18th 1920). During the following 2 weeks the growths decreased in size and 241 days after b. o. p. only an ulcer was seen on the painted area covered by a crust of blood. From this ulcer a conical-shaped cutaneous horn now developed, measuring 15 mm in height and 17 mm in basal diameter 35 days later. At its base extensive infiltration was found.

The horn increased very quickly in size (see Plate I figs. 6—9).

August	18.	292	days	after	b.o.p.	its height was	24	mm. its diameter	22	mm.
August	28.	302	—	—	—	—	29	- —	21	-
Sept.	10.	315	—	—	—	—	33	- —	23	-
Sept.	23.	328	—	—	—	—	36	- —	23	-

The mouse fell off more and more, lost its hairs and died on September 28th 1920, 333 days after the first painting, its weight being then 21.7 grammes.

Post mortem examination showed the conical-shaped tumor to consist of firmly concreted necrotical masses, covered on their surface by a hard crust. On microscopical examination its base presented itself as a keratinising carcinoma

(type: epithelioma malignum), growing invasively into the muscular layers of the back. Towards the surface, the tumor tissue passed into closely packed strands of horny cells with faintly staining nucleus, or into horny masses without nuclei. Everywhere leucocytes and lymphocytes were abundant.

The axillary lymphatic glands and the lungs were cut in serial sections. Metastases were not detected.

Mouse No. 9. (named I_{13}). White female mouse, weight 20 grammes, painted from June 30th 1919. 153 days after b.o.p. fissures of the skin were observed in addition to some thickening, and 38 days later a nodule somewhat larger than the head of a pin had developed, but after a fortnight it again disappeared, leaving in its place an excoriated area with slight marginal thickening. The paintings were now discontinued, 205 days after b.o.p. During the succeeding time a small ulcer with partly thickened elevated margin, resembling a rodent ulcer, was observed and constantly increased in size.

The mouse died on May 17th 1920, 322 days after b.o.p., weight 15 gr. The ulcer then measured 5 mm by 10 mm. Beyond the local changes no pathological findings were seen, an axillary gland was enlarged nearly to the size of half a pea.

Microscopical examination of the painted area showed the thickened wall-like border of the ulcer to consist partly of a strongly keratinising carcinoma of the type: epithelioma malignum, partly—on the limit of this carcinoma and distinctly separated from it—of a small nodule situated in corium and built up by strongly polymorphonucleated faintly staining sarcoma-like cells, several of which were plurinuclear, and small giant cells (see Plate III fig. 17 and 18). In this place it proved impossible to distinguish between the stroma and the real tumor parenchym. Unquestionable epithelial cells or keratinised cells were nowhere found, and the covering epidermis was normal. Foci of lymphocytes, leucocytes or inflammatory processes of other kinds were not detected. In preparations stained according to Mallory's method, a distinct fine fibrillar reticulum presented itself deep blue, but also in some of the neighbouring areas such fine fibrillæ could be found here and there. Typical spindle cell sarcoma was not detectable.

In the enlarged axillary lymphatic gland metastatic deposits of a typical strongly keratinising carcinoma were observed (see Plate III fig. 19). No signs of sarcoma. The lungs were cut in serial sections. No metastases.

Mouse No. 10. (named I_{14}). White female mouse, weight 19 gr painted from June 30th 1919. After losing of hairs and slighter

thickening of the skin, a small papillomatous nodule had developed on the 114th day after the first painting. On December 1st, 40 days later, this had, however, again vanished, leaving in the painted area only a dermatitis with crust and fissures. On December 20th (173 days after b.o.p.) a small nodule had again developed and during the following time increased in size, being as big as a pea on January 21st 1920 (205 days after b.o.p.) and resembling a cutaneous horn. The paintings were now discontinued. On January 23rd was noted: tumor takes its rise exactly from the middle of the painted area, it is of the size of a pea and ulcerated at the base, its diameter being there 7 mm. Its height measures 5 mm (see Plate IV fig. 22). During the following time the growth increased very rapidly, and on February 4th 1920 measured 12 mm by 5 mm (see Plate IV fig. 23). The ulceration at the base was now more pronounced, rugged and rough on its surface. On February 18th most of the ulcerated area was found to be covered by horny masses, the primary cutaneous horn being now 13 mm in height (see Plate IV fig. 24). On March 5th extensive deep infiltration was noted; tumor measured now from side to side 14 mm in basal diameter, its height being 15 mm from the base to the point of the cutaneous horn (see Plate IV fig. 25). The mouse was killed on March 13th 20, 257 days after b.o.p. (see Plate IV fig. 26). Its weight on death was 19 gr.

Post mortem examination showed tumor to consist of a superficial horn-like part, built up by keratinising cells (cutaneous horn) and a whitish, softer basal part (see Plate IV fig. 27) reaching far down into the subcutaneous tissue and the upper muscular layers, but without growing invasively into the deep muscles of the back.

Parts of the basal region of tumor were used for transplantation (see below pag. 27). The remainders weighed 130 centigrammes.

There was no swelling of the regionary lymphatic glands. No metastatic deposits in the lungs, and no other pathological findings.

Microscopical examination showed tumor to consist partly of thick layers of all but necrotical strongly keratinising cells building up the superficial cutaneous horn, partly of invasive downgrowth of strands and nests of flat cells admixed with numerous strongly keratinising epithelial pearls (see Plate V fig. 28). The epithelial elements were situated in a stroma of connective tissue that in some places was only scarce, in others very rich in cells and built up by closely packed spindle cells (see Plate V fig. 29). In some areas these elements would be so predominant as to make the tumor tissue appear as a spindle cell sarcoma (see Plate V fig. 30 and Plate VI fig. 32), only here and there admixed with a few epithelial

cells and strands of epithelium. In other places, in which the epithelial elements were more abundant, the structure presented a picture similar to that of a carcino-sarcoma (se Plate V fig. 31), and, in fact, the tumor tissue represented the pure epithelial type only in smaller limited areas, where the carcinoma partly belonged to the type: Epithelioma malignum.

The axillary and cervical lymphatic glands and the lungs were cut in serial sections. No metastases were found.

As will be seen from the above reports, the initial processes in these longest lived 10 mice, as well as in those mentioned before, consisted in losing of hairs, more or less pronounced thickening and roughness of the skin, in some cases accompanied by crust or fissure formation.

After this, in all the mice, more or less warty small growths developed, arising at different points of time and at the earlist to be seen about 4 months (mouse No. 10), at the latest about 8 months (mouse No. 7) after the first painting. These growths gradually assumed the form of process-like or warty papillomata, but, as a rule, after some time fell off, either leaving a bleeding slightly rough ulcer (see mouse No. 4 and No. 9), or being replaced by new, in some mice multiple growths of the same kind. The rate of growth of these papillomata was extremely rapid, they were covered by thick layers of keratinising epithelium, and on the surface of the ulcers deposits of secretion and necrotic horny cells were found. By these processes in several cases (especially in mouse No. 4, No. 8 and No. 10) conical-shaped cutaneous horns of such enormous dimensions developed, that their size nearly corresponded to one third or half of the total size of the mouse (see Plate I, figs. 3 and 8—9).

As later predominant phenomena, furthermore, in most cases an extensive, often wall-like thickening and infiltration could be seen at the base and margin of the large strongly

keratinising papillomatous growths and cutaneous horns, and of the ulcers, due to the falling off of the papillomata. Such more or less pronounced infiltrations could at the earliest be demonstrated (on palpation or inspection) with certainty about 7 months after the first painting, and, as a rule, increased considerably until the death of the mice, in some cases then presenting the size of a pea or more.

In accordance with the statements of YAMAGIWA, ICHIKAWA and TSUTSUI it was proved in our experiments that the growth of such infiltrations, developed at later junctures, like the growth of the large papillomata and cutaneous horns, not only did not cease, but actually continued with unchanging energy until the death of the mice, even if the tar paintings were discontinued. The table below demonstrates this fact, as far as 6 of the mice are concerned. Noted in days passed since the first painting, the moment of suspension of the painting is contained in the 1st column, the day of death of the mice in the 2nd column, and finally, in the 3rd column the days passed between these two junctures, viz. the period in which the papillomata, cutaneous horns and ulcers have continued their growth with undiminished power.

Mouse No.	Painting suspended	Death of the mouse	The changes increased (until the death of the mouse) for:
4	276	302	26 days.
10	205	257	52 —
6	249	320	71 —
5	224	315	91 —
8	231	333	102 —
9	205	322	117 —

The accompanying photographs will further illustrate the enormous dimensions of the papillomata and cutane-

ous horns, and their great increase after suspension of the paintings (see Plate I, figs. 1—9, and Plate IV, figs. 22—26).

Microscopical examination of the changes met with in 8 of these mice (No. 1—8) showed the papillary growths and cutaneous horns — especially in the upper parts most distant from their base — to consist, to a great extent, of deposits of horny and necrotical masses in which faintly staining epithelial cells and fine spurs of degenerating and decomposed connective tissue could indistinctly be traced here and there. In deeper layers the structure was more intact and often distinctly papillary, ramified spurs or septa of connective tissue being situated in enormous layers of keratinising epithelium of typical structure with admixture of lymphocytes, leucocytes and remainders of necrotical epithelium. In the deepest basal parts and infiltrated borders of the growths as well as in the thickened ground of the ulcers, typical flat celled carcinoma was found, representing in mouse 2 an early (see Plate II, fig. 12), in the others a more advanced stage. In mouse No. 4, in which, on death, the lesions presented the aspect of a rodent ulcer, the carcinoma disclosed but little tendency to keratinisation, whereas in the other 7 mice it contained numerous horny globes and epithelial pearls, and in most of the cases more or less perfectly corresponded to epithelioma malignum of typical structure. In 7 animals the invasive downgrowth reached down into the cutaneous muscular layers, penetrating it to some extent, while in the 8th case (mouse No. 2) it had only invaded the connective tissue of corium.

The adjacent part of the skin partly showed collateral hyperplasia of the epithelium, partly more or less pro-

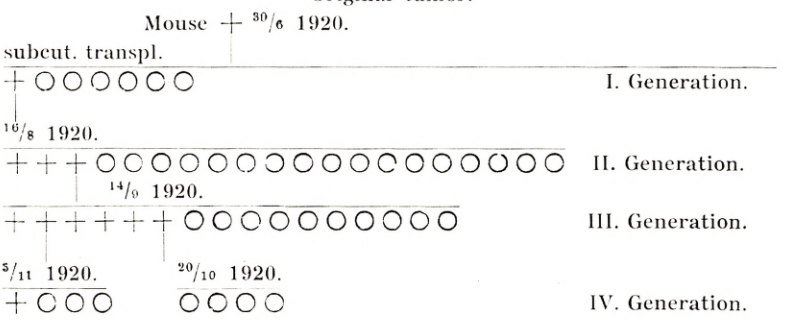
nounced inflammatory processes chiefly consisting of foci of lymphocytes, leucocytes and slighter proliferation of the elements of the connective tissue. Often the amount of mast cells was strongly increased.

In mouse No. 6 an axillary lymphatic gland was found to be the seat of extensive metastatic deposits of strongly keratinising carcinoma of the same structure as the primary tumor (see Plate III, figs. 15 and 16). Neither in lungs, lymphatic glands, nor in other organs of any other of these 8 mice were metastases detected.

Attempts were made in 3 cases to transplant the developed carcinomata (mouse No. 1, No. 5 and No. 7), but in 2 cases without success (mouse No. 5 and No. 7). It ought, however, to be noticed that for these transplantations only such a small number of mice was available that the negative result of the experiments, in reality, could not surprise, and the less so as transplantation of strongly keratinising tumors will frequently meet with great difficulties.

On the other hand, subcutaneous transplantation of the carcinoma of mouse No. 1 gave positive results. These experiments may be seen from the following schematic table (see page 24), in which the mice inoculated successfully are marked with +, the mice inoculated unsuccessfully with O.

As will be seen from the table, tumor could not by far be classed among the easily transplantable tumors, and its propagation in the mice inoculated only took place so slowly that subcutaneous nodules as large as hemp seeds or kernels of nuts did not develop till 4—6 weeks after transplantation. Nevertheless, it is justifiable to ascribe to this tumor a transplantability which must be regarded as



very remarkable, considering that the tumor was a carcinoma with strong tendency to keratinisation, (see Plate IV, figs. 20 and 21). That, as a rule, keratinising tumors will present far greater difficulties with regard to transplantability than did the carcinoma here recorded, is seen from the observations of MURRAY[1] and HAALAND[1] mentioned in one of FIBIGER's previous papers.[2]

All in all, the changes found in all these 8 mice must be characterised as combinations of strong development of keratosis with papillomatous growth, cutaneous horns and carcinomata.

According to the above observations regarding the development of the process in the live mouse, the supposition seems obvious that the carcinoma appeared as a secondary and final process, as a complication to primarily developed papillary growths. Nevertheless, we wish to emphasize distinctly that the microscopial examinations hitherto made by us do not as yet admit of any definite fixation of the consecutive order of the processes, nor of any decision as to whether or not the development of carcinoma be —

[1] Third and Fourth Scientific Report of the Imperial Cancer Research Fund 1908 and 1911.

[2] Det Kgl. Danske Videnskabernes Selskabs Biologiske Meddelelser I_{14} 1919.

especially or at all — bound to the base of the papillomata, and neither do we — at the present stage of the investigations — see our way to form any judgment as to whether any special importance must be assigned to the epithelium of the hair follicles as an original starting point for the development of carcinoma, as it has been maintained by YAMAGIWA and ICHIKAWA[1] with regard to the tumors developed in tar painted rabbits.

For the solution of these questions investigations will be required which are not — like those of this paper — mainly confined to the advanced stage of the processes, and carried out with the chief aim of identifying their nature without a thorough study of the nicer details.

Besides carcinomatous changes as the above described, processes of a different kind were found in 2 mice (No. 9 and No. 10).

The presence of a typical keratinising carcinoma in mouse No. 9 was already ascertained beyond doubt by the microscopical examination (see page 18), and furthermore, was proved by a strongly developed carcinomatous metastasis in an axillary lymphatic gland (see Plate III, fig. 19).

But more consideration was required to determine the nature of the small tumor growth (see Plate III, figs. 17 and 18) adjacent to the carcinoma and situated in the corium, the structure of which tumor brought to mind that of certain types of polymorphous cell sarcomata, among others that of some sarcomata, found in rats (CLUNET[2] (X-ray sarcoma) BULLOCK and ROHDENBURG[3] (cysticercus-sarcoma).

[1] l. c.

[2] Recherches expérimentales sur les tumeurs malignes. Paris 1910.

[3] Journal of Cancer Research. Vol. II. 1917.

Haaland[1] who, while studying the secondary development of sarcoma from the stroma in transplanted originally pure carcinomata in mice, has described tumor structure of a similar kind, points out the difficulties one finds, at certain stages of the development of such sarcomata, in distinguishing between the elements of the latter and those of the carcinoma. After thorough examination, we must maintain our previously quoted[2] identification of the tumor in question as a polymorphous cell sarcoma and the total tumor growth as a carcino-sarcoma.

On the other hand, any discussion about the nature of the growth observed in mouse No. 10 is precluded. In this case we are placed before a mixed tumor built up in some places only of a keratinising squamous cell carcinoma, in others only of a typical spindle cell sarcoma, and finally, in certain areas of a mixture of both these components, as will be seen from the subjoined figures (Plate V, figs. 28—31, Plate VI, fig. 32).

As these demonstrate, the diagnosis: carcino-sarcoma must be considered conclusive solely on the base of the histological examination, but it is furthermore supported by the outcome of the transplantation experiments which are illustrated by the following table and by the figures (Plate VI, figs. 33—37).

These inoculations like those mentioned above were effected by means of a hollow needle like the one employed in the Imperial Cancer Research Fund's laboratories. Only 2 mice were inoculated intraperitoneally (with positive result), all the other mice subcutaneously.

It will be seen from the table that transplantation of tumor tissue from this mouse has been carried on—up till now—for 9 months and 11 generations[3]). In the first transplantation inocula-

[1] l. c.

[2] Compt. rendus de la Société de Biologie. 1920.

[3] Further transplantations have been performed up till now (February 1921) with the same success in 2 generations.

	Transplantations made from: (number of mice)	Carcino sarcoma	Pure spindle cell sarcoma	○	Total
I. Generation (from)	1. ($^{13}/_{3}$ 1920	6	15	3	24
II. — a. —	2.	0	21	2	23
b. —	4.	0	7	6	13
b_1. —	4.	0	10	6	16
b_2. —	4.	(0)	(0)	(16)	(16)
III. — —	6.	0	22	6	28
IV. — -	5.	0	20	9	29
V. — —	2.	0	9	5	14
VI. — —	2.	0	10	4	14
VII. — —	3.	0	14	4	18
VIII. — —	4.	0	12	8	20
IX. — —	1.	0	4	4	8
X. — —	1.	0	5	1	6
XI. — —	2. ($^{15}/_{12}$ 1920)	0	8	2	10
		6	157	60+(16)	223+(16)

tion was made subcutaneously into 25 mice, one of which died already on the tenth day after transplantation. In 21 of the surviving 24 mice, tumors developed, being in 6 cases carcinosarcomata of the same type as the primary tumor (see Plate V fig. 29 and Plate VI fig. 36), whereas, in 15 mice, tumors were produced which on microscopical examination (most of them cut totally in serial sections) appeared as pure spindle cell sarcomata (see Plate VI fig. 37).

In 10 succeeding transplantations into, all in all, 199 mice, 142 of which gave positive result, only pure spindle cell sarcomata of the same homogeneous type developed.

The transplantations II b, II b_1 and II b_2 were all made with material from the same 4 mice, but the transplanted material used in transplantation II b_1 and in transplantation II b_2 had been conserved for respectively 7 and 14 days at a temperature of $\div$ 12—14° C. As will be seen from the table, conservation of the material for 7 days at this low temperature did not diminish the positive results of the transplantations, whereas transplantations with the same material having been exposed to the same low temperature for 14 days, gave only negative results. That these last negative results were not due to spontaneous immunity of the inoculated mice was shown by re-inoculations of 13 of these 16 mice with a fresh (not-frozen) material which in 12 mice gave positive results (3 of the 16 mice died too early after the 1st inoculation, and thus were not re-inoculated).

The tumor has thus been transplanted with positive results into 163 out of 223 mice (73 %).

The growth of the inoculated tumor pieces took place at an extraordinarily quick rate, so that in numerous mice, already after 2—3 weeks, tumors as large as dates or plums had developed (see Plate VI figs. 33—35), disclosing, however, at this juncture a tendency to necrosis and hemorrhage. Not unfrequently these tumors were the seat of extensive necrosis and ulceration after 4—6 weeks.

Thus, the carcino-sarcoma of this mouse (No. 10) proved to be easily transplantable, but only in a small number of the inoculated mice of the 1st generation did carcino-sarcoma develop, pure spindle cell sarcoma being produced in the majority of the mice of this generation and in all later generations.

With this case a new observation is added to the well-known examples already at hand, concerning the power of the sarcomatous tissue of overgrowing quickly and totally superseding the carcinomatous tissue on transplantation of mixed tumors in mice. Bashford[1] has reported 2 cases, in which spontaneous carcino-sarcoma in mice already on the 1st transplantation gav rise only to pure spindle cell sarcomata and continued to grow as such in all later generations; and the numerous renowned observations of secondary development of sarcoma in transplanted carcinomata in mice (Ehrlich, Apolant, Loeb, Bashford, Haaland, Russel, Clunet, a. o.) have also long ago proved the supremacy of the sarcomatous tissue which sets in sooner or later.

Apolant[2] has shown that the influence of cold (÷ 10° C.) for 15 days upon artificial mixtures of carcinoma and spindle cell sarcoma from mice may injure the sarcomatous

[1] Fourth Scientific Report of the Imperial Cancer Research Fund. 1911. pag. 165—166.

[2] Zeitschrift für Krebsforschung Bd. VI. 1908.

tissue to such an extent as to make only carcino-sarcoma or pure carcinoma develop on transplantation of the tumor mixture, whereas, on transplantation of the tumor mixture which has not at all or only for a few days been exposed to the action of the cold, either pure sarcoma or sarcoma with minimal admixture of carcinoma will develop.

As will be seen from the explanation adjoining the table above, similar experiments were made with our tumor for the purpose of injuring the tumor tissue by exposing it to the action of cold, to such an extent as to give rise to a further development of carcinomatous elements possibly present. These experiments, however, gave only negative results.

The development of carcino-sarcoma has, as commonly known, been explained — especially on the base of the above-mentioned observations of sarcomatous growth in transplanted, originally pure carcinomatous tumors — as a result of an influence exercised by a primary carcinoma upon the stroma of connective tissue, and, no doubt, it cannot be precluded that also the carcino-sarcomata in our two cases (No. 9 and No. 10) may in their earliest stages originally have been pure carcinomata produced by the tar painting.

To this explanation, however, the objection may be raised that the majority both of carcinomatous and of sarcomatous elements in the examined parts of the tumor of mouse No. 10 were dispersed in mutually separated, only to some extent confluent areas, and that the same arrangement in a still more pronounced form was refound in the tumor from mouse No. 9, in which, in fact, the mixture of both components was so slightly pronounced that the tumor might also be explained as a growth com-

posed of a carcinoma in contact with a quite adjacent neighbouring sarcoma (see Plate III, fig. 17). It must furthermore be taken into consideration that in a mouse painted with tar by Tsutsui[1] a spindle cell sarcoma developed which, according to an adjoined illustration, seems to have contained no carcinomatous elements whatever. If the sarcomatous growth be due in this case to the action of a primary carcinoma, the latter must have disappeared completely by the time when the sarcomatous tumor had developed. It seems, however, just as likely, that the tar painting in this case may have caused primary development of sarcoma without any preceding carcinomatous growth, in analogy with the fact that influence of other kinds that generally produce development of carcinoma, may exceptionally give rise to sarcomatous growth, for which special peculiarities of the influenced elements of the connective tissue may possibly be made responsible.

J. Clunet[2], Marie, J. Clunet and Raulot Lapointe[3] have thus in 2 cases produced sarcoma in rats by means of X-rays in analogy with the cases of Roentgen sarcoma observed in man (Porter and Wolbach[4] a. o.) although the X-rays, as known, will generally only give rise to carcinoma. Also the reports on aniline tumors, which are, as a rule, carcinomatous, contain one observation of sarcoma (Rehn[5]).

Thus, the production of carcino-sarcomata, according to our view, must not be ascribed only to a secondary development of sarcoma impelled by a primarily developed

[1] l. c.
[2] l. c.
[3] Bull. de l'Association Française pour l'Étude du Cancer. 1912.
[4] Zeitschrift f. Krebsforschung Bd. XIII. 1913. pag. 559.
[5] Archiv f. klin. Chirurgie. 1895. Bd. 50.

carcinoma, but may also be due to the fact that the development of the carcinomatous components of the tumor tissue and the development of the sarcomatous components may both be co-ordinate effects of one and the same factor which has influenced epithelium and connective tissue coincidently. It seems to us that this explanation can hardly be precluded, neither in the cases here recorded where tar painting gave rise to the tumor growth, nor in cases of spontaneous primary carcino-sarcoma of unknown origin.

Hence, the total result of the above 2 series of experiments will, in the first place, generally speaking be that in agreement with the statements of TSUTSUI it does not cause any difficulty to produce cutaneous carcinoma in mice experimentally on painting their skin with coal tar for a longer period every 2nd—3rd day.

In all 10 mice which survived the 1st painting for at least 243 days, a malignant tumor developed, being in 8 cases a typical — more or less — keratinising carcinoma, in 2 mice, carcino-sarcoma. In 2 mice carcinomatous metastases were found in the lymphatic glands, in 2 cases (mouse No. 1 and mouse No. 10) tumor proved to be transplantable.

This outcome of the 2 series of experiments has now been confirmed by the result of a 3rd, made by one of us (FRIDTJOF BANG). This series was carried out as an introduction partly to a further study of the histological processes and their development, partly to a more definite fixation of the point of time, at which the cancer begins.

For this purpose, in the 3rd series the microscopical

examination of the changes was not put off till the death of the mice, diagnostic excisions being performed in several cases (as done by YAMAGIWA and ICHIKAWA in their experiments on rabbits) as soon as the presence of deep downgrowing infiltration was proved by means of palpation or inspection.

For the rest, this series of experiments did not differ from those dealt with in the preceding pages. The mice were painted every 2nd—3rd day in the same way and with the same coal tar from the same supply as in previous experiments. The results were in perfect keeping with those above reported, the initial changes being also in these mice losing of hairs, thickening of the skin, slight inflammatory changes, small fissures or crust formation, succeeded by development of papillomatous growths and cutaneous horns, and as a final stage infiltration and ulcers.

The series of experiments comprises 20 white mice among which the 13 longest lived presented carcinomatous growths (proved solely by diagnostic excision in 2 animals, by diagnostic excision and post mortem examination in 3, and by post mortem examination in 8). Metastases were found in the axillary lymphatic glands of 4 mice and in the lung of 2.

A transplantation experiment was effected in one case and with positive result.

The tumor was a cornifying carcinoma with a great metastatic nodule in an axillary lymphatic gland. The implanted tissue—in 4 out of 6 mice inoculated with the primary tumor, in 2 out of 6 mice inoculated with the lymphatic gland—grew very quickly and in about 3 weeks developed into tumors of about the size of a pea or the kernel of a hazel nut, the histological structure of which tumors corresponded completely to the structure of the original tumor. Most unfortunately these tumors soon showed signs of infection, and transplantation, therefore, was not continued.

In all cases the tumors produced were more or less keratinising carcinomata, often of the type of epithelioma malignum. Unquestionable pure sarcoma or carcino-sarcoma was not found, although the possibility of an admixture of sarcomatous elements could not be precluded in one mouse.

It seems to us unnecessary to give a detailed report of the development of the changes in each animal of this series, partly because, in nearly all respects, it would be a repetition of previous statements, partly because a further account will appear when the material has been subjected to a more thorough histological study.

The table below (see page 34) is given in order to afford a general view of the total result of the whole 3 series of experiments. The 1st column contains the amount of days passed from the 1st painting until the time when the nature of the changes was proved, thus being in the case of 44 mice synonymous with the day of their death, while it indicates the day of diagnostic excision in the case of 1 mice of the 3rd series. These 2 animals both belong to the group of the longest lived mice of the 3rd series.

The skin affections are divided into 1) slight, 2) papillomatous and 3) development of carcinoma.

As seen from the table all 15 mice which survived the 1st painting only for at most 89 days presented but slight changes, whereas in 28 out of 30 mice which survived the 1st painting for 103—333 days papillomata and cutaneous horns had developed, being in no less than 24 individuals accompanied by development of carcinoma (in 22 individuals) or carcino-sarcoma (in 2 individuals). Thus, development of carcinoma (carcino-sarcoma) was found

Days passed after 1st painting	Number of mice	Slight changes	Papillomatous growth	Carcinoma (or carcinosarcoma.)
	I—II series of experiments:			
23—89	10	10	..	..
103—123	4	2	2	..
184	1	..	1	..
243—333	10	..	..	10
	III series of experiments:			
29—72	5	5	..	..
180—182	2	..	1	1
209—267	13	..	..	13
Total:	45	17	4	24
	All three series of experiments:			
180 days or longer	26	..	2	24

altogether in 24 out of 26 mice which survived the 1st painting for at least half a year (180 days).

Thus, in our investigations tar cancer has been produced with greater frequency than in the experiments made by TSUTSUI.

Furthermore, our experiments have shown that, like the tar carcinomata in rabbits (YAMAGIWA and ICHIKAWA) the artificially produced tar carcinoma may also in mice produce metastatic deposits in the lymphatic glands. Metastases in the lungs we have found in 2 of 13 mice, the lungs of 12 of which were cut completely in serial sections.

Finally, we have succeeded in giving the proof of the transplantability of the tar carcinomata produced artificially in mice.

The reason why development of malignant growths was found in a relatively greater number of mice in our experiments than was the case in TSUTSUI's experiments, cannot be given, but it might perhaps be found in a dif-

ference between the tar used by TSUTSUI and that used by us, or in a difference as to susceptibility of the Japanese and the Danish mice.

As mentioned above, development of carcinoma was observed in our experiments at the earliest about half a year (182 days) after the beginning of painting, but examination of the carcinoma in this mouse, then dead, rendered it probable that development of cancer may take its rise at a still earlier juncture, and in some new investigations on this question made by one of us (BANG) and not yet finished, carcinomatous growth — up till now — has been found in several mice 5 months, or still earlier, after the 1st painting.

On the whole, the initial processes of carcinoma development in mice are in perfect keeping with those which precede the development of cutaneous carcinoma in workers who have been exposed to the action of soot, tar, crude paraffin and pitch.

Also in these workers the dermatitis will be characterized by keratosis, and development of papillomatous growth — as will be known — is a common finding, large cutaneous horns being also observed in chimney sweeps (CURLING[1], WADD[2], JOHNSON[3]) although of course of relatively far smaller dimensions than those found in mice and rabbits.

Furthermore, the histological structure of the growths presents conformities to that of the above professional

[1] Treatise on Diseases of the testicle, cit. by LEBERT: Ueber Keratose. Breslau. 1864.

[2] Cases of diseased prepuce and scrotum. London 1817. cit. by STOEHR: Ueber den Schornsteinfegerkrebs der Engländer. Würzburg 1822.

[3] The Lancet. 1844. Vol. II.

carcinomata in man, these being also, as a rule, strongly keratinising types, belonging to the group of epithelioma malignum.

The successful results of the experiments made by Yamagiwa, Ichikawa, Tsutsui and by us renders it possible that continued investigation may solve the old question as to what chemical compounds or mixtures of compounds must be made responsible for the occurrence of this group of professional cancers.

In our experiments we have not yet entered upon this question which will probably require widely ranging investigations, but we might add a single item worthy of interest.

In consequence of the statements of Bayet and Slosse[1] in 1919 as to their having procured the definite proof that arsenic was the cause of tar cancer and the real cause too of cancer of chimney sweeps, briquette- and paraffin-workers, we have caused an analysis to be made of the tar employed in all our experiments. This analysis, kindly carried out by the Pharmacological Institute of the University of Copenhagen[2] gave as a result, that the tar in question contained arsenic in such small quantities that it could only be called traces (about 0,0003 per cent.).

That such a slight admixture of arsenic should actually be the active cancer-producing element in tar, seems rather dubious to our view, and let it further be added, that tests made in the laboratories of the Lister Institute have failed to detect any arsenic in pitch (H. C. Ross 1917).[3]

The question as to the special nature of the active sub-

[1] l. c.

[2] Also in this place we wish to express our thanks to the Director of the Institute, Prof. Dr. J. Bock.

[3] l. c.

stances, however, is not only of great interest to the hygiene of manufacture and to dermatology. The tracing out of such chemical compounds as might possess the power of giving rise to such excessive cell proliferations as those in question, presents the greatest interest also to biology and cancer research in general.

The continued and undiminished growth of the carcinomata after the suspension of the tar paintings corroborate previous clinical observations and does not lack analogies. Also other carcinomata (as f. inst. X-ray cancer, aniline cancer) will continue their growth even if the etiological factors, active at the earliest rise of the tumor, have disappeared, in the same way the Spiroptera cancer continues its growth even if the Spiropterae decrease in number or quite disappear (FIBIGER)[1], and it will be seen from the investigations of C. O. JENSEN[2] that neither does the plant cancer described by ERWIN SMITH cease its development, after *Bacterium tumefaciens* has died off in tumor.

These facts call our attention to a circumstance which augments the difficulties in exploring the causation of carcinomata, viz. that the etiological factors which were originally active, may have disappeared at the point of time when the carcinoma is observed, these factors being thus untraceable.

Thus, a further experimental investigation with the aim of ascertaining the shortest time necessary for the action of the tar to produce development of carcinoma, and how long after discontinuance of the irritant carcinoma may develop, — will be of the greatest interest.

[1] Det Kgl. Danske Videnskabernes Selskabs Biologiske Meddelelser I, 11.
[2] Landbohøjskolens Aarsskrift 1918.

The production of carcinoma by so simple a method as tar painting, finally, represents an important step forward in experimental cancer research. Up till now, only one method has been known by which to produce carcinoma experimentally with certainty, namely the transmission of *Spiroptera neoplastica (Gongylonema neoplasticum)* which in 50—60 per cent of black and white rats (unfrequently in mice) gives rise to development of carcinoma of the stomach, and in a few cases furthermore of the tongue. On the transmission of spiropterae carcinoma has now been produced (Fibiger) in more than 100 experimental animals (rats and mice) at the Anatomo-Pathological Institute of the University of Copenhagen and, as previously stated, this method has rendered it possible to yield a contribution to the study of several of the problems of carcinoma.

This method, however, has this inconvenience, that development of carcinoma takes place in an organ, the stomach, that is not within easy reach of examination as long as the animal is alive, whereas tar carcinoma is produced on the skin, where the pathological processes and the propagation of the carcinoma may be followed from day to day in the live animal. Tar carcinoma also seems to be more frequently produced, in so far as it can hardly be due to an accident that tar carcinoma in these experiments occurred in no less than 24 out of 26 mice examined, at the earliest, half a year after the beginning of the paintings. That all sorts of tar should be equally active, is however, on the face of the matter, hardly probable.

But experimental tar carcinoma in mice, according to our experiments, furthermore, seems to possess a peculiarity which gives it a special place among the carcinomata

hitherto found in mice, viz. its liability to metastasis formation in the lymphatic glands. It is a well-known fact that carcinoma in mice only very rarely will present metastatic deposits in these glands. As an example need only be mentioned that among 273 malignant tumors in mice examined by HAALAND[1], only 4 were found in which glandular metastasis could be observed with the naked eye, and microscopical examinations also have proved the rare occurrence of such metastases.

In distinction from this, we have found metastatic deposits in axillary lymphatic glands of no less than 6 out of our 24 tar painted carcinomatous mice. These carcinomatous lymphatic glands were all much enlarged and in several cases the carcinoma was visible already on naked eye examination.

Hence, the experimentally produced tar carcinoma in white mice may be said to present such great analogies to the carcinoma in man that among all known carcinomata in mice it may be considered the one which bears the closest resemblance to human carcinoma.

And experimental production of tar carcinoma will not only prove a control and an excellent supplement to the Spiroptera-method, but in some respects even exceeds the latter, and especially will render it less difficult, experimentally to examine and follow the effects of therapeutic methods, and to study the possibility of inducing immunity.

That it has now come within the reach of cancer research experimentally to produce with certainty carcinoma of quite homogeneous anatomical structure by means of two methods so different as the transmission of a nematode and repeated painting with an antiseptic substance

[1] l. c.

like tar, is a fact which yields a support to the modern view that the carcinomata cannot be due to one single identical cause, but that several or numerous factors of different origin and different nature may be active in inducing the epithelial cells to unlimited carcinomatous proliferation.

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

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Færdig fra Trykkeriet den 23. Marts 1921.

PLATES

PLATE I.

Fig. 1. Mouse No. 6 Papillomatous growth, 238 days after the beginning of the tar painting. Natural size.

- 2. The same mouse. 289 days after b. o. p., 40 days after suspension of the tar painting. Natural size.
- 3. Mouse No. 4. Cutaneous horn, 292 days after the b. o. p. Natural size.
- 4. Mouse No. 5. Papillomatous growth (cutaneous horn) 224 days after b. o. p. The tar painting was now suspended. Natural size.
- 5. The same mouse. 65 days later. Natural size.
- 6. Mouse No. 8. Cutaneous horn, 290 days after b. o. p., 59 days after suspension of the tar painting. Natural size.
- 7. The same mouse, 68 days after suspension of the tar painting. Natural size.
- 8. The same mouse, 76 days after suspension of the tar painting. Natural size.
- 9. The same mouse, on death, 102 days after suspension of the tar painting. Natural size.

Fig. 1.

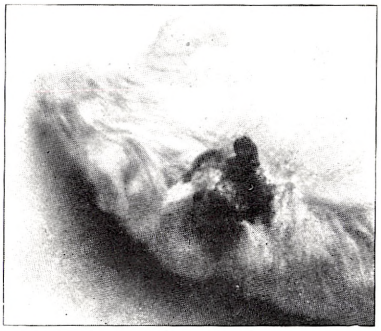


Fig. 2.

Fig. 3.

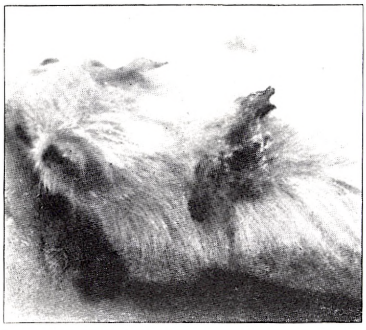


Fig. 4.

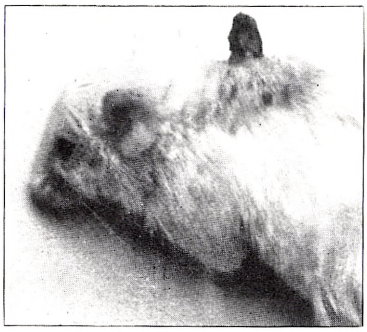
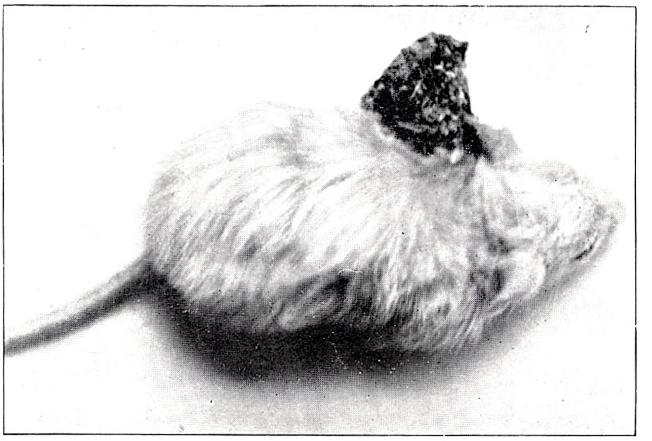


Fig. 5.



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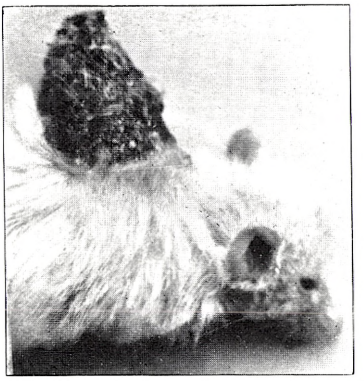


Fig. 7.

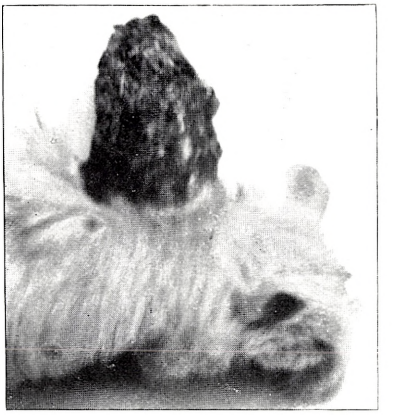


Fig. 8.

Fig. 9.

PLATE II.

Fig. 10. Papilloma in mouse, named II_{18}. $\times \frac{3}{1}\frac{2}{}$.

- 11. Papilloma in mouse, named I_2. $\times \frac{3}{1}\frac{2}{}$.
- 12. Carcinoma. Initial stage. Mouse No. 2, named I_9. $\times \frac{4}{1}\frac{5}{}$.
- 13. Carcinoma (type: Epithelioma malignum). Diagnostic excision. Mouse belonging to the 3rd series of experiments. $\times \frac{3}{1}\frac{2}{}$.
- 14. Carcinoma, representing very distinctly the type of Epithelioma malignum. Mouse No. 5. $\times \frac{4}{1}\frac{5}{}$.

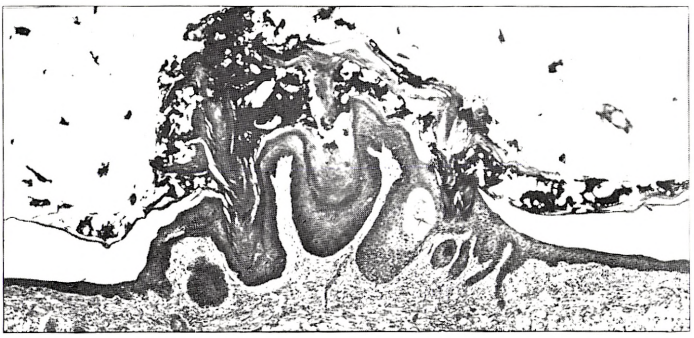


Fig. 10.

Fig. 11.

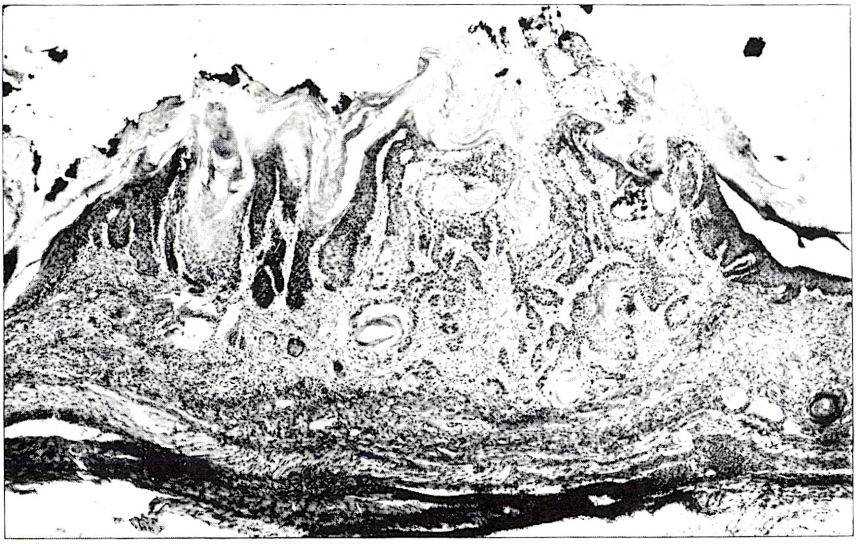


Fig. 12.

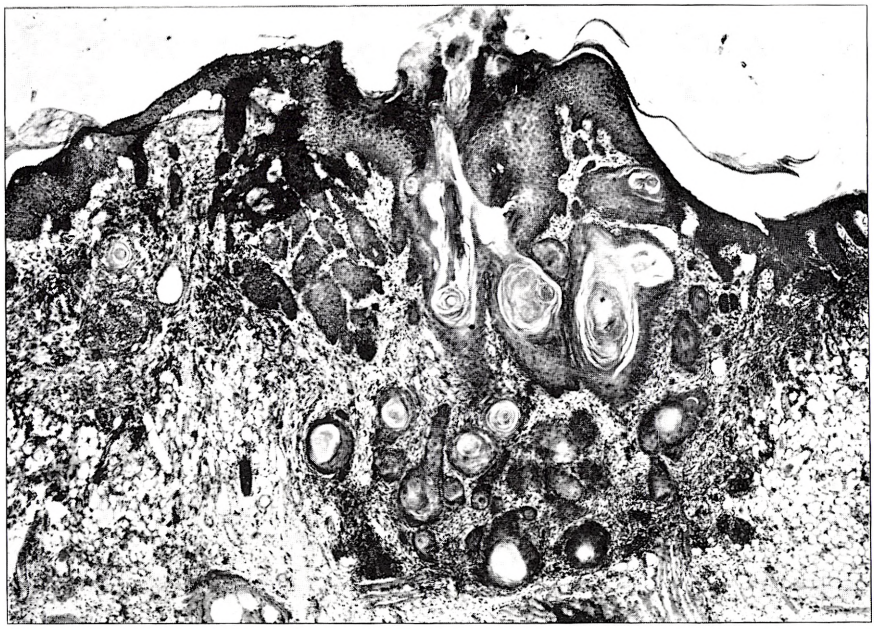


Fig. 13.

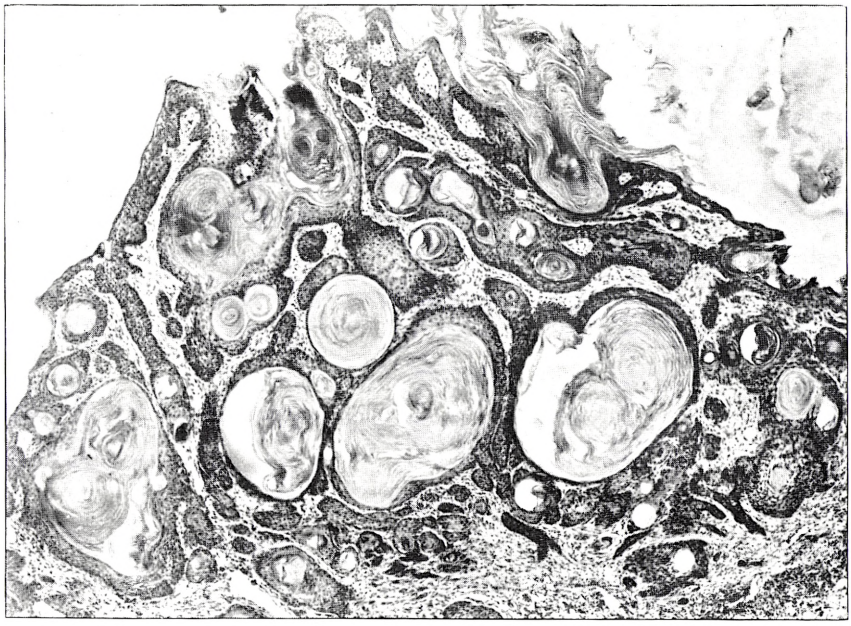


Fig. 14.

PLATE III.

Fig. 15. Keratinising carcinoma. Mouse No. 6. $\times \frac{45}{1}$.

- 16. Metastasis of the same tumor in an axillary lymphatic gland. $\times \frac{45}{1}$.

- 17. Carcino-sarcoma. Mouse No. 9. To the left carcinoma (type: Epithelioma malignum). To the right the polymorphous celled sarcomatous nodule. $\times \frac{45}{1}$.

- 18. The sarcomatous tissue from the tumor in mouse No. 9. $\times \frac{112}{1}$.

- 19. Carcinomatous, very strongly keratinising metastasis in an axillary lymphatic gland in the same mouse. (No. 9.) $\times \frac{45}{1}$.

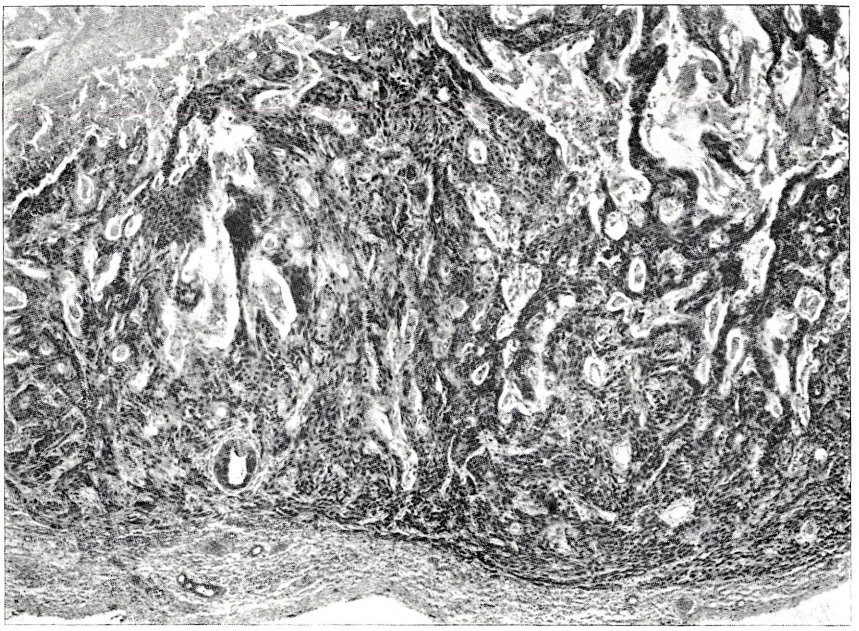


Fig. 15.

Fig. 16.

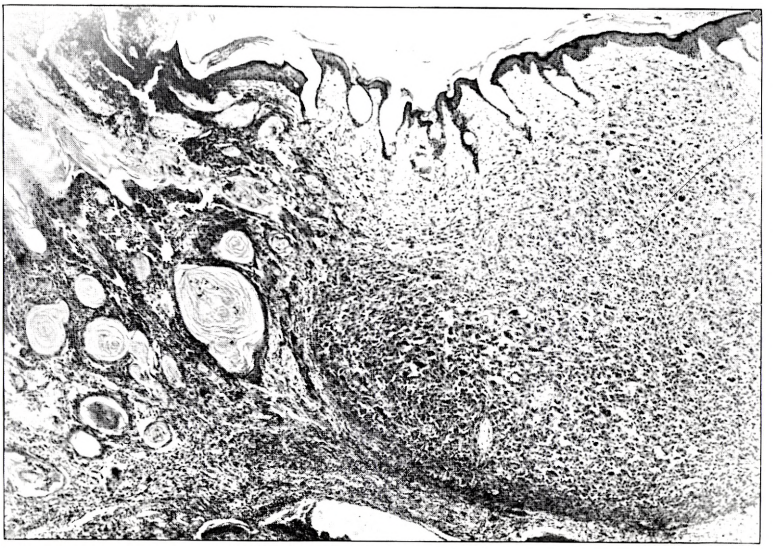


Fig. 17.

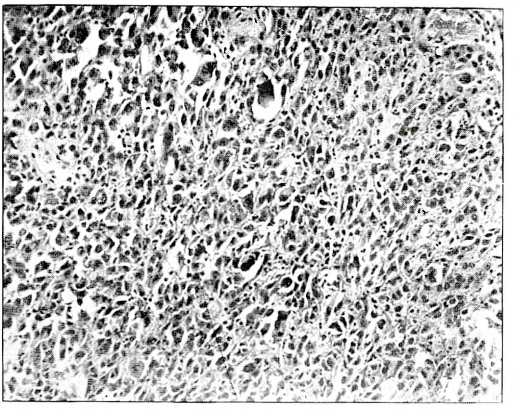


Fig. 18.

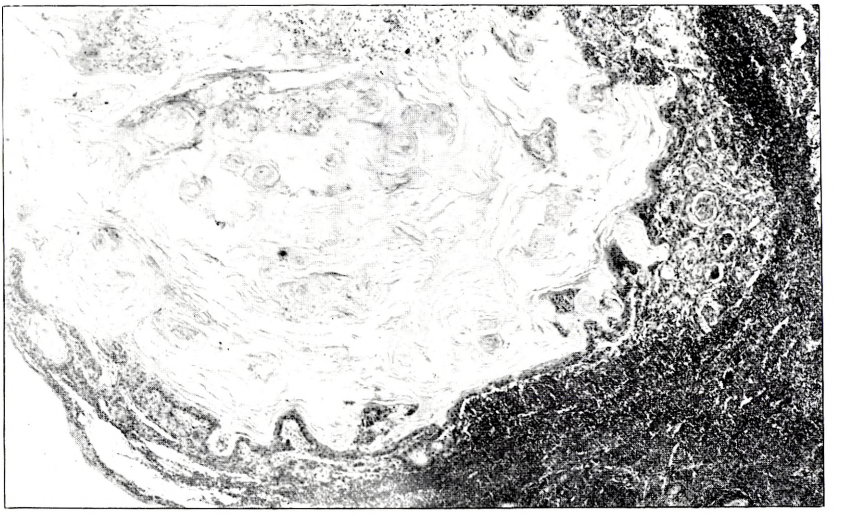


Fig. 19.

PLATE IV.

Fig. 20. Carcinoma (type: Epithelioma malignum) in mouse No. 1. $\times \frac{4.5}{1}$.

- 21. The same tumor transplanted. $\times \frac{4.5}{1}$.
- 22. Mouse No. 10. Cutaneous horn, 208 days after b. o. p. The paintings were suspended 3 days before. Natural size.
- 23. The same mouse, 14 days after suspension of the painting. Natural size.
- 24. The same mouse, 28 days after suspension of the tar painting. Natural size.
- 25. The same mouse, 44 days after suspension of the tar painting. Natural size.
- 26. The same mouse on death, 52 days after suspension of the tar painting. Natural size.
- 27. The same mouse. Cutaneous horn. The one half of the cutaneous horn and its carcinosarcomatous base, cut vertically. Natural size.

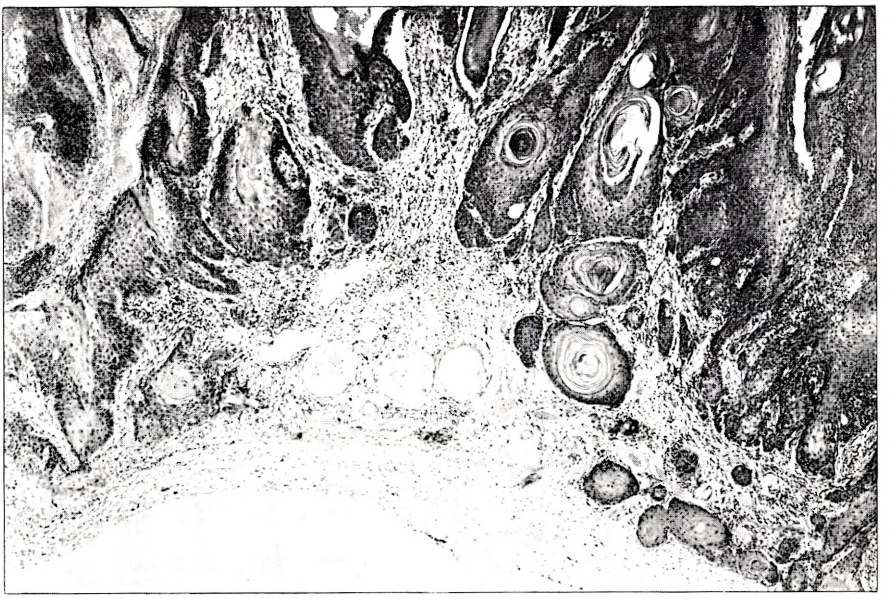


Fig. 20.

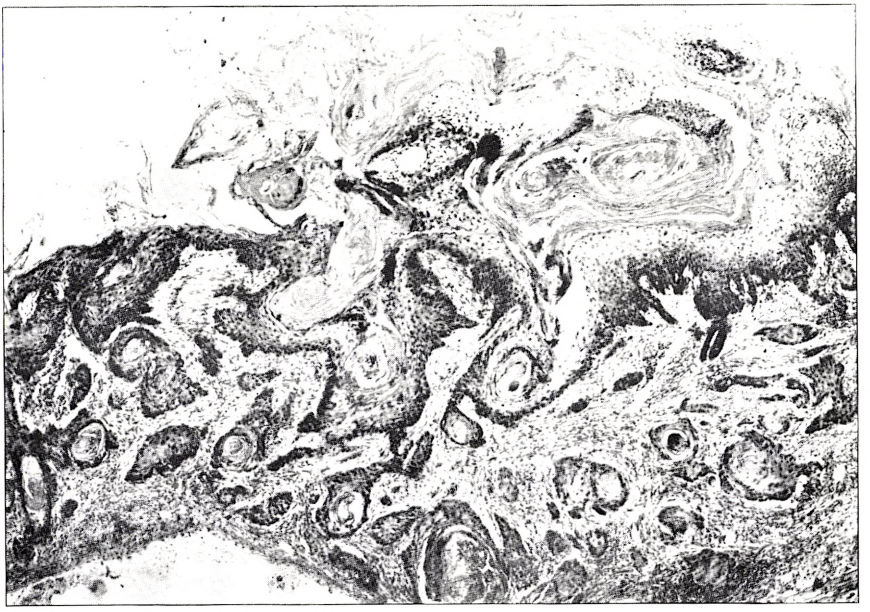


Fig. 21.

Fig. 22.

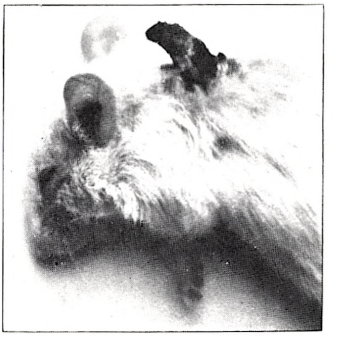
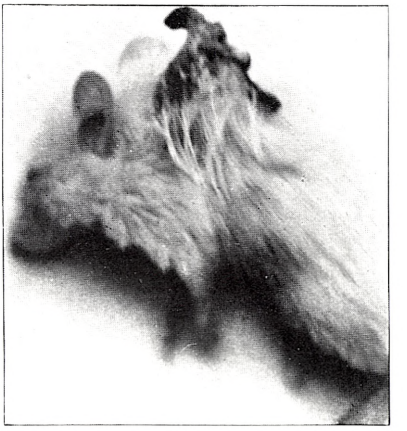


Fig. 23.

Fig. 24.



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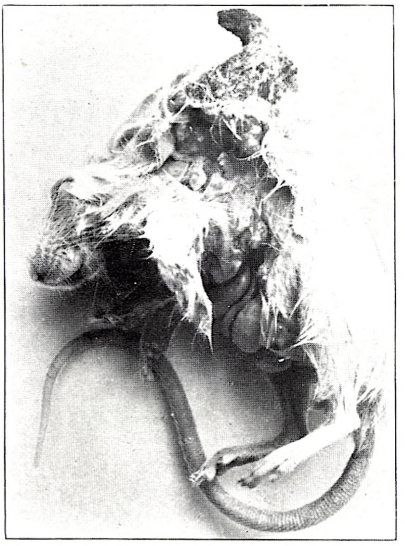


Fig. 26.

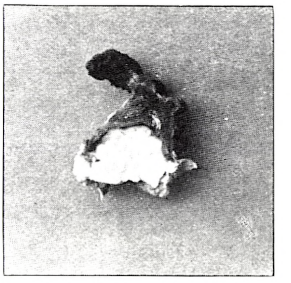
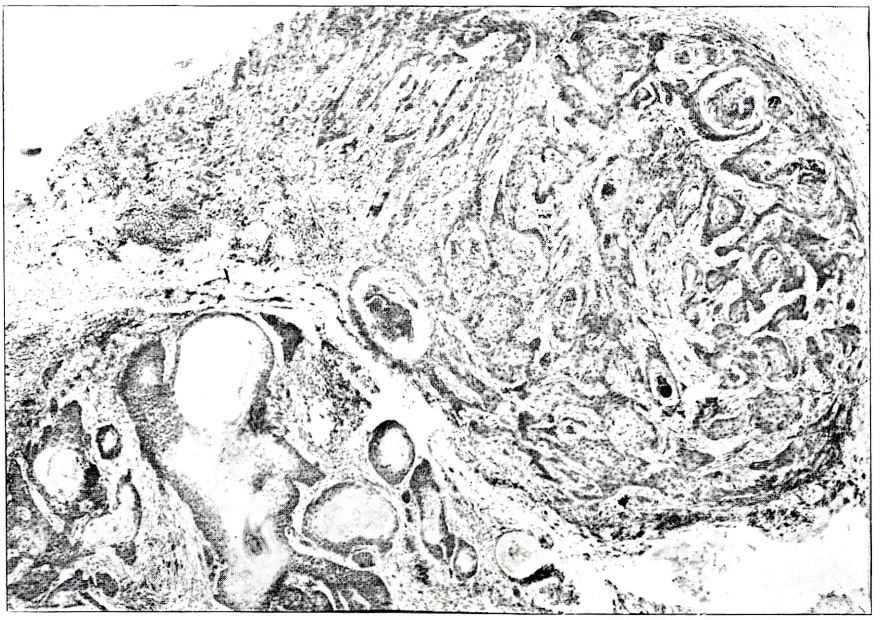


Fig. 27.

PLATE V.

Fig. 28. Mouse No. 10. The keratinising carcinomatous part of the tumor. $\times \frac{45}{1}$.

- 29. The same mouse. Mixture of carcinomatous and sarcomatous components of the tumor. $\times \frac{40}{1}$.
- 30. The same mouse. A spindle celled sarcomatous part of the tumor. $\times \frac{45}{1}$.
- 31. The same mouse. Carcinomatous and sarcomatous areas adjacent to each other. $\times \frac{45}{1}$.



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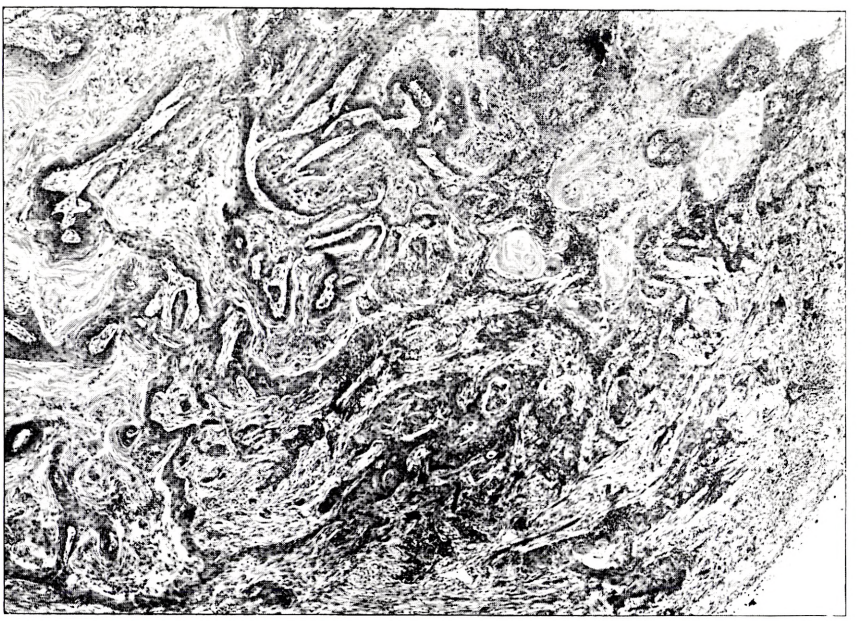

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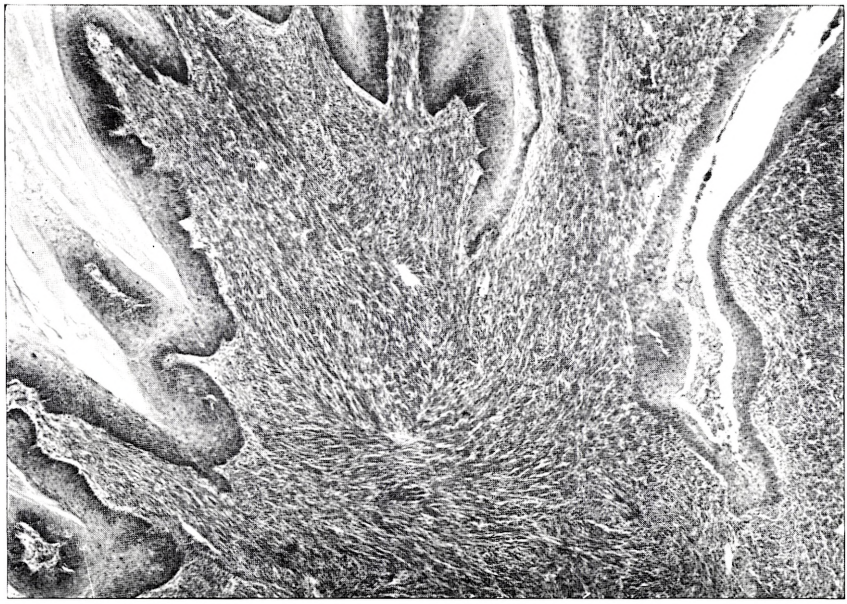

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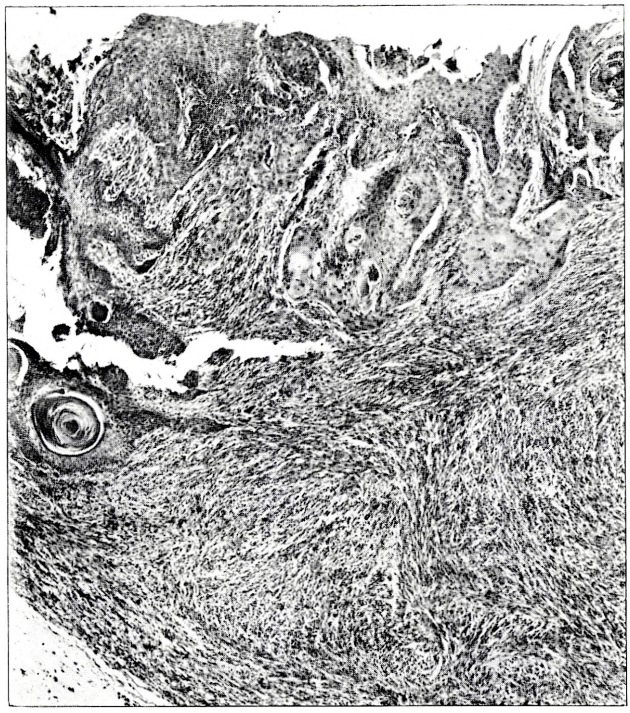

Fig. 31.

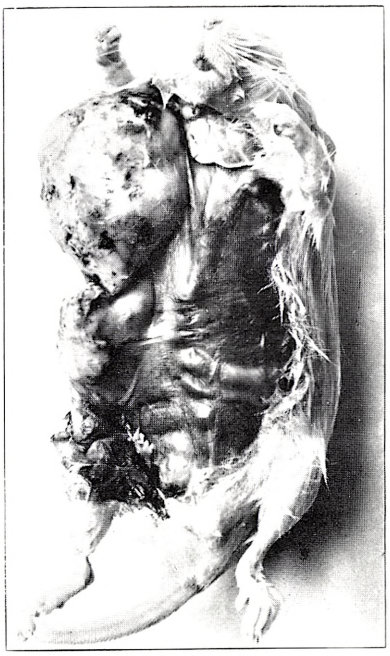

Fig. 33.

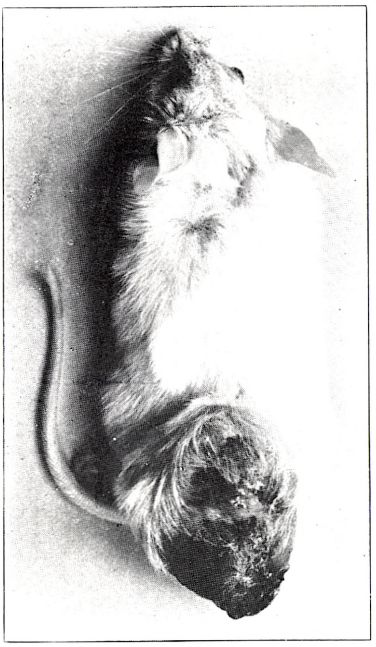

Fig. 34.

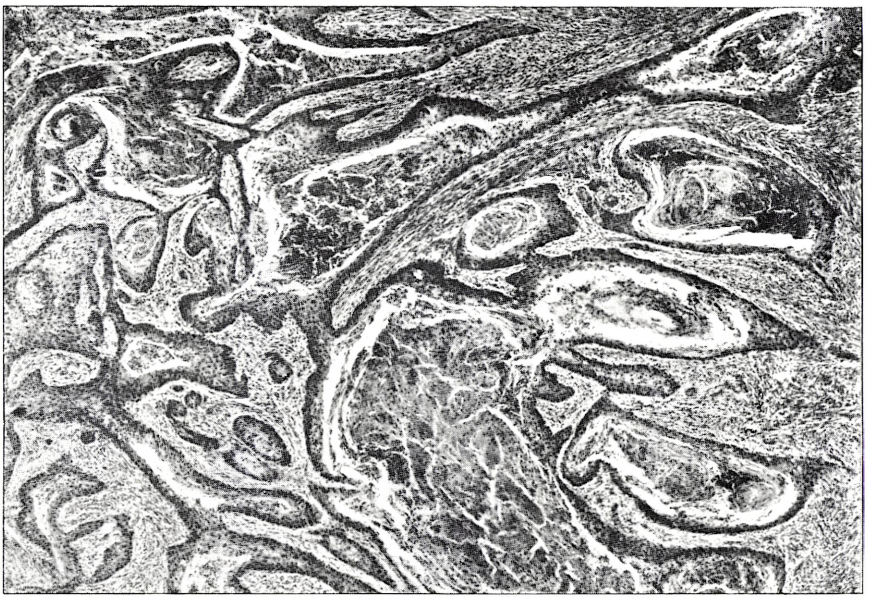

Fig. 36.

PLATE VI.

Fig. 32. Mouse No. 10. Spindle celled sarcomatous part of the tumor. $\times \frac{100}{1}$.

- 33. Mouse with subcutaneous tumor (pure spindle celled sarcoma), developed after transplantation of tumor tissue from mouse No. 10. Second generation. Natural size.
- 34 and 35. Mice with subcutaneous spindle celled sarcomatous tumors, developed on continued transplantation of tumor tissue from mouse No. 10. 8th generation. Natural size. The inoculations have been made at the root of the tail.
- 36 Subcutaneous carcino-sarcoma in a mouse, to which the carcino-sarcoma of mouse No. 10 has been transplanted. $\times \frac{15}{1}$.
- 37. Subcutaneous pure spindle celled sarcoma in another mouse, to which the carcino-sarcoma of mouse No. 10 has been transplanted. $\times \frac{100}{1}$.

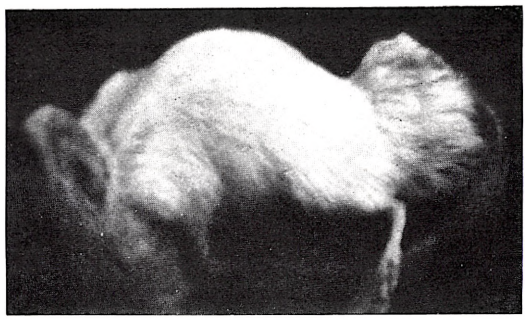

Fig. 35.

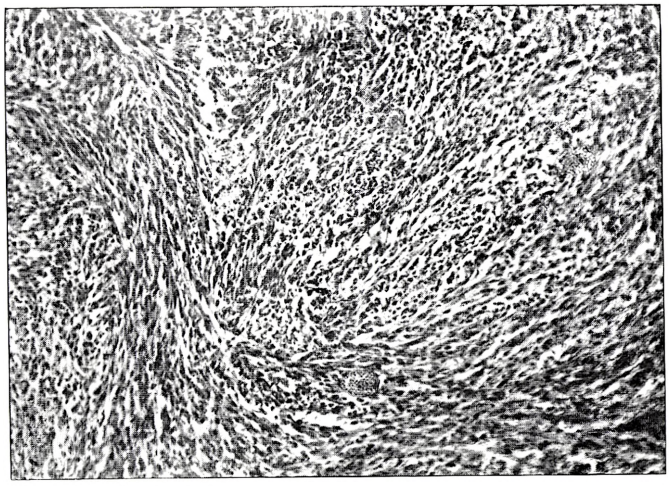

Fig. 32.

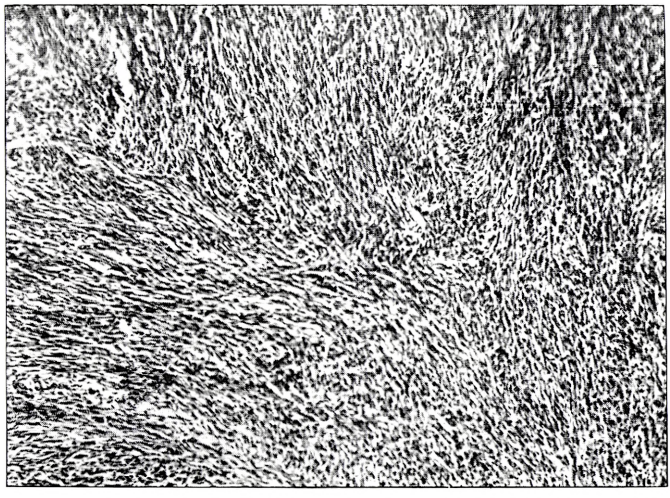

Fig. 37.

DET KGL. DANSKE VIDENSKABERNES SELSKABS SKRIFTER

NATURVIDENSKABELIG OG MATHEMATISK AFDELING

8DE RÆKKE

Kr. Ø.

I., 1915—1917 10,75

1. PRYTZ, K., og J. N. NIELSEN: Undersøgelser til Fremstilling af Normaler i Metersystemet grundet paa Sammenligning med de danske Rigsprototyper for Kilogrammet og Meteren. 1915 1,55
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3. CHRISTIANSEN, M.: Bakterier af Tyfus-Coligruppen, forekommende i Tarmen hos sunde Spædkalve og ved disses Tarminfektioner. Sammenlignende Undersøgelser. 1916 2,25
4. JUEL, C.: Die elementare Ringfläche vierter Ordnung. 1916. .. 0,60
5. ZEUTHEN, H. G.: Hvorledes Mathematiken i Tiden fra Platon til Euklid blev en rationel Videnskab. Avec un résumé en français. 1917 8,00

II., 1916—1918 (med 4 Tavler) 11,50

1. JØRGENSEN, S. M.: Det kemiske Syrebegrebs Udviklingshistorie indtil 1830. Efterladt Manuskript, udgivet af OVE JØRGENSEN og S. P. L. SØRENSEN. 1916 3,45
2. HANSEN-OSTENFELD, CARL: De danske Farvandes Plankton i Aarene 1898—1901. Phytoplankton og Protozoer. 2. Protozoer; Organismer med usikker Stilling; Parasiter i Phytoplanktonter. Med 4 Figurgrupper og 7 Tabeller i Teksten. Avec un résumé en français. 1916 2,75
3. JENSEN, J. L. W. V.: Undersøgelser over en Klasse fundamentale Uligheder i de analytiske Funktioners Theori. I. 1916.... 0,90
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5. JUEL, C.: Die gewundenen Kurven vom Maximalindex auf einer Regelfläche zweiter Ordnung. 1917 0,75
6. WARMING, EUG.: Om Jordudløbere. With a Résumé in English. 1918 3,65

III., 1917—1919 (med 14 Kort og 12 Tavler) 26.00

1. WESENBERG-LUND, C.: Furesøstudier. En bathymetrisk-botanisk zoologisk Undersøgelse af Mølleaaens Søer. Under Medvirkning af Oberst M. J. SAND, Mag. J. BOYE PETERSEN, Fru A. SEIDELIN RAUNKIÆR og Mag. sc. C. M. STEENBERG. Med 7 bathymetriske Kort, 7 Vegetationskort, 8 Tavler og ca. 50 i Texten trykte Figurer. Avec un résumé en français. 1917 22,00
2. LEHMANN, ALFR.: Stofskifte ved sjælelig Virksomhed. With a Résumé in English. 1918 3,15
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V., (under Pressen).

1. BJERRUM, NIELS, u. KIRCHNER, AAGE: Die Rhodanide des Goldes und das freie Rhodan. Mit einem Anhang über das Goldchlorid. 1918. 3,50

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