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FROM THE
UNIVERSITY OF PENNSYLVANIA MEDICAL BULLETIN,
MARCH–APRIL, 1907.

Reprinted from the University of Pennsylvania Medical Bulletin, March–April, 1907.

FURTHER OBSERVATIONS ON THE ENDEMIC OCCURRENCE OF CARCINOMA AND ON THE INOCULABILITY OF TUMORS.

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About seven years ago I published, with Dr. George Jobson,[1] some observations on the endemic occurrence of carcinoma in cattle. We found a ranch in Wyoming in which a relatively large proportion of cases of carcinoma of the inner angle of the eye in cattle had been observed in the course of the last ten years. These observations corresponded to those made on human beings by Behla and others some time before. We left it undecided whether or not that endemic occurrence had to be explained by the presence of a micro-organism. We noted, especially, that no peculiar conditions which differed from those of the neighboring ranches, which were practically free from cancer, existed on that ranch in Wyoming.

I[2] published further observations on the endemic occurrence of cancer in rats which were affected with sarcoma. From 1900–1903 I found in a few cages which were standing side by side in the Polyclinic of Chicago, in the course of three years, three rats all of which had cystic sarcoma of the

[1] Medicine, April, 1900.
[2] Centralbl. f Bacteriol., 1904, vol. xxxvii.

thyroid gland. The main facts may be stated as follows: In a few cages, which were kept very clean, tumors were observed among animals which received a certain regular food which usually had been boiled. All the tumors were found on the same part of the body, and showed the same macroscopic and microscopic structure. These tumors preserved, in the main, their character during the course of many transplantations. It was especially noticed that such tumors were found in animals a relatively long time after the last animal affected, with such a tumor, had been removed from the laboratory. It was further found that in other cases in which an endemic occurrence of tumors had been observed, the tumors had about the same structure and the same seat in each instance, in contradistinction to the endemic occurrence of tumors in man in which the tumors observed at the same locality had a usually different character in different individuals. I laid especial stress upon this difference between the endemic occurrence of cancer in man and in animals.

I furthermore found that if new rats were kept in the same cage with the rats which had been inoculated with tumors no spontaneous development of tumors was found, neither did the feeding of new animals with fresh tumors lead to a new formation of tumors in those rats. I pointed out the possibility that a hereditary factor is at the base of such endemic occurrence of cancer. I further concluded that if an infectious agency should be the cause of the endemic occurrence, the facts communicated render it very probable that tumors differing in structure and position are caused by *different* microörganisms.

In the following I intend to report on some further observations on the endemic occurrence of cancer in mice. In this case no certain proof could as yet be obtained of the infectious nature of the endemic occurrence; certain facts point even to the probability that hereditary influences are at work. In the latter part of December, 1906, I obtained from the

same breeder six white mice which were affected with tumors. These tumors proved to be adenocarcinomas or carcinomas. Simultaneously three cages were received in which a number of mice affected with tumors had lived for some time. Approximately thirty white mice from a different source were put into those cages without any previous cleaning of the cages or without change of the bedding used for the tumor mice by the breeder. The new mice lived approximately three months in two of those cages[1] and no tumors developed among them. It can, of course, not be excluded that tumors may develop later. Personal inquiries made at the place where the tumor mice had been bred did not give any indication that the endemic occurrence could be attributed to certain infected cages. It appeared rather to depend upon hereditary conditions. The breeder believes to have observed cases in which a number of tumors were observed in different generations of the same family, although that family had changed cages repeatedly. Moreover, the cages were frequently washed. There was no special cage to which infection could be attributed. Tumors could be found in mice only eight months old. Many cases occurred in one small room and a few in the other small room. In each the mice were akin and considerably inbred, but unrelated or only very distant relatives of those in the other room. In each room they were bred two or three years with no change of stock except in the tumor-room, where at one time, new males all taken from one family, were introduced. The only difference in environment of mice in the two rooms was that in the tumor-room no sunshine was admitted, while the other was bathed in sunlight.

I now wish to give a somewhat detailed report of the tumors found in six mice which I received early in the winter of 1906-1907 from the breeder of mice referred to above.

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[1] Most of the mice kept in one of the three cages were lost by accident about three weeks ago. Only three mice were saved. About a dozen new mice were added to the three old ones. April 2, among those mice, a female mouse with a tumor on the side was found.

1. *The first mouse* had a large tumor on the right side of the head and the upper part of the neck. The tumor was nodulated, somewhat hard, and had white, necrotic areas inside. No metastasis was visible to the naked eye. A little nodule was present, however, at the side of the large tumor on the neck. Microscopic examination showed the tumor cells, which were of an epithelial character, to grow usually in solid masses. The pressure of the cells upon each other caused them to be somewhat elongated. In other places, however, a distinct alveolar character was present, the central cells becoming necrotic or entirely dissolved, a central cavity being formed in this way. The colloid masses in the centre of the alveoli are much smaller than in the tumor of the second mouse and are of a different character. Wide areas of the tumor become necrotic; mitoses are present. Sometimes we find in the centre of the alveoli tumor cells which are much larger than the surrounding cells and have a large nucleus. Inside the alveoli these cells swell and may occasionally have two nuclei. Still later the nuclei and cytoplasma become dissolved. Sometimes a large number of tumor cells may swell simultaneously and degenerate, and then larger cavities are formed which are lined with tumor cells. In some places near the tumor we find collections of small mononuclear cells forming little packages. This tumor penetrates into the surrounding striated muscle and destroys it. At one place the tumor penetrates into the subcutaneous fat tissue. In a neighboring lympahtic gland a myeloplaque can be seen on microscopic examination; many mononuclear eosinophile cells are present in the fat tissue around the lymph glands. No tumor cells can be seen in this lymphatic gland. Macroscopically, the lung did not show any nodules. Microscopically, we find disseminated, in different parts of the lung, a number of tumor nodules which are of different size, but usually quite small. In the centre of some of the cell nests we find large, red-staining cells with a large vesicular nucleus. These cells probably form colloid material. Sometimes no

lumen is visible in these cell nests; at other times an alveolar structure is present. These tumor cells are sometimes distinctly situated in the alveoli of the lung. No relation can be seen between the bloodvessels and the tumor nodules. This tumor was transplanted December 22, 1906, subcutaneously into thirteen rats. Besides, it was inoculated into tumor mouse No. 3.

Tumor mouse No. 3 inoculated with a little piece of tumor mouse No. 1 died thirteen days after the inoculation, January 5, 1907. Microscopic examination of the transplanted piece shows a necrotic mass in the centre. In the periphery, strings of tumor cells grow in large number. They at first form solid masses; through degeneration of some tumor cells a few small cavities are formed. In this way an alveolar appearance is occasionally produced in these cell masses. Some new-formed strings of tumor cells penetrate directly into the surrounding tissue without the preparatory work of any granulation tissue.

Another mouse examined, eleven days after the inoculation, showed a subcutaneous nodule with necrotic tissue in the centre. Tumor alveoli, in which the cells are a little shrunken, are present in the periphery of the necrotic mass. In the lung is found a nodule consisting of masses of epithelial-like cells filling out the alveoli of the lung. A neighboring bronchus shows adenomatous outgrowth. It is not certain whether this nodule in the lung was produced through the proliferation of the bronchial epithelial cells or through the proliferation of the alveolar epithelium of the lung. We certainly had not to deal with a proliferation of tumor cells which were inoculated directly from the first tumor mouse.

A third mouse inoculated with this tumor died twenty-six days after inoculation. A little subcutaneous nodule attached to the skin was found. This nodule consists mainly of necrotic tissue, but a few rows of tumor cells, which, however, are somewhat shrunken, are present.

2. *The second mouse* died suddenly during the night, in the latter part of December, and this tumor could, therefore, not be used for inoculation. The tumor, of about the size of a hazelnut, belongs to the type of papillary adenocarcinoma, in which the cells are usually much higher and the canals which they line wider and more irregular than in the other varieties of adenocarcinoma. The tumors form papillary prominences into the canals. Desquamation of masses of such cells into the lumen of the canal can take place, and their cytoplasm contributes to the formation of the colloid mass in the centre of the tubules. At one end of the tumor we find ducts and alveoli made up of smaller cells somewhat of the structure of the mammary gland. Some of the alveoli are lined by several rows of epithelial cells. At some places the tumor cells seem to penetrate into the fibrous connective tissue and to split the latter into smaller fibers. Some of these cells stain markedly with eosin. At other places part of the tumor cells form thin vacuoles in their cytoplasm and some alveoli can be lined by such finely vacuolar cells.

3. *The third tumor mouse* into which a piece of the first tumor mouse had been implanted, was found dead on January 5, thirteen and one-half days after the inoculation. Nine nodules, which were partly white, partly hemorrhagic, were found, mostly in the subcutaneous tissue in different parts of the body. These tumors were not used for transplantation. One subserous nodule was situated on the right side of the body near the thigh. It consisted of a dark, hemorrhagic, and a white, not hemorrhagic part. Microscopically the tumor consisted of alveoli containing some colloid material lined by one row of cells and of some more solid parts in which apparently the tumor cells were pressed closely together so that the alveolar arrangement disappeared. No mitosis could be seen; this may readily be explained if we take into consideration that the animal was dead for some time before the tumor was fixed. In the dense connective tissue near the ordinary alveolar tumor tissue there were gland-like, wider ducts which

contained colloid masses. The latter had usually a concentric structure. This tumor nodule includes a lymph gland in the marginal sinuses of which large phagocytic cells can be seen which apparently take up lymphocytes. Another nodule was found between the right thigh and the genital organs. A part of this tumor was hemorrhagic. The alveoli here are a little larger and contain colloid. In the dense connective tissue we again find wide ducts with a dense colloid material. Parts of the tumor degenerated; in others hemorrhages were present. The tumor consists of alveolar and somewhat more solid masses of tumor cells. These more solid areas are merely tumor alveoli in which the cell proliferation has taken place more actively, and as a result of this proliferation the crowding of the cells takes place. This can be equally well observed in other tumors of this series. A third nodule was found in the subcutaneous tissue of the right humerus. This tumor appeared as a cyst with gray-white material inside. Microscopically large areas of necrosis were found in the centre. The fourth nodule was situated between the tail and the left thigh, subcutaneously. It was hemorrhagic and showed microscopically typical tumor structure with large degenerated areas. In the dense connective tissue we again find the gland-like cystic ducts containing colloid and lined by one row of flat cells. The tumor cells are here partially shrunken and large hemorrhagic areas are present. The lining epithelium of a cyst can be entirely lost at one side. The wall which separates two cysts can be perforated. The cytoplasm of desquamated cells is included in the colloid mass. There is much blood pigment present in the cells and in the colloid. A fifth subcutaneous nodule was found situated on the left side near the forearm and was partly necrotic. The tumor is, in part, degenerated and hemorrhages are visible under the microscope. In the dense connective tissue we again see balls of concentrically arranged colloid. The alveoli are somewhat shrunken. The sixth nodule, which was on the left side of the trunk subcutaneously, was very dark, a result of hemorrhage.

There were also white parts present. This tumor had, in every respect, microscopically the same structure as the other nodules. The same colloid material was present in the ducts of the dense connective tissue. Much degeneration can be seen. Hemorrhages are present; in the peripheral sinuses of a neighboring lymph gland we see large phagocytic cells which have sometimes two nuclei. Even if these large cells in the lymph sinuses should be tumor cells, which is not probable, we would have to deal merely with a secondary invasion of the lymph glands inasmuch as the tumor nodule did not take its origin from a metastasis in the lymph gland. The seventh nodule was found under the skin of the neck. The nodule showed the typical microscopic structure.

There were large areas of degeneration present. Again, in some of the sinuses of a neighboring lymph gland relatively large cells could be seen which originate probably through the proliferation of the endothelial cells, but their character as tumor cells cannot be entirely excluded. Near the spinal column three little transparent nodules were found. They showed the same structure; the glandular ducts filled with the colloid masses in the connective tissue could be seen. The colloid bodies are even visible between the striated muscle into which the tumor penetrates. A little lymph gland free from tumor is situated in the neighborhood of these three tumor nodules. Firmly attached to the skin a little nodule could be seen in the abdominal wall. Microscopically we here find necrotic material encapsulated by connective tissue. In the subcutaneous tissue nearby, glandular ducts which contain the typical colloid and alveolar granular tumor tissue are present. The largest tumor of this mouse had about the size of a walnut, and was situated near the left thigh. It was softer than the tumors of mouse 1 and 2 and was partly hemorrhagic. In the kidney of this animal a cyst was present, the contents of which could not be determined. In the lung nothing special could be seen.

4. *In the fourth mouse* the tumor was partly hemorrhagic and soft. The tumor consisted of simple alveoli which were arranged in rows between the bloodvessels. The blood channels are very wide and merely lined by endothelium. The small alveoli were separated by edematous connective tissue. A small number of mitoses could be seen. The structure of this tumor had some distant resemblance to that of a perithelioma. In other places of the tumor the alveoli without lumen are closely pressed toward each other. At such places there are more mitoses present and no dilated capillaries are seen. Wherever we find many mitoses, the connective tissue is not seen to be edematous; and where we find edematous connective tissue between the alveoli the mitoses are not very numerous. The lymph glands which were examined microscopically were found to be normal. In the lung, which appeared to the naked eye to be normal, small metastases were found on microscopic examination. The tumor cells of the metastatic nodules had a somewhat alveolar arrangement but sometimes no distinct lumen was present. Some connective tissue surrounded the tumor nodules; small nodules of round cells were also present.

Twenty-one mice were inoculated with material from this tumor, on January 8. Thirteen days after inoculation one inoculated mouse was killed. Microscopically, a mass of necrotic tumor tissue was found which was surrounded by connective tissue.

5. *In the fifth mouse* one tumor was found situated on the left side of the chest near the leg; this tumor was very large and began to break through the skin; it was quite hard. On the right posterior part of the body a somewhat smaller tumor was found. It was a little softer and had not yet penetrated through the skin. No metastasis was visible to the naked eye. The tumor on the posterior part of the body, which had not yet broken through the skin, showed alveoli with or without red-staining colloid material in the centre. A few small areas were seen in which the cells were

pressed together; some of these cells were in mitotic division, otherwise no mitosis could be seen. At places where the cells were pressed together, the alveolar character was no longer well pronounced. The tumor tissue can penetrate deeply into the neighboring tissue. In the fat, which surrounds the tumor, the blood capillaries are well visible and in the neighborhood of the bloodvessels we see canals lined with high cells and with a colloid material in the centre. The alveoli of this tissue have a somewhat larger lumen than could be seen in the former tumors. Some areas of necrosis are present.

January 19 seventeen mice were inoculated with this tumor. Two days later one inoculated mouse died. In the centre of the nodule the section shows necrosis. In the periphery living tumor cells are present with well-preserved alveoli which are perhaps a little shrunken. Four and one-half to five days after the transplantation seven tumor mice died, probably as a result of exposure to cold. The transplanted pieces were examined microscopically. No. 1 was mostly necrotic. At the margin, tumor alveoli were present which were well preserved but somewhat shrunken. No. 2 shows the same picture. In some peripheral alveoli vesicular nuclei are well preserved. No. 3 shows the same. Especially noticeable is the fact that some of the transplanted alveoli, which were on all sides surrounded by connective tissue, were better preserved than some of the more peripheral alveoli. No. 4 shows, likewise, at the margin, alveoli which are well preserved and only a little shrunken. In No. 5 the same condition is found. No. 6 is almost entirely necrotic. At the margin a few shrunken alveoli are present. No. 7 is entirely necrotic.

The second tumor of mouse 5, which was a little ulcerated, shows in the whole the same structure as the first one. There are, however, more glandular ducts present in the connective tissue which separates the tumor lobules. The tumor lobules themselves are composed of large or small alveoli which contain a colloid mass in the centre. A number of mitoses are present in the tumor cells. Where

the tumor cells are pressed together the mitoses are more numerous and at such places alveoli, containing a lumen, can hardly be seen. The tumor penetrates at certain places into the surrounding striated muscle and destroys the muscle tissue.

This tumor was transplanted, January 19, 1906, into thirteen mice. Three days after inoculation one inoculated mouse died. The greater part of the inoculated tumor was necrotic. At the margin, alveoli were present which were rather shrunken, but which were otherwise well preserved. No polynuclear leukocytes could be seen. Fourteen days after transplantation another mouse was found dead. The transplanted piece was entirely necrotic. The connective tissue penetrated into the necrotic material.

No metastases were found in the lung of mouse No. 5 on microscopic examination.

The sixth mouse had three tumors which were situated on the left side of the body between the anterior and posterior extremities. The tumor situated near the left hind leg developed only a few weeks after the mouse had been received. This last tumor was quite hemorrhagic and was not used for transplantation. The tumor situated anteriorly contained much dark, hemorrhagic fluid. Part of it was used for transplantation. The second tumor was not hemorrhagic. Microscopically this tumor was found to consist of small laveoli with a lumen in the centre; besides there were present more solid parts in which no lumen was visible between the cells. Both parts showed mitoses, but they were more numerous in the more solid parts. The structure of this tumor was almost identical with that of the tumors of the third mouse. Ducts lined with several rows of epithelium were also present. Mitoses could be found in the cells lining these ducts. At some places, however, the ducts were lined with only one row of epithelium.

One white mouse and two gray mice were inoculated with material from this tumor.

The second tumor of this mouse was not hemorrhagic. Microscopically, we find masses of densely-packed cells with mitoses. Usually no lumen is visible between the cells. At other places, however, distinct alveoli with a lumen in the centre are present. In the connective tissue glandular ducts can be seen. The structure of the first tumor differs from the second tumor by the prevalence of the densely packed cells and the smaller number of well-formed alveoli. The difference between the first and second tumor depends mainly upon the large amount of densely packed cells in the second tumor.

A piece of this tumor was inoculated into seven white mice, three of which were pregnant on January 23. At the same time ten white mice were inoculated with tumor material which had been heated previously for an hour to 39°. One of the pregnant mice died two and a half days after inoculation. Microscopic examination showed the transplanted piece to be almost entirely necrotic. In the periphery there are areas of shrunken, partly disorganized tumor cells. In a mouse killed two days, seventeen hours after inoculation, the inoculated piece showed necrotic material in the centre. In the periphery there were some well-preserved ducts present, the cells of which were only little altered or shrunken.

The third and more recent tumor was very hemorrhagic. Very irregular, degenerated areas can be seen in this tumor. As a result of the degeneration, large cavities are formed. Many hemorrhages are seen in the sections. Mitoses can be found in the tumor cells. In the fat tissue surrounding the tumor, gland-like ducts which form a part of the tumor tissue are visible.

The third tumor seems to have originated in a fat lobule in which the tumor cells form gland-like ducts, and which proliferate as is indicated by the presence of mitoses. In the centre of the ducts concentrically laminated colloid masses can be seen. At one place in the fat lobule a collection of lymphoid cells is present. In the axillary lymphatic glands we find marked proliferation of the endothelial cells of the sinuses.

In the lungs little nodules are formed by collections of round cells on the pleura. Near such epipleural nodules in the lung tissue, other proper nodules are formed through a proliferation of alveolar epithelium. A round, cyst-like body which was cut almost entirely into serial sections, and was found to consist of tissue closely resembling the structure of the body of the epididymis, was discovered in the axilla of this mouse. Desquamated cells and some rod-like bodies which resembled spermatozoa could be seen in the lumen of the canals. No trace of the testicle was present. Instead, we found areolar connective tissue. The arrangement of the ducts around this areolar tissue was very similar to that of the epididymis around the testicle.

It will now be necessary to discuss some of our observations and experiments with reference to conclusions reached by other investigators. We found indications that the endemic occurrence of cancer is, in part at least, caused by hereditary conditions. Certain predisposing conditions transmitted to different members of the same family may be one of the factors which determines the endemic occurrence of cancer. On the other hand, no definite indication could be obtained tending to prove that the presence of an organism in the cage was responsible. The cages were frequently cleaned. The mice in different rooms were kept in the same way. They were given the same food. Their cages were cleaned by the same person. Nevertheless, the endemic occurrence of cancer was mainly confined to one room. The mice kept in this room belonged to a certain number of families which interbred among each other, but were kept entirely separate from mice in the other room. There are indications that young mice descending from the same parents developed tumors although they were kept in different cages. If we, therefore, have facts which suggest that hereditary influences are important, we cannot ignore certain observations made by other investigators which suggest very strongly that transmission of a microörganism is responsible for the endemic occurrence of cancer among animals. Here

we must mention the observations of Morau,[1] who believes that certain insects carried the infecting organism from one mouse to another and were thus responsible for the endemic occurrence observed in his cages. Recently Haaland[2] reported from the Institut Pasteur, observations which seem equally to suggest a microörganism as the cause of the endemic occurrence of cancer in mice. It was found that if mice afflicted with cancer were put into cages containing mice obtained from places at which no cancer had been observed in the course of a year, cases of cancer developed among the mice which had been hitherto free from tumors. On the other hand, if they put mice which were free from tumors into cages in which a number of tumor mice had been raised, tumors could not be observed to originate among the new mice if the tumor mice had been removed before the new mice had been put into cages. Very interesting are the recent observations of Gaylord and Clowes.[3] Toward the end of the third year of my transplantations of the cystic sarcomas of the thyroid of rats, a number of rats affected with sarcoma were kept for several months in some cages in the New York State Cancer Laboratory in Buffalo. Two years after my rats had been removed, another rat which had a fibrosarcoma of the right abdominal aspect was found in one of the cages previously inhabited by my rats. A little more than a year later two other sarcomas were detected among new rats kept in the same cages. One of those was a fibrosarcoma on the right side of the abdominal aspect. The other was a cystic sarcoma of the thyroid region. This observation is certainly very striking and suggests the transmission of microörgan-

[1] Recherches expér. sur la transmissibilité de certains neoplasmes. Arch. de médec. expér., 1894. I could not find any statement in the original papers of Morau regarding the action of insects. If it should exist, it must have escaped my notice. Several writers, however, as E. G. Borrel, mention the views of Morau.

[2] Les tumeurs de la souris, Annales de l'Institut Pasteur, 1905.

[3] Evidences of Infected Cages as the Source of Spontaneous Cancer, Jour. Amer. Med. Assoc., January, 1907.

isms as a cause of the endemic occurrence of cancer. As yet we cannot, however, regard this explanation as absolutely proven, inasmuch as the sarcoma of the thyroid of rats is found at different places in America. At least, I myself obtained a rat affected with sarcoma of the thyroid which was found in the laboratory of an insane asylum in Iowa. The other sarcomas of the thyroid of rats which I have used in my experiments were obtained from a different source in Chicago. In Europe, Velich found a fibrosarcoma situated on the leg of a rat. Firket, and v. Eiselsberg likewise found sarcomas in rats. Nevertheless, it is not likely that the observations which indicate the transmission of a microörganism as the cause of the endemic occurrence of cancer in animals are to be explained as accidental occurrences, and it is possible that several factors are active in producing the endemicity of malignant tumors. Morau,[1] on the other side. also found indications of a hereditary transmission of carcinoma in mice. Eberth and Spude[2] found tumors in several mice of the same family. They interpret this occurrence evidently as due to a hereditary disposition. It is, however, doubtful whether in their case the influence of microörganisms can be excluded with certainty. Doubtful also is the interpretation of the endemic occurrence of carcinoma of the thyroid gland found in the trout and which was recently very carefully described by L. Pick.[3] Fish kept in certain tanks acquire this disease,which in this case is not limited to fish of the same family; if heredity plays any part at all in this case, it is certainly not the only important factor. On the other hand, Pick does not bring forward any fact which excludes the possibility of an infectious origin. He thinks it most probable that the primary condition present in the trout is struma of the thyroid. He believes, in fish as in man, goitre to be caused by certain conditions of the water and to occur endemically. The occurrence

[1] Morau, C. R. Académie des sciences, 1893.
[2] Ueber familiäre Endotheliome, Virch. Archiv, Band cliii.
[3] Berl. klin. Woch., 1905, vol. xlii.

of carcinoma is then merely a secondary condition following the goitre. He believes that there is as little cause to assume a microörganism as the cause of carcinoma of the thyroid of the brook trout as it is in the case of the endemic occurrence of goitre in man. From these different observations we might, in a tentative way, draw the conclusion that several factors co-operate in the causation of the endemic occurrence of cancer in animals. A further elucidation of those factors promises results of great importance for the understanding of the cause of malignant tumors in general.

Our observations recorded above are of interest in some other respects. In the first place, we find that among six mice affected with spontaneous tumors, and being sent by the same breeder, three mice had multiple tumors. One mouse had two, another mouse had three, and the third mouse nine at different parts of the body. Of these three mice, two had been kept longer in the laboratory than the others. It is quite possible that multiple tumors would have developed in some of the other mice, if they had been somewhat longer under observation. In one case at least it was distinctly possible to follow the development of a new tumor in a mouse previously affected with two other tumors. Picture No. 1 shows mouse 6 with three tumors. The one situated posteriorly was not present when the mouse was sent to Philadelphia. It seemed to develop quite suddenly and appeared at first to be in close proximity to the second of the three tumors.

These observations show: first, that we have here to deal with an endemic occurrence of so-called multiple tumors; secondly, that the structure of the tumors which were multiple (tumors in mice 3, 5, and 6) was not identical but very much alike, much more so than the structure of the tumors found in mice 1, 2, and 6, which differed in some respects from each other, and from the tumors of the other set of mice. The multiple tumors, which were present in different mice, were almost identical in structure in the same mouse. Some

minor differences were probably due to some accidental conditions such as hemorrhage. There exists, therefore, an endemic occurrence of multiple tumors. Whether this endemic occurrence of multiple tumors is due to the predisposition of the animals affected, or whether it is due to a special character

FIG. 1.—Mouse 6 with three tumors. The third tumor developed shortly before the death of the animal.

of the tumor, cannot be determined at present, but it is hoped that transplantation of these tumors may answer this question. Another question arises, however: In this case do we have actually to deal with multiple primary tumors or are those tumors secondary metastases of one primary tumor into

the subcutaneous tissue, or into the tissue adjoining the latter? It is certainly an unusual thing for carcinoma to cause the formation of multiple metastases rather at distant places in the skin than in the internal organs. Furthermore, we could not find any evidence of metastases in the lymph glands. In not a single instance was there any indication that one of these multiple subcutaneous tumors originated in a lymph gland. The lymph glands were in each

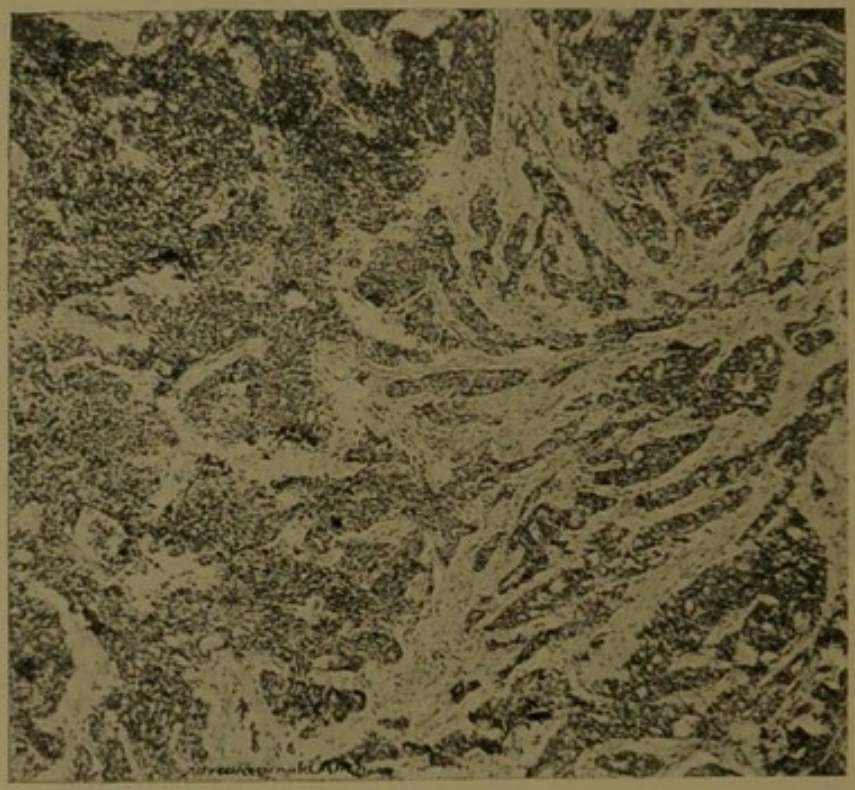

FIG. 2.—Section through tumor of first mouse. The tumor consists mostly of solid masses of cells. At some places an alveolar arrangement is visible.

case free from tumors. There were cells visible in the sinuses which were probably proliferated endothelial cells or mononuclear leukocytes. We would, therefore, have to assume that the metastases took place through the bloodvessels. It would, however, be hard to explain why the bloodvessels should carry tumor cells into other parts of the skin rather than into the internal organs, which is the ordinary situation of metastases in carcinoma.

The multiple subcutaneous tumors are scattered over very wide areas of the body, and they are found at such places where the existence of normal mammary-gland tissue can with great probability be excluded. We have, therefore, the following alternative: Either we have to deal with multiple metastases into the subcutaneous tissue, a very unusual occurrence for carcinoma if the dissemination by the lymphatics can be excluded, or we have to deal with a real multiplicity of primary tumors. In the latter case it becomes very unlikely

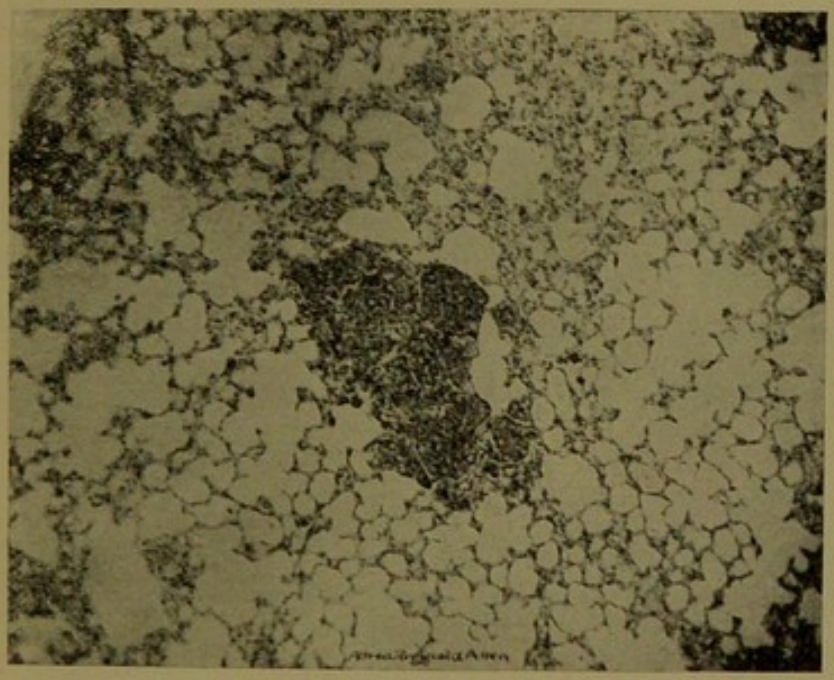

FIG. 3.—Metastasis in the lung of mouse 1.

that all tumors take their origin from the mammary glands. Such an origin would, for instance, be extremely unlikely in the case of the little tumor which was found directly at the side of the root of the tail, or in the case of the nodules found near the spinal column. Careful studies led especially Apolant to the conclusion that all subcutaneous adenocarcinomas of the white mouse take their origin in the mammary gland. The description of Apolant leaves no doubt as to the great similarity of these tumors in American and in European mice.

Macroscopic metastases into inner organs could not be seen in any of the mice, but on microscopic examination small metastatic nodules could be found in mice No. 1 and 4; two animals in which no multiplicity of tumors existed. The metastases in the lung consist of very small disseminated lobules which at first are almost free from connective tissue, the tumor cells growing into the alveoli. Later, the tumor nodule is invaded and surrounded by connective tissue. The way by which the lung is invaded cannot be clearly seen.

FIG. 4.—Section through a body resembling the epididymis, found in axilla of mouse 1.

Emboli of tumor cells are not visible in the pu'monary vessels. Haaland was able to demonstrate the presence of microscopic metastases in cases in which macroscopically no metastases were present. We could likewise confirm another observation of Haaland's. We found that in the lung of mouse No. 6, besides the epipleural nodules of lymphoid cells and of proliferated pleural endothelial cells, nodules were present which seemed to consist of proliferated alveolar epithelium. In a similar way in a mouse inoculated with the tumor of mouse No. 1 which died eleven days after inocula-

tion, a little nodule was found in the lung which consisted of proliferated epithelium of an adjoining bronchus or of the epithelium of the alveoli of the lung. Such tumor-like formations cannot be regarded as due to metastases of tumor cells; it will, however, be advisable to suspend judgment about their significance until further control examination of lungs of normal mice, and of the lungs inoculated with other than tumor material, have been made.

Our tumors infiltrated the surrounding muscle and fat tissue in a similar way as does carcinoma in man.

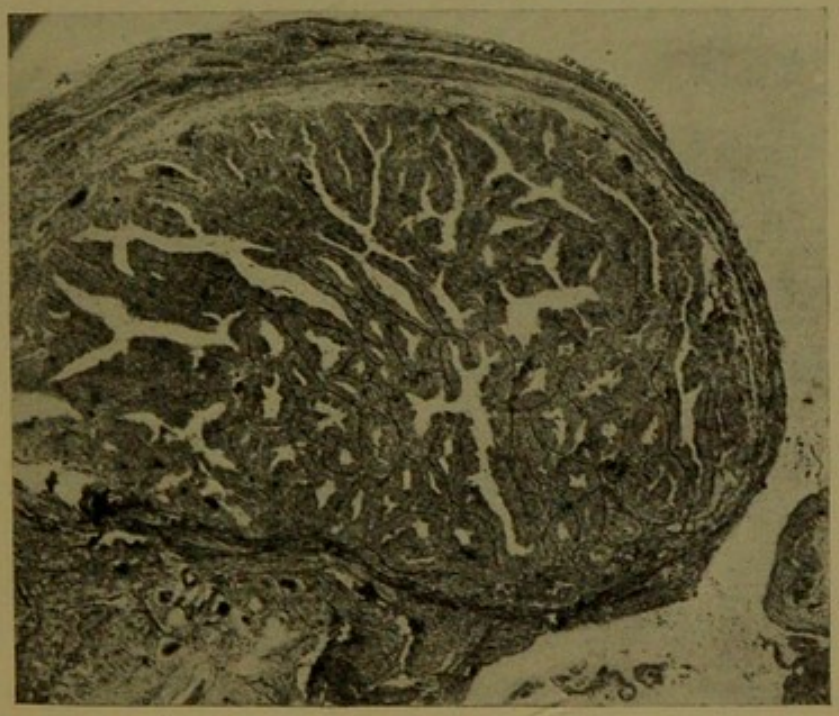

FIG. 5.—Section through adenocarcinoma of mouse 2. Papillomatous structure. No colloid material present in this part of the tumor.

Of especial interest is the fact that in the axilla of mouse No. 6 which had several spontaneous tumors, and which, like the other mice, was a female, we found a round body the structure of which closely resembled the epididymis. In this connection it may be stated that in two mice which we received from another breeder I found cysts situated at the neck of the animals. These cysts were lined by cuboidal epithelium and contained a fluid. One of these two cysts,

which was under observation during several months, enlarged gradually, until the cyst ruptured subcutaneously and its contents escaped. The wound closed and the cyst began to fill again. Transplantation of parts of the cyst wall into other mice did not lead to the formation of new cysts. The same

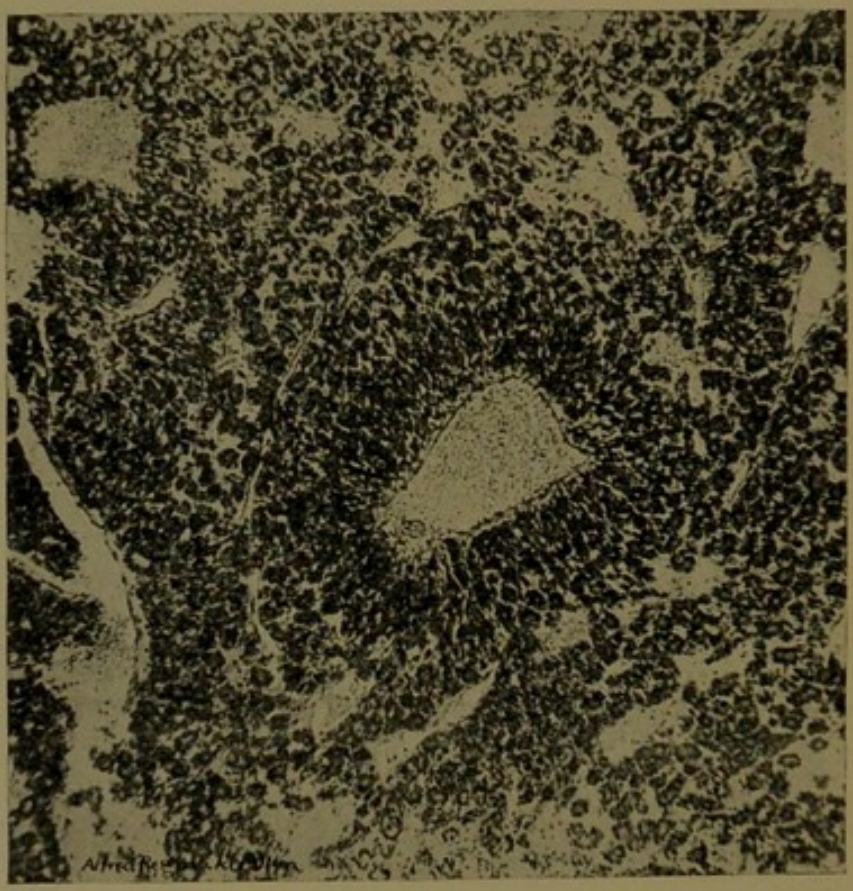

FIG. 6.—Section through tumor (adenomatous part of an adenocarcinoma) of mouse 4. Perithelioma-like arrangement of the alveoli. Blood capillaries are much dilated.

breeder who sent these two mice to the laboratory has supplied us with two mice which were affected with adenocarcinomas; in one animal the tumor was situated in the subcutaneous tissue of the neck, in the other it was attached to the thigh. One of these tumors was transplanted successfully. We see, therefore, that in mice which are either themselves afflicted with tumors or in mice bred at places where tumors are occasionally found among other mice, certain peculiarities

occur which, in part at least, may be due to congenital malformation.

Inoculations with the tumors found in four mice were carried out into a very large number of other white mice. A number of these animals died accidentally from exposure to cold. The transplanted pieces were examined microscopically. An active proliferation of inoculated tumor cells could not be seen on microscopic examination, with the exception of one case which shall be referred to shortly. Of all the mice which remained alive, only one developed a tumor. The one mouse in which, on microscopic examination, the development of a tumor was seen in the periphery of the inoculated piece was in the case of tumor mouse No. 3 which had been inoculated with material from the tumor mouse No. 1. This mouse died thirteen days after it had been inoculated. I was led to inoculate a mouse which carried a spontaneous tumor by the consideration that animals already affected with a spontaneous tumor might be a more favorable soil for the growth of an inoculated tumor of a similar character. An experiment done, approximately six years ago, in which I transplanted an adenoma of the mammary gland of a rat simultaneously into other rats, and into the rat affected with the tumor demonstrated the great difference between the fate of the transplanted piece in the rat affected with the tumor and in other rats. In the latter the piece very soon degenerated; in the former it was preserved and enlarged even during pregnancy. In this case the pregnancy of the animal may have been a complicating factor. Such a complication did not exist in the recent experiment. The fact that the mouse carrying the spontaneous tumor was successfully inoculated with a tumor of another mouse and that, on the other hand, among all the other inoculated mice only one single mouse developed a tumor, is hardly an accidental occurrence, but seems to demonstrate that there exists a certain relationship between the predisposition of the animal which favors the development of a primary tumor and that kind of predisposition which per-

mits transplanted tumors to grow. Experiments carried on with Dr. S. Leopold on a carcinoma of a mammary gland of a dog during the winter 1906–1907 showed equally well that the pieces of tumor transplanted into the animal originally affected with the tumor may remain alive, although pieces transplanted into other dogs became necrotic.

In my first papers on the experimental inoculation of tumors I carefully distinguished between these two sets of factors. The experiments recorded here suggest that after all there exists a connection between these two factors and that some of the conditions which permit transplanted tumors to develop are identical with or related to some of those conditions which contribute to the development of a primary tumor in an animal. This predisposition is, however, only one of the factors which participate in the production of a primary malignant tumor in an animal.

One more conclusion, as to the inoculability of tumors, may be drawn from these experiments, namely, that multiplicity of tumors does not necessarily correspond to a high degree of inoculability of such tumors into other animals. In a former paper[1] I pointed out another condition, namely, that a connection between the rapidity of growth of the primary tumor and the inoculabili y of tumors does not need to exist. Tumors which grow relatively slowly in the original mou e and in the first generation of inoculated mice may, nevertheless, yield 100 per cent. of successful inoculations, and rapidly-growing tumors, on the other hand, may only be inoculated with great difficulties. The inoculability of tumors depends, therefore, on a number of different conditions, and the energy of growth of the original tumor is merely one of the factors which are of importance. It is only by careful experimental analysis of the different factors which determine the growth of tumors that we hope to come nearer to a full understanding of the direct cause of tumors. We have to be satisfied to add one small

[1] Zeitschr. f. Krebsforschung, vol. v.

piece of evidence to another. The difficulty of obtaining sufficient material favorable to experimentation excludes, in many cases, the carrying on of as large a number of experiments as could be desired.

Some of the observations and conclusions may be summarized as follow :

1. The observations recorded here suggest that hereditary transmission of certain predisposing conditions from parents to offspring is one of the factors causing the so-called endemic occurrence of cancer among animals.

2. It is shown, experimentally, that various kinds of tumors remain better preserved when inoculated into the animal in which the tumor had developed originally, or into an animal affected with the same kind of tumor than when inoculated into other animals of the same species. In the latter they may become necrotic; in the former they remain alive or grow. This has been found in three sets of experiments in the rat, mouse, and dog. Further experiments must determine to what extent this rule generally holds good.

3. No parallelism need to exist between the tendency of tumors to become multiple and their inoculability into other mice. Originally slowly growing tumors may be easily inoculable. The energy of growth of the original tumor is only one of the factors which determines the degree of inoculability of a tumor.

4. An endemic occurrence of multiple tumors among mice is described. As many as nine tumors may be found under the skin in different parts of the same mouse. The tumors found in the same animal are almost identical. If these tumors represent primary multiple tumors and not metastases, the conclusion is inevitable that many of these tumors are not derived from the mammary glands. Metastases into lymphatic glands could never be found.

5. In confirmation of Haaland's observations, microscopic metastases can be found in the lung in a certain number of cases when macroscopically no metastases exist. Besides the

metastatic nodules, different nodules are occasionally formed in the lung through the proliferation of the alveolar or bronchial epithelium.

6. An unusual anomaly is described in a mouse affected with a tumor. A structure closely resembling the epididymis was found in the axilla of a female mouse. Other mice sent by a breeder, among whose stock tumors had been found, showed in each case the development of a large cyst in the neck. The possibility of a relationship of these anomalies to the formation of certain tumors in mice must be taken into consideration.