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STUDIES UPON A TRANSPLANTABLE RAT TUMOR.

Originally Regarded as a Sarcoma; Probably a Teratoma from Which an Adeno-carcinoma Developed.

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Plates I–XVI.

By far the greater number of studies of transplantable tumors have been made upon mice, in which species tumors not infrequently arise spontaneously. These tumors consist for the most part of the epithelial neoplasms which take their origin from the mammary glands, as was originally pointed out by Livingood, and present the histological appearances of adenoma and adeno-carcinoma, the dividing line between the two being indefinite. Other types of tumors have been found very rarely in mice. Livingood described a sebaceous adenoma; Ehrlich, a chondroma; Haaland and Tyzzer, lympho-sarcomata; Bashford, Murray and Haaland, epidermal cancer; and, more recently, Jobling has met with an instance of what appeared to be Hodgkins's disease, and two examples of papillary cystoma of the ovary. In several instances, namely, in the cases reported by Ehrlich and Apolant, Leo Loeb, Bashford and Liepman, adenomata have been described which through hypertrophy of the stroma and elimination of the epithelial elements have changed slowly or more quickly into spindle-cell sarcomata. Once the sarcomatous condition has become established, it has persisted through many transplantation generations.

The transplantable mouse tumors have been studied with great profit, and the high degree of success which has attended these studies and the considerable advance made in uncovering the biological conditions underlying tumor growth have resulted in large part from the happy circumstance that a fair percentage of spontaneous tumors of mice are transplantable into individuals of the same race

or species, and also from the facts of the small size, rapid maturation and brief life history of the mouse, which favor the making of observations on a large and sufficient scale to yield data of scientific value.

The number of tumors of rats, which have been studied in a manner similar to the tumors of mice, is small, in spite of the fact that rat tumors had been transplanted successfully before mouse tumors. Moreover, the variety of rat tumors thus far transplanted exceeds the variety of transplantable mouse tumors, for while nearly all the mouse tumors thus far described have been epithelial neoplasms derived from the mammary gland of the female mouse, far more sarcomata have been encountered in rats than epithelial tumors, and both have sprung from diverse parts of the body.

In 1889 Hanau transferred successfully an epidermal cancer of the rat through two generations. The only previously successful experiments had been made by Novinski and by Wehr. In 1876 Novinski implanted a cancer of the nose of a dog into two others, in one of which a lymphatic gland metastasis appeared; and in 1889 Wehr inoculated successfully the so-called carcinoma of the penis of the dog. As we now know, the latter tumor is not a cancer, but probably a round cell sarcoma.

The instances in which adenomatous tumors of the mammary gland of the rat of a transplantable nature have been recorded are very few, the chief one consisting of the instance reported by Michaelis and Lewin. It is noteworthy that this tumor lost, after a small number of passages, its adenomatous and took on a sarcomatous character. We wish to add another word to the subject of the discrepancy in the histological structure and place of origin of mouse and rat tumors. The records of the spontaneous tumors of animals are very imperfect and take account of little more than the transplantable examples. We know from our own experience that simple adenomatous and fibromatous tumors arise not so very infrequently from the mammary gland of the female rat, although they have not up to the present time been transplanted successfully. We have records of three such examples of benign fibro-adenomatous neoplasms, as well as of a lipoma of the subcutaneous tissues.

We have brought together in brief form the recorded instances

of transplantable rat tumors. As stated, the first report was by Hanau (1889), who observed three instances of epithelial cancer among some white rats raised in the laboratory. The cancer affected the skin of the external genitals. The transplanted tumor arose in the region of the vulva, and produced inguinal and axillary gland metastases. Portions of the latter were used for transplantation, and the secondary nodules showed the histological structure of the original tumor. In one animal in which the inoculation was into the scrotum, there were multiple peritoneal nodules, and masses within the diaphragm, and one mass in the posterior mediastinum.

In 1890 von Eiselsberg reported an instance of a spindle-cell sarcoma of a mixed gray and white rat, which arose in the periosteum of the scapula. It was transplanted successfully into the peritoneal cavity of another rat.

In 1892 Firket reported an example of spindle-cell sarcoma of the peritoneal cavity of a white rat. A series of successful transplantations was made, and the original histological structure of the tumor was always reproduced. An effort was made to implant the tumor upon guinea-pigs, but unsuccessfully.

In 1898 Velich transplanted a sub-periosteal spindle-cell sarcoma of the thigh of the white rat through several generations. One series of rats was inoculated into suppurating sinuses without result. Another was inoculated with blood from the field of incision of the tumor, also without result. In one rat the grafted tumor penetrated into the depth of the tissues, compressed the spinal cord, and produced motor paralysis. Two guinea-pigs were inoculated without result. Rats inoculated with fluid derived from the tumor, but free from visible particles, yielded no tumors. Suspension of tumor particles in salt solution before injection into the peritoneal cavity did not prevent tumor formation. From the intra-peritoneal masses subcutaneous tumors were developed in other rats. A second graft was successfully implanted in a rat in which a growing tumor already existed. Pieces of tumor kept sterile and outside the body for twenty-four hours and for three days before inoculation produced tumors, but after four days none developed. A progressive decrease in the energy of tumor growth from the first to the ninth generation was observed, and no further transplantations were

obtained after the ninth generation. An instance of spontaneous inoculation of the jaw of a rat was observed in an animal which gnawed the tumor of a rat kept in the same cage. By wounding a rat at the back of one of the canine teeth and allowing it to gnaw a tumor, a nodule was made to develop in the wound, and since the tumor gnawed was situated in the subcutaneous tissue of the same rat, the result is an illustration of the development of a second tumor in an animal possessing a growing tumor.

Leo Loeb in 1901 transplanted through many generations a cystic sarcoma of the thyroid gland of the white rat, which was inoculable under the skin and in the abdominal cavity and by means of the cystic fluid derived from the tumors. The fat of the implanted fragments was studied. Of the grafts many of the cells degenerated, but a number retained a normal appearance and mitoses were observed in the periphery as early as fifteen or twenty days after implantation. Of these some probably belonged to the tumor cells. In from five to eight days a capsule of connective tissue had surrounded the graft. The center of the graft at this time showed shrunken cells with pyknotic nuclei and blood vessels with desquamated epithelium, but not all the cells were necrotic. Mitoses were visible as late as the twelfth to the fifteenth day, at about which time the tumor cells invaded the capsule. In order to ascertain the demarcation between host and tumor cells the grafts were enclosed in gauze sacs, and in spite of suppuration about many of the sacs, a part of the grafts developed, but the result was not definitive. It was noted that when the graft became infected its growth was retarded. After arrest of growth, excision of a part sometimes led to renewal of development, and replantation of the excised part to another part of the body was sometimes followed by growth of the new graft. In some cases in which the excision failed to awaken growth in the original tumor the graft derived from it, implanted in another animal, yielded a tumor. Local metastases were produced, but systemic metastases did not develop even when the tumor invaded a blood vessel. The examples of contact inoculation were most pronounced from intra-peritoneal implantations, and the separate nodules even penetrated the diaphragm and projected into the pleural cavity. The histology remained fixed during fifteen months

of transplantation. Attempts to implant the tumor into guinea-pigs, hens and mice failed. In guinea-pigs, at first mitotic division of the tumor cells occurred, but the grafts did not increase in size and the emigration of leucocytes was more abundant than about the grafts in rats. The opinion is expressed that in the production of tumors by grafting the new tumor is derived from the cells of the graft and not from the cells of the host; and Loeb, therefore, views these tumor cells as resembling germ cells in their capacity for unlimited reproduction.

In 1902 Loeb described several more rat tumors. One was an adeno-carcinoma originating apparently in the pancreas and not transplantable. Another depended from the neck, originated in the thyroid gland and was divided unequally by a fibrous band into halves, one of which was an adeno-carcinoma and the other a cystic sarcoma. A single sarcomatous metastasis existed in the lung. The sarcoma was transplantable. A third rat showed an adenoma of the mammary gland. It was excised and fragments were implanted beneath the skin of the same animal and of other rats. A month later the fragment implanted in the original rat had increased to eight times its former size, when the animal gave birth to young, after which in a remarkably short time the transplanted tumor shrank in size. No growth took place in the other rats inoculated. A fourth tumor was a cystic sarcoma of the thyroid gland, which had produced local and inguinal lymphatic gland metastases. Portions of this tumor kept on ice for five days grew on transplantation, while other portions kept in the thermostat or at variable room temperature failed to grow. Loeb concluded, erroneously, as we now know, from these experiments, that two agencies are required to cause growth of the grafts—the tumor cells and a tumor-producing factor. The latter he believed did not lose power on exposure to temperatures of 2° to 4° C., while he believed it less certain that the sarcoma cells remain alive at those temperatures. He found that injections of tumor pulp suspended in salt solution and of filtrates through paper, asbestos and unglazed porcelain gave negative results. The tumor-producing factor was conceived to be rendered inactive by salt solution. Loeb considered that two factors were concerned in immunity to tumor grafts, one being a state of

resistance to the transplanted tumor cells and the other to the tumor-producing agency.

In 1903 Loeb described in a paper dealing with the endemic occurrence of tumors in animals still another example of cystic sarcoma of the thyroid gland of the rat.

Gaylord and Clowes in 1907 described three rats which developed tumors while being kept in cages previously employed by Loeb in his experiments with a transplantable sarcoma of the thyroid gland. In 1904 a rat was discovered showing a fibro-sarcoma of the abdominal wall and axillary region, and in 1905 two rats were found showing respectively fibro-sarcoma of the abdominal wall and spindle-cell sarcoma of the thyroid gland. The last tumor was transplantable.

In 1907 Jensen reported an instance of transplantable spindle-cell sarcoma of two gray-brown rats which had previously—and before the tumors developed—been inoculated with an acid-fast bacillus derived from a pseudotuberculous disease of cattle. Rat 1 showed nodules of varying size in the peritoneal cavity, and small nodules in the liver and lungs. The growth was infiltrative. Rat 2 showed small nodules in the lungs and none elsewhere. From Rat 1 inoculations were made into wild rats and rats obtained from Berlin, but without results, and into several brown and white rats of the laboratory stock, some of which developed the tumor. Later certain Berlin and London rats were inoculated with occasional success. Gray rats, however, were found refractory. The tumor from Rat 2 could also be transplanted to the laboratory stock, but not to the rats of foreign origin.[1]

In 1907 Michaelis and Lewin published their studies of a transplantable carcinoma of the rat which in later generations became converted into a spindle-cell sarcoma. The original tumor developed in the mammary gland. The first transplantations yielded about 50 per cent. of tumors and produced lung metastases. Some of the tumors underwent complete retrogression. It was noted that young rats were more subject to the implantations than old ones, and that the white, gray, mixed and black rats were all susceptible

[1] Professor Jensen kindly sent us living rats possessing tumors, which we have propagated successfully through many generations in American rats.

by subcutaneous and by intra-peritoneal inoculation. Even before the conversion of the tumor into sarcoma it had been noted that the microscopical characters of the growth varied considerably and sometimes presented appearances of pure adenoma; at other times, of alveolar and solid carcinoma; and at still other times, on account of the rich development of the stroma, of sarcoma. Moreover, there sometimes developed cystic spaces carrying papillary outgrowths, and the individual cells varied so greatly in respect to size, quality of protoplasm and nature of nucleus as to excite wonder that they could all arise from a single tumor. One tumor of the third generation is stated to have exhibited in places the appearances of typical cancroid. The overgrowth of stroma which eventually gave rise to a sarcoma began in the fifth generation and in the seventh generation the sarcoma had been perfected. By choosing the most rapidly growing strains, and by double inoculations at intervals of a few days, the number of successful implantations was made to reach 100 per cent. Rats with growing tumors could be successfully implanted with a second graft. Rats which had recovered spontaneously from growing tumors were resistant to the several types of this tumor, namely, the adenoma, epithelioma and sarcoma, and to Jensen's sarcoma. The percentage of spontaneous recoveries was about ten. The blood of the rat gave a considerable degree of immunity and a reciprocal immunity was produced between mice and rats by inoculating with the rat tumor, on the one hand, and mouse carcinoma, on the other.[2]

Apolant in 1908 reported very briefly upon twenty-four rat tumors which had been collected by Ehrlich's laboratory. They consisted of one fibro-lipoma, two fibromata, three adeno-fibromata, thirteen sarcomata, and five tumors which were designated mixed tumors of the "Flexner type."[3] He speaks also of two forms of rat sar-

[2] Dr. Lewin kindly sent us specimens of living rats with tumors of the sarcomatous variety, which we have propagated successfully in American rats through a number of generations.

[3] It is perhaps questionable, in view of the transformations through which the rat tumor we are describing passed, whether Apolant is correct in speaking of the tumor as a mixed one, and in giving it such a specific cognomen. If it is true that the tumor we are describing represents merely a type of mixed tumor, which is not uncommon in the rat, it would be quite significant, we think, par-

comata according to their microscopical structure. One of these presented the characters of an ordinary spindle-cell sarcoma, whereas the other possessed the characters of a round cell sarcoma with lymph-adenoid structure. The latter type was not homogeneous, but showed in some parts a cellular structure resembling that of the lymphatic glands, in which there was a reticulated tissue composed of spindle and branching cells, in the meshes of which lay the round cells, and in other parts a looser tissue composed essentially of the reticulated tissue. Moreover, in still other places the structure approached the type of the spindle-cell sarcoma. It is stated that this tumor possessed from the beginning a high degree of energy of proliferation, and yielded a high percentage of successful inoculations. In the course of the implantations of this tumor it exhibited more and more the character of a spindle-cell sarcoma, until finally it became a pure tumor of this type. The reverse process was observed in still another tumor, which, at the beginning, presented the usual characters of the spindle-cell sarcoma of rats, and gradually became transformed into a tumor presenting lymph-adenoid structure.

HISTORY OF THE TUMOR.

A full grown, white, male rat of the laboratory stock died spontaneously on January 5, 1906. The autopsy performed soon after death revealed a tumor the size of a walnut attached to the left seminal vesicle and projecting into the abdominal cavity. There were no adhesions to other viscera. The tumor was approximately spherical in shape and was covered with peritoneum. The surface was roughened by irregular granulations. The consistence was firm and the mass cut with difficulty. There were no visible metastases. The cut surface of the tumor was not uniform; it was in general dead white in color, but certain areas were more opaque and of a yellowish tinge.

ticularly in view of the manner in which the highly organized type of tumor has been developed in our example. On the other hand, we are inclined to believe that the facts to be presented concerning the structure of our tumor with reference to the original moiety of adeno-carcinomatous structure will suffice to put it in a place quite by itself.

A small piece of the tumor was cautiously removed, cut into bits with sterile scissors, suspended in sterile normal salt solution and injected through a needle with a large bore into the abdominal cavity and beneath the skin of three white rats, the histories of which will be given later.

The remainder of the tumor was preserved for histological examination. In the course of the studies of the tumor carried out during the past few years the original mass has been subjected to repeated microscopical examinations.

In our first publications[4] regarding this tumor we described it as a polymorphous sarcoma. As the following description will show, the original tumor cannot really be brought into harmony with the morphology of any of the established types of sarcoma. It would appear certain now that the original tumor should not have been regarded as a single type. In the course of transplantation it has passed through several forms of structure corresponding with sarcoma and simple and adeno-carcinoma, and has come to be established in the last form. When the carcinomatous forms made their appearance the changes in the structure of the tumor were so remarkable, and apparently so abrupt, that the origin of the new elements from the previously perceived ones in the original tumor seemed exceedingly doubtful. Hence the original mass, which had been preserved, was subjected to reëxamination by sections made from all parts of it. The result was the discovery of certain structures of an epithelial character that pointed to the existence of an adenomatous moiety in the original tumor. These epithelial elements consisted in part of glandular structures of a normal type, and in part of such atypical formations as are seen in adeno-carcinoma. Certain of these glandular bodies of normal appearance seemed indeed to be participating directly in the production of

[4] Flexner and Jobling, Infiltrating and Metastasizing Sarcoma of the Rat, *Jour. of the American Med. Assoc.*, 1907, xlviii, 420; Infiltrierendes und metastasenbildendes Sarcom der Ratte, *Cent. f. allg. Path.*, 1907, xviii, p. 257; Remarks on and Exhibition of Specimens of a Metastasizing Sarcoma of the Rat, *Proc. of the Soc. for Exper. Biol. and Med.*, 1906–07, iv, 12; On Secondary Transplantation of a Sarcoma of the Rat, *ibid.*, 44; On the Promoting Influence of Heated Tumor Emulsions on Tumor Growth, *ibid.*, 156; Restraint and Promotion of Tumor Growth, *idem*, 1907–08, v, 16; Metaplasia and Metastasis of a Rat Tumor, *ibid.*, 52; Further Notes on a Rat Tumor, *ibid.*, 90.

the atypical formations indicative of carcinoma, thus indicating possibly the origin of the carcinomatous elements from several distinct foci.

It now appeared, therefore, as if the original growth within the seminal vesicle first consisted of a morphologically simple tumor made up of one kind of tissue, that by invasion of the inner or epithelial wall of the vesicle and by inclusion of certain epithelial structures became, as it were, infected with these epithelial cells, which upon removal from their normal relations and control assumed malignant properties. According to this view the epithelial moiety was inconspicuously present in the part of the tumor that was originally transplanted, and it gradually divested itself in the course of the transplantation of the prevailing simple tissue until it became itself dominant.

It may be questioned whether the part of the tumor originally described did not possess a greater complexity of structure than could properly be attributed to a so-called polymorphous sarcoma. Indeed, it was pointed out originally that the relation of cells and connective tissue within the original tumor was such as to permit its being described as imperfectly alveolar. The sections exhibited a connective tissue framework, varying in density, containing spindle cells and supporting other cells far richer in protoplasm. The latter cells occupied spaces of variable size and form, so that sometimes a single cell and sometimes several cells were thus enclosed. This relation of large cells to stroma was particularly well shown in sections stained by Mallory's aniline blue and fuchsin method.

The complexity of the original mass does not end here, for the inclusions at one point were such as to arouse the feeling that the tumor was, in fact, originally a teratoma or embryoma, and the subsequent transformations, first into a tumor resembling polymorphous sarcoma, and next into an adeno-carcinoma, represented changes of secondary nature.

HISTOLOGY OF THE ORIGINAL TUMOR.

Under the low power of the microscope the sections showed considerable diversity. Tissue taken from the free part of the mass, away from its attachment to the seminal vesicle, showed irregular

bands and whorls of cells. Two principal types of cells were distinguishable: (*a*) polymorphous cells rich in protoplasm, with single vesicular nuclei or with multiple or large polymorphous nuclei; and (*b*) elongated and spindle-shaped cells possessing solid nuclei and closely associated with the connective tissue framework. The arrangement was such that the cells rich in protoplasm lay within areas composed of spindle cells and connective tissue fibrils. Blood vessels were fairly numerous and polymorphonuclear leucocytes richly infiltrated the tissue. Under the medium power of the microscope the distinctions in the two types of cells became more evident. The large polymorphous cells made up usually by far the larger part of the tissue, and often were so fused together as to suggest syncitial formation. In addition, there were present a number of multinucleated giant cells and cells with lobed gigantic nuclei distinct from the fused small cells. Areas existed containing a preponderance of the spindle cells, but they never occurred strictly independently of the larger cells. On the other hand, the spindle cells and fibrillar connective tissue tended to increase and diminish together. The collections of polynuclear leucocytes were larger in the degenerated foci, but they were not limited to the larger areas of degeneration. The quantity of connective tissue in the tumor was likely to be underestimated from sections stained in hematoxylin and eosin (Plate I, Fig. 1). The sections stained by the Mallory method showed the interstitial tissue to be much more abundant than was suspected, and the polymorphous cells to be surrounded by it.

Sections prepared from those parts of the original mass which were attached to the seminal vesicle exhibited besides what has been described still other structures. In the first place, portions of the wall and epithelial membrane of normal appearance were included. Moreover, there occurred glandular structures which exhibited evidences of proliferation. These later inclusions were of tubular nature, although the lumen of the tube was likely to be filled with projecting outgrowths of the walls. These formations tended to be surrounded by structureless proper membranes, but sometimes the latter were imperfect, so that groups of epithelial cells passed from the tubule into the adjacent tissue. Where this proliferation was going on the tubule had lost its regular and characteristic struc-

ture, and there developed small alveoli, consisting of polymorphous cells surrounded by spindle-cells and connective tissue, such as composed the mass of the tumor (Plate I, Fig. 2). In another part large tubules lay in hyalin connective tissue, which separated them from the usual tumor tissue, so that the formation of definite alveoli enclosing epithelial cells derived from the tubule was readily made out (Plate I, Fig. 3).

There was also discovered still another example of carcinomatous formation of striking and unmistakable character, which originated close to the epithelial wall of the vesicle. Sections through a part of the original growth, at a point showing to the naked eye small cavities and under the microscope cystic dilations of the inner glandular cavities of the vesicle, exhibited also irregular spaces lined with cubical epithelium and containing lumena in which there were desquamated cells and polynuclear leucocytes. These carcinomatous acini partially surrounded the cyst-like spaces which were included in the thickened fibrous wall of the vesicle supporting the tumor (Plate II, Fig. 4). These carcinomatous acini occurred also among the tumor cells proper, as well as in the fibrous stroma. In certain places spindle cells and fibrillated connective tissue encroached on the acini, compressing them and bringing about degeneration of the cubical cells, and the acini had in places been transformed into solid alveoli and thus made to resemble the cell groups shown in Fig. 1. Sections stained by Mallory's method showed the stroma to be abundant and to bear that relation to the acini and alveoli that is seen in a glandular organ.

We come now to a structure discovered within the mass, the explanation of which is impossible excepting on the basis of a developmental error. This structure consisted of a large space or tube, the walls of which were more or less corrugated and branching and covered with a layer several cells deep of high epithelium. Owing to the branching nature of the corrugations a certain number of elongated glandular tubular cavities appeared in the sections to be contained within the wall. The supporting connective tissue was richly infiltrated with small round cells. Adjacent to the large tube there lay a typical compound serous or mucous gland, and outside that structure a certain amount of smooth muscle existed (Plate II, Fig. 5).

The considerable variety of epithelial formations present in the tumor led us to examine microscopically the internal and external genital organs, and the seminal vesicles especially, of a number of white rats. Although there was some lack of agreement between the glandular structures contained in the tumor and those occurring normally in the vesicle, yet many of them appeared to be identical. A closer identification was, on the other hand, not possible with any other of the glandular parts of the internal genital organs. We failed entirely to identify the large tube with its associated serous or mucous gland with any structure which we discovered in the sections of the normal genital organs. It must, therefore, be concluded to have been an accidental and erroneous embryonic inclusion.

The foregoing description of the histological characters of the tumor indicates that it was not a simple but a complex formation, in which all the parts were not equal. This inequality was shown first in the body of the tumor, in which the polymorphous and spindle cells were united in a heterogeneous fashion, but it was shown even before in those parts in which the carcinomatous alveoli had developed (Plate III, Fig. 6). This absence of homogeneousness has an especial significance as regards the understanding of the interesting series of transformations through which the tumor passed in the course of transplantation through several thousand rats in the past three years.

It is not possible to establish the original tumor as a simple outgrowth from the seminal vesicle, as it was at first believed to be. As has been detailed, the more searching study of the original mass revealed epithelial structures differing materially from those of the seminal vesicles. The question, therefore, arises whether the tumor should not be regarded as a teratoma. If it is to be so regarded, then it follows that a part only of the epithelial structures contained within the tumor had been derived from the seminal vesicle, the other parts and perhaps the chief part of the epithelial inclusions having been derived from structures which under normal conditions would go to form adjacent epithelial organs.

It is also impossible to establish the precise origin of the cancerous transformation which occurred in parts of the tumor, and it cannot be stated with assurance that the transformation took place at many

points, rather than at one point. The obvious interpretation to be put upon the appearances described in connection with those sections from which Figs. 2, 3 and 4 have been prepared is to the effect that they represent points at and structures with which this transformation is proceeding. We rather tend to the opinion that the condition represented in Fig. 2 speaks for an active transformation of the glandular tubules into carcinoma, and yet the appearances are not wholly conclusive, since the reverse process, namely, that of cancerous invasion of the tubules from without, can not be excluded.

TRANSPLANTATION GENERATIONS.

In order that the vagaries of the tumor may be followed, and its properties established, some account of different transplantation generations will be given. To make the account as brief as possible, merely characteristic examples will be chosen. The first transplantation generation turned out luckily, since owing to inexperience only three rats were inoculated. Small morsels cut with scissors were suspended in salt solution and injected into the peritoneal cavity and beneath the skin of three adult white rats on January 6, 1906. The first of these was killed on February 8, and no tumor was found. The two remaining rats developed intraperitoneal but no subcutaneous tumors. Rat 2 was killed on March 19, at which time a nodule the size of a small bean was attached to the abdominal parietes. Rat 3 died on June 26, nearly six months after inoculation. There was no emaciation, but the skin over the abdomen was tense and the abdominal cavity contained a tumor the size of a walnut, attached to the abdominal wall and to the great omentum. The tumor resembled the original in its gross appearances, although the central portion was necrotic but firm. Two smaller pea-sized nodules also occurred in the omentum; each kidney contained several metastases up to three millimeters in size; the lungs were studded with nodules ranging from a millet seed to a bean in size; a large nodule at the inferior border of the lungs united them to the mediastinum and diaphragm and extended into the pericardium; the inferior part of the pericardial sac was thickened into a cup-shaped depression for the apex of the heart;

the left ventricle was invaded from the pericardium and contained a nodule the size of a pea extending into the ventricle, while the visceral epicardium was generally thickened and white. On section the lungs were found to contain disseminated nodules, and the costal pleura contained several nodules.

It is interesting that Rat 3, which succumbed to the tumor developing within the peritoneal cavity, should have shown extensive metastases, while the original animal, which also showed an intraperitoneal growth, was not the subject of metastasis. Microscopical examination of the partially developed nodule from Rat 2 showed that the growth was proceeding from the periphery, while the center was already necrotic. The clearest picture illustrating the mode of growth was found at the point at which the abdominal muscle was being invaded. The cells of the outermost layer were small and round and resembled the usual small cell infiltration observed at the growing edge of malignant tumors in human beings. Mingled with them were oval epithelioid cells derived from the muscle and the interstitial connective tissue, and a moderate number of polynuclear leucocytes. Adjacent to this layer was a layer of capillary vessels separated from one another by oval and elongated cells and a fine fibrillar tissue. This one, which was very cellular, passed over into the fibrous layer, which was more cellular at the periphery than at the center. This fibrous strand was not strictly homogeneous, but included islands of large cells corresponding with the polymorphous cells, rich in protoplasm, of the original tumor. In the central fibrous tissue small islands of these cells were undergoing necrosis. There were present a certain number of multinucleated masses of protoplasm, constituting giant cells, or resembling syncytium. Sections stained by Mallory's method brought out the fact that almost everywhere in the tumor the connective tissue was so arranged as to form definite spaces enclosing oval and polyhedral cells with large vesicular nuclei. The tumor was, therefore, not a homogeneous structure anywhere, but was made up of two elements, connective tissue and cells independent of but enclosed in it.

The microscopical appearance of the tumor nodules in Rat 3 will be described. The large omental nodule showed a cortex merely of

living tissue, and center consisting of hyalin and fibrous tissue. The most peripheral zone was the most cellular, but also contained much fibrous tissue. The cells consisted chiefly of the large polymorphous cells, with vesicular nuclei occurring singly and in groups. The center, which was hyalin and fibrous, still showed areas of the polymorphous cells. These cells and the cells forming the capillaries and the stroma were quite distinct. The smaller omental nodules represented developments within the adipose tissue. The most recent invasion was at the periphery, which consisted of areas of the polymorphous cells embedded in hyalin connective tissue. These nodules were inactive and the centers, already hyalin, enclosed atrophying cells of the large type.

The lung nodules might or might not involve the pleura. When the intrapulmonary nodules were small they surrounded the medium-sized blood vessels and consisted of the large cells occupying the perivascular lymph spaces, from which they spread into the alveoli. Apparently the alveolar epithelium was lost and the spaces were entirely filled from the larger tumor cells. Unless the pleura was invaded there was no increase in the connective tissue, but invasion of the pleura at once led to a new growth of connective tissue, which together with the tumor alveoli quickly underwent hyalin and other forms of necrosis. However, in the hyalin tissue a certain number of columns of the large tumor cells persisted. Usually the lower border of the tumor in the pulmonary parenchyma preserved its integrity. In addition to the usual cells, multinucleated giant cells occurred in the pulmonary nodules. Sections carried through the large nodule at the hilum of the lung from which the pericardium was involved showed appearances similar to those of the large intraperitoneal mass, but in addition there were present a remarkable number of mono- and multinucleated giant cells, some of which were of the megacaryocytic type (Plate III, Fig. 7). These cells often included mononuclear and polynuclear leucocytes. Some of the massive giant cells included scores of smaller cells, probably leucocytes, in process of fragmentation. The giant cells were irregularly distributed. The great blood vessels were surrounded by tumor, but were patent. An intermediate stage was present in this part of the lungs, between the

early condition in which the alveoli were occupied by tumor cells, and the later one, in which the tumor had become hyalin and fibrous. This intermediate stage was characterized by a thickening, through spindle cells and fibrillated tissue, of the air vesicles, which contained mononuclear cells of the type of the tumor cells, suggesting when viewed under the low power of the microscope an alveolated tumor of the epithelial type. Mitotic figures in the large cells were frequent. The nodules in the parietal pleura were invading the intercostal muscles on the one side and projecting into the pleural cavity on the other. They lacked the organoid characters described and were composed of spindle cells coursing longitudinally and transversely, and of small round cells next to the muscle, and were invaded with polynuclear leucocytes.

The masses in the kidneys, the largest of which measured six by four millimeters, developed in the cortex. These nodules, some of which were wedge-shaped, were produced by an invasion of the tubules of the kidney and of the perivascular connective tissue with the large polyform tumor cells, which had taken the place of the proper epithelium of the tubules. Among them a few multinucleated giant cells were present. The glomeruli persisted much longer than the tubular structures and the intertubular connective tissue was increased by a layer of fine connective tissue fibrils and spindle cells. Polymorphonuclear leucocytes were distributed throughout, and accumulated most numerously in minute foci of necrosis. When the nodules projected beyond the capsule they consisted chiefly of connective tissue containing a certain number of large cells occupying definite spaces suggestive of lymphatics. When these lines of cells were longitudinally distributed, they sometimes extended across the field of the microscope, and when they were cut obliquely or transversely, they resembled alveoli.

The nodule in the ventricular wall consisted of a solid tumor composed of a fibrillated stroma, spindle cells and capillary vessels, in which the large tumor cells were embedded in an irregular manner. There was absence of alveolation, and the tumor cells proper formed small groups or occurred singly, and were closely surrounded by the stroma. Giant cells were also numerous. At the extending edge of the nodule a zone of small round cells

appeared, from which capillaries penetrated the muscle fibers. These fibers were separated and compressed by spindle and small round cells, a formation disclosing the invasion of the tumor cells proper, which passed between the muscle fibers in parallel lines. The cells that appeared to be the most recent consisted of multinucleated masses of protoplasm. What was taken to be the coronary sinus had been invaded by the large cells, which were unprovided with a stroma. At one point the lumen was bridged by the tumor cells and at other places a fibrinous thrombus had developed, into which the tumor cells were pushing (Plate IV, Fig. 8). The general layer of epithelium was thickened and infiltrated with a mixture of the large tumor cells and spindle cells, and was excessively vascularized. It is patent that this transplantable tumor possessed a considerable degree of invasive power, while it exhibited, on the other hand, relatively slow growth. Death was caused apparently not by interfering with the nutrition of the animal, but rather by mechanical causes, chiefly probably connected with the extensive lesions of the lungs.

The structure of the tumor, as revealed by the first transplantation nodules, was sufficiently varied to make classification very difficult. The tumor was not, apparently, homogeneous in nature, as are the usual sarcomata, but rather of heterogeneous composition, such as is found among the epithelial tumors. The point should be emphasized that all the metastases discovered were such as could best be produced through the general blood current. There was no evidence of involvement of the lymphatic glands anywhere, but that the lymphatic vessels of the viscera could be invaded was indicated by the conditions observed in the lungs and the capsule of the kidney. Direct infiltration of the serous membranes through implantation occurred in the peritoneal cavity and in the epicardium.

In the course of the different generations considerable variations in histological structure and metastatic properties, which it is not necessary to describe in detail, were presented by the tumor. On the other hand, it is desirable to present the important facts regarding the peculiar behavior of the tumor. The generations represent a continuous series, while different strains within a generation, expressed by letters of the alphabet, do not represent a uniform

series, but indicate merely the different sets of inoculations, usually from different nodules, within a generation. A given generation will, therefore, contain one or more strains according to the circumstances governing the transplantations. These facts are of some importance, in view of the circumstance that the tumors were often histologically dissimilar within a given generation, as will be shown.

ABSTRACT OF PROTOCOLS OF THE RATS WITH TUMORS.

In this section there will be presented a selected series of protocols, briefly abstracted in order to bring out the varying and changeable properties of the tumor.

Of the second generation, strain *a*, Rat 7 survived four months and presented a large ulcerating subcutaneous mass and pulmonary and diaphragmatic metastases. Rat 8, inoculated at the same time as 7, developed a subcutaneous tumor which ulcerated later, as a result of which it survived a month longer and showed only pulmonary metastases. Rat 6, of the same series, survived two weeks longer than Rat 7, and showed, besides the subcutaneous nodule, kidney metastases.

Rat 64 of the fourth generation (*a*) survived five and one-half months. The tumor which first developed was partially excised for inoculation purposes, but it recurred and eventually caused death. It invaded the muscles and peritoneum and projected into the abdominal cavity posteriorly. Metastases were present in the sternum, cartilage of the ribs, diaphragm, left thoracic wall to a marked degree, right thoracic wall to a small degree, the anterior mediastinum, the lungs and left kidney. The slower progress of the tumor was the result of the excision, since other rats of this series not operated upon succumbed within three to four months of the inoculation. Other rats showed lung metastases and one, Rat 66, a metastasis in the auricular appendage. Rat 47 is of special interest in this connection. It was inoculated into the peritoneal cavity with a single fragment of tumor and survived about four months. The main tumor, which developed from the fragment, was situated in the omentum, but secondary nodules were present elsewhere in the abdominal cavity (Plate XIII, Photograph 1). One of these, measuring 1 by 2 centimeters, was attached to the ensiform cartilage, which proved to be a point of predilection for the development of intra-peritoneal nodules. A second point of predilection, as it afterwards proved to be, was the space between the liver and the diaphragm, next to the cardiac extremity of the stomach. In this instance the stomach had been invaded by the tumor, which had passed to the mucous membrane and led to a partial digestion of the membrane and production of a cup-shaped depression. A single pin-point metastasis existed in the lungs. Rat 46 was also inoculated into the peritoneal cavity. It survived about three months. The original fragment developed in the pelvis to a tumor measuring 3 by 2 by 1½ centimeters, which had invaded the muscle of the abdominal wall. A series of

secondary nodules developed within the omentum and extended upwards to the transverse colon and in the gastro-hepatic omentum, reaching the diaphragm and binding it to the lesser curvature of the stomach. The cardia of the stomach was invaded over its entire extent at the diaphragm by the tumor, which passed into the cavity and replaced the mucous membrane, which became altered to form a cup-shaped depression (Plate XIV, Photograph 2). Separate small nodules appeared over the peritoneal covering of the stomach, and the lungs showed a number of small metastases. Rat 61 survived a subcutaneous inoculation about three months. The superficial tumor which developed at the lower border of the thoracic wall measured 3 by 4 centimeters. It penetrated the muscles and projected into the abdominal cavity, pushing the retro-peritoneal tissue before it. As a result the right kidney and adrenal gland were forced forwards, the kidney was extensively invaded with tumor, the right adrenal gland was converted into tumor, and a mass of tumor extended in the gastro-hepatic omentum towards the lesser curvature of the stomach, over which it spread and into which it penetrated. An elevated boss-like nodule appeared in the mucous membrane and was connected with the mass in the wall; while on the opposite (left) side a larger ulcerated area existed, probably representing an implantation tumor upon the mucous membrane through contact (Plate V, Fig. 9). Rat 67 survived five months and was inoculated on two separate occasions. The first tumor developed in the subcutaneous tissues and ulcerated. The second fragment was introduced into the lumbar muscles, developed there and extended upward, invading the sternum and ribs. The left pleural cavity was quite filled with a mass which compressed the left lung entirely. At the same time it grew downwards, displacing the diaphragm inferiorly, and upwards to the apex of the thoracic cavity, pushing through the ribs and intercostal muscle and came to lie beneath the superficial fascia. The pericardium and mediastinum were overgrown and the right pleura, which was invaded, led to the binding of the lung to the ribs. Rat 51 survived an intra-abdominal inoculation about four months. A main mass 5 by 5½ centimeters developed and secondary small nodules were produced. The mesentery was invaded, thickened, and caused to retract, so as to bring the small intestine into a close small coil. The right kidney was surrounded by a mantel of tumor; the diaphragm, and from it the pleuræ, were invaded, and ascites existed. The ascitic fluid measured 25 centimeters and contained masses of tumor cells.

As will be observed, in the course of the fourth generation, in which a considerable number of rats was inoculated, a considerable variety of effects were produced. What is particularly striking is the invasiveness exhibited by the tumor and its tendency to develop frequently in the region of the diaphragm, adjacent to the right lobe of the liver and the stomach, and to avoid invading the former and choosing regularly to invade the latter. In no instance during this generation was a lymphatic gland metastasis observed.

In the fifth generation some interesting examples were observed. Rat 325 (*g*) was inoculated into the muscles of the back and survived 2½ months. The tumor which developed measured 2 by 2 centimeters. It invaded the ribs and spinal column in the dorsal region, produced paraplegia, and, projecting into the thoracic and abdominal cavities, displaced forward the liver and kidney. The

lungs and mediastinum contained nodules. Rat 327 was killed after three months, at which time there existed besides the subcutaneous nodule and the lung metastases a mass in the region of the axillary gland which measured 0.4 by 0.4 centimeter. It should be remarked that this was the first example of apparently a lymphatic gland metastasis. Rat 276 survived 2½ months. A subcutaneous nodule of the right side had penetrated the abdominal cavity, pushed the kidney and adrenal gland forward, displaced the spinal column to the right, and given rise to a secondary nodule compressing the ureter, which above the nodule was dilated to the size of a goose quill, and was associated with a hydronephrosis in which the kidney was enlarged to double its normal size. There were other nodules in the omentum and serous covering of the spleen and liver, in the mediastinum, and between the heart and the lungs. Rat 196 (*d*) survived three months. The original ulcerated subcutaneous nodule involved the skin and abdominal muscles and penetrated the lower ribs into the abdominal cavity. In the subcutaneous tissue above the main tumor was a separate smaller nodule connected with the original tumor by a white cord. The second nodule was in turn connected by a similar white cord with a third nodule, situated in the axillary space and representing probably the much enlarged axillary glands. The white cords were taken to be lymphatic vessels invaded by tumor. Rat 197 showed, as the result of a second implantation, tumor invasion of the inguinal lymphatic gland on the side of the inoculation, and other metastatic nodules. Rat 230 exhibited invasion of the ribs, penetration of the thoracic cavity and the spinal canal, associated with paraplegia. The infra-clavicular lymphatic glands on both sides were invaded. The superior lobes of the lungs were studded with nodules, the caudal lobe was the seat of a diffuse infiltration, a lymphatic gland above the diaphragm was enlarged by tumor to the size of a pea, and the kidney contained a nodule 2½ centimeters in size. Rat 231 showed a similar condition to the last, and in addition, a retro-peritoneal and bronchial gland, as well as the left axillary gland, contained metastases (Plate XIV, Photograph 3; Plate XV, Photograph 4). Rat 93 (*a*) survived 3½ months. A secondary graft developing subcutaneously involved the axillary gland, which became enlarged to a size of 1 by 2 centimeters and from which a growth surrounded the clavicle, penetrated into the pleural cavity, and encircled the great vessels of the heart. The lungs presented nodules, but the heart did not. Rat 88 survived an intra-muscular inoculation two months. The main tumor forced its way into the thorax on the left side, pressed against the vertebral column, which was displaced anteriorly and to the right. The ribs corresponding to the main portion of the tumor had disappeared, the lower half of the left pleural cavity was obliterated by the mass, which invaded the diaphragm, penetrated into the abdominal cavity, grew into the cardia of the stomach, the superior pole of the kidney and the adjacent adrenal gland and involved the lymphatic glands at the superior surface of the liver. Rat 418 (*h*) developed a subcutaneous tumor which ulcerated and led to axillary and inguinal lymphatic gland metastases. In the inguinal region a chain of enlarged glands or nodules extended from the margin of the tumor to the angle of the leg. Pulmonary and mediastinal metastases occurred. Rat 424 (*i*) was inoculated intra-peritoneally with ascitic fluid from Rat 51. Multiple intra-abdominal nodules de-

veloped in the omentum at the ensiform cartilage and between the spleen and the left kidney. A chain of nodules extended from the pelvis to the stomach, the wall of which at the expanded esophageal part was invaded and the mucosa ulcerated (Plate XV, Photograph 5). Rat 425 developed an intra-abdominal tumor growing about the stomach, spleen, left kidney and adrenal gland. A nodule had passed completely through the spleen, and the left adrenal gland was lost in the growth. Rat 426 exhibited two large and many small intra-abdominal tumors. The inoculation was made with ascitic fluid in the manner of Rat 424. The left kidney was replaced by tumor, except for a thin shell of renal tissue surrounding a large cyst.

In the fifth generation, therefore, the tumor began to produce lymphatic gland metastases, and at the same time continued to be deeply invasive of tissues in its locality, to be disseminated by implantation, and to produce, as before, abundant distant metastases. In the succeeding generations the properties of the tumor as now developed did not undergo any striking alteration. A few notes may be made, therefore, with reference to certain points which appeared from time to time. For example, it was noted in the seventh generation that seven additional examples of gastric invasion occurred. The invasion always took place at the dilated esophageal extremity, and from the tumor growing between the liver and the diaphragm. The kidneys were not infrequently the seat of metastasis. Sometimes one kidney would be quite converted into tumor (Rat 597, seventh generation), another example of compression of the ureter, succeeded by hydronephrosis, was noted in the tenth generation. The pulmonary metastases were much the most common of all. As a rule, when lung nodules existed, the mediastinum was invaded and usually the parietal pleura and the intercostal muscles became infected. Sometimes an entire lung was invaded from the hilum (Rat 1775, tenth generation), and compression of a branch of the pulmonary artery by tumor gave rise to infarction of a lobe of the lung (Rat 401, seventh generation). The myocardium was invaded in several additional instances. A mass extended from the lungs into the wall of the heart (Rat 553, sixth generation). A metastatic nodule appeared in the left ventricle (Rat 401, seventh generation) and implantation nodules occurred in both auricles and in the left ventricle (Rat 2392, ninth generation). The lymphatic metastases continued to appear. They were usually in the axillary and inguinal glands, and arose from subcutaneous inoculation. However, other superficial and even the deeper glands were sometimes involved. The retro-peritoneal glands (Photographs 3 and 4) were several times involved, and once a chain of these glands was affected, along with the axillary gland (Rat 1648, eleventh generation). A pelvic lymphatic gland was once involved, but afterwards suppurated (Rat 3035, fourteenth generation). The bronchial lymphatic glands, together with the axillary glands (Rat 1860, tenth generation) and once the bronchial, trachæal and clavicular glands were invaded jointly (Rat 1617, eleventh generation). The inguinal, axillary and mesenteric glands were once jointly invaded (Rat 2048, tenth generation). In one instance, a tumor nodule pressing upon the common bile duct produced jaundice (Rat 1338, ninth generation). In two instances the wall of the thoracic duct showed small nodules (Rats 1753, tenth, and Rat 2273, eleventh generation).

So much has been stated of the tendency of the large tumor masses to set up metastases that it remains to be added that the tumor could grow to a considerable size, produce marked local destruction of tissue, without at the same time yielding any discoverable metastases.

The object of presenting such a considerable number of protocols is to bring out the fact of the malignancy of the tumor under discussion, and the great variety of effects which it produced according to the circumstances of its development. The tumor was found to recur after what appeared to be complete surgical extirpation, to grow through the skin, muscle, fascia and bone, and to penetrate into the circulation, thus setting up secondary or metastatic foci at a distance. It should be noted that until the fifth generation there had been no instance of lymphatic gland metastasis, and during that generation and in subsequent generations this form of local metastasis became common. As will be observed a little later, the acquisition of the property enabling the tumor to invade the lymphatics was associated with a significant histological alteration in its structure. The growth within the abdominal cavity tended to become multiple, even when they arose from a single inoculated fragment, a result to be expected in view of the conditions in the peritoneal cavity which favor the secondary implantation of tumor cells. It is, however, noteworthy that these implantations were so common between the diaphragm and the stomach, which structures tended themselves always to be invaded. Of all the intra-abdominal organs, the stomach was most frequently penetrated by the tumor, which tended to appear in the mucous membrane and to set up ulceration quite as tumors do in man which originate in the mucous membrane. It is further remarkable that the gastric nodules always developed in the esophageal segment of the stomach, and that the mucosa was subject to infection directly through contact with tumor on the opposite side of the viscus. It is worth considering, therefore, whether the tumor can be implanted on the normal mucosa. We have abundant evidence to show that rats cannot be infected by feeding upon the tumor. It was repeatedly observed that when fragments of the tumor were inoculated into the muscles they tended to develop towards the interior, and when implanted beneath the skin, towards the surface of the body.

Cartilage and bone offered no impediment to the growth of the tumor. The deposit by preference of the tumor emboli in certain organs, such as the lungs and kidneys, is a distinctive feature of the tumor, as is the tendency of the lung nodules to lead to infection of the mediastinal tissues and the walls of the thorax, the latter apparently through contact. Metastases were never developed in any of the internal organs or lymphatic glands except through blood infections, although they might be produced by direct extension from neighboring parts. It is probable that the nodules in the heart muscle were all the result of ingrowth from the lungs, and it is remarkable that with the frequency of the pulmonary metastases the bronchial lymphatic glands were so rarely affected. The superficial lymphatic glands were much oftener affected than the deep ones. Small and medium-sized and ulcerated tumors, in which only a thin membranous remnant of tissue remained, might produce many metastases, and large, well developed tumors might produce no metastases. It should be noted that in one instance a nodule compressed the common bile duct and led to jaundice; in two instances small nodules were observed in the thoracic duct.

HISTOLOGY OF THE LATER TRANSPLANTATIONS.

It is not our intention, in considering the histology of the transplantation tumors, to describe exhaustively the successive generations. The effort will be made, however, to present the facts of the histology in such a manner as to bring out the significant and fundamental histological changes through which the tumor has passed, and with which have been correlated certain properties of metastasis, and to make clear minor fluctuations in structure that are being slowly eliminated. In another section there will be discussed the manner of proliferation of the tumor fragments, so that this point can be passed over now.

The second and third generations of the tumor showed no remarkable variations of the structures already described (see page 19). A variation was found according as the tumor was proliferating or had come to a temporary standstill. According to one or the other, two quite independent types could be distinguished:

(1) a proliferating tissue, producing stroma and columns of epithelial-like cells (Plate V, Fig. 10; Plate VI, Fig. 11; low and high power); (2) a stationary tissue composed of capillaries and spindle cells and few epithelioid cells (Plate VI, Fig. 12). The two types were grossly so different that had it not been for their common origin and certain resemblances between the epithelial cells they could not be considered as related. What should be perhaps emphasized is the fact that in retrogressing hyalin fibroid tissue associated with the stationary nodules the most persistent cells were certain epithelioid elements occupying compressed spaces in the hyalin mass (Plate VII, Fig. 13).

In the fourth generation (*a*) the appearances were various. An omental nodule in Rat 45 was composed wholly of elongated, closely packed cells, without arrangement into alveoli, and the nodule was quite solid except where islands of adipose tissue remained free of tumor. In Rat 49 the pancreas had been invaded and the tumor had produced obliteration of the splenic vessels, causing necrosis of the spleen. In respect to histology, this tumor was imperfectly alveolated or fibrous. The growth in the pancreas was highly fibrous and included isolated tubules resembling ducts, lined with superficial epithelium, doubtless the remains of the excretory ducts of the gland. The walls of the obliterated splenic artery and vein, which contained a hyalin and leucocytic thrombus, showed inflammation and the passage of the large tumor cells from without into the lumina (Plate VII, Fig. 14). In other sections the splenic artery showed an obliterating endarteritis of high degree, the spleen, a wedge-shaped growth of tumor about the vessels, and the liver, a superficial invasion attended with necrosis of adjacent hepatic lobules. In Rat 50 the small intrapulmonary nodules consisted of the air vesicles, in which the normal lining had been substituted by the large tumor cells. The pleural nodules were larger and the epithelial masses within them exhibited an acinous arrangement. When the pleural mass projected beyond the membrane the number of living tumor cells was small and it was composed chiefly of hyalin fibrous tissue, or a homogeneous tissue into which the large pale degenerating tumor cells with large hypochromatic nuclei were being fused. The portions of the nodule immediately within the

pleural membrane and extending superficially into the lung were composed of large, irregular alveoli containing epithelioid cells, often enclosing clefts suggesting lumena. Some of these alveoli had become dilated into small cysts enclosing cell detritus. The large epithelioid cells were dividing rapidly by mitosis, while no mitoses were seen in the stroma. The tumor, which was extending into the parietal pleura and the intercostal muscles, preserved its alveolar character, although the amount of stroma being produced was highly excessive. The manner of gastric invasion was characteristic (Plate VIII, Fig. 15). In the first place the growth was limited to the dilated esophageal segment. The section viewed from the inner surface of the organ showed a cup-shaped depression over the central and part of the peripheral portion of the growth, which rested on necrotic tissue devoid of nuclei. The mucous membrane was persistent at each side of this crater. The main or central mass of tumor was highly fibrous and resembled in structure a cicatrizing, scirrhous carcinoma in man. This dense fibrous portion still showed a small number relatively of compressed areas of cells, originally the large epithelioid cell. A sharp but iregular line of demarcation composed of fragmented leucocytes and bacteria marked the limits of living and dead tissue. The extreme edges of the tumified mass showed more tumor tissue proper, less fibrous tissue, and some remains of the smooth muscular wall of the organ. Where the mucous membrane was still preserved, the epithelioid tumor cells occupied spaces between the connective tissue fibrils that formed the submucosa and penetrated the papillary layer. As soon as the papillary layer was invaded, two kinds of pathological changes took place in the mucosa. The first affected the inner or superficial face of the epithelium; the continuity of the horny layer was disturbed and collections of fluid in vesicles formed. These vesicles penetrated only a short distance into the substance of the interpapillary epithelial processes, but they projected for a much greater distance into the cavity of the organ. The contents of the vesicles were homogeneous and contained leucocytes and fragments of keratinized epithelium. The epithelial layer below was much disturbed. The second change consisted of a deepening of the interpapillary processes, which also became narrowed and penetrated

more deeply into the tumor-infiltrated sub-mucosa. Moreover, they had divided irregularly into digits. The epithelial cells composing them had become paler and richer in protoplasm and were actively dividing by mitosis. As the edge of the crater was approached the dilatation was most marked and the horny layer imperfectly formed and rapidly disintegrating. In other words, the epithelial layer of the esophagus, at the point of beginning invasion of the tumor, was caused to undergo atypical proliferation. Rat 61, which also showed invasion of the gastric wall, was interesting in virtue of the fact that it brought out the difference in vulnerability of the esophageal and glandular portions of the organ. The growth was mainly confined in the muscular wall and the peritoneal coat, both of which were lost in the mass. The mucous membrane of the esophageal segment was invaded and ulcerated, the invasion ceasing a fraction of a millimeter before reaching the glandular segment. At the point of cessation in the esophageal territory the muscularis mucosæ formed the boundary line, although the tumor cells had penetrated between it and the muscular tunic, which had now become widely separated. In the normal organ the demarcation between the two anatomical parts—the esophageal and glandular portions of the organ—is distinct and indicated by a finger-like projection of sub-mucous connective tissue and smooth muscle fibers, as well as by the change in epithelium. The squamous epithelium ends on the glandular side of the projection, although the esophageal layer has lost its papillary character and become thinner before it disappears. The limits of the tumor were shown also on the outer surface of the stomach, since the peritoneal and muscular tunics, corresponding with the glandular part of the organ, showed no invasion, and the omental adhesions were limited wholly to the mass in the esophageal region.

The kidney nodules were developed chiefly in the cortex, the proper struture of which was replaced by the tumor cells. The latter invaded the tubules and took the place of the normal epithelium, from which they were distinguished by their greater density of protoplasm and greater richness in chromatin of the nuclei. The tumor cells formed a lining similar to the old, but sometimes a double one. The inter-tubular tissue was increased; it had become homo-

geneous and rarely showed the presence of the large tumor cells, but the compression and obliteration of the tubules, the epithelium of which was replaced by tumor cells, through the new connective tissue growth, sometimes produced appearances resembling an extratubular invasion. A certain amount of glomerular obliteration had taken place through hyalin thrombosis. The central mass of the tumor, except small islands immediately surrounding blood vessels, was entirely necrotic, and the walls of the vessels had been invaded and the endothelium had sometimes been displaced inwards by the tumor cells, although the lumena were still patent.

The subcutaneous tumors became attached very late to the skin, because of the great resistance to invasion exercised by the corium (Fig. 10). Examples show that the growth in the corium tends not to be continuous, but to be initiated apparently by large epithelioid cells, which are carried to a distance apparently in the tissue spaces, where they proliferate and start the production of a young stroma. The stroma of the tumor proper is sometimes more subject to degeneration than the epithelioid cells. An example of this condition was presented by Rat 53, in which the stroma was necrotic and freely invaded with leucocytes undergoing fragmentation, while the epithelial strands retained their form and appearance, except for the fact that the protoplasm was more strongly basophilic than usual. The numerous blood vessels were dilated and many contained hyalin or agglutinative red corpuscular thrombi.

From the description of the foregoing examples it would appear that the tumor had acquired in the fourth generation the unmistakable characters of a carcinoma, yet in point of fact the carcinomatous type was not really established in this generation, since the tendency for the epithelioid cells to be in close relationship with the stroma had not been entirely lost. Rat 57 illustrates this point.

The tumor when about one centimeter in diameter was in part excised for purposes of transplantation. The cortical zone, which was employed, showed under the microscope elongated bundles of highly cellular connective tissue enclosing small groups of epithelioid cells. The young tissue consisted of spindle cells which were particularly massed at the periphery, while the central part was more fibrous or even hyalin. While the epithelioid and connective tissue elements

were intermingled at the periphery, the former were well defined in the fibrous stroma. At the death of the rat, a month later, the recurrent nodule proved to be highly fibrous, and showed epithelioid cells in rows and islands in process of compression and atrophy.

We shall now consider a few examples of the fifth generation, which, as will be recalled, was characterized especially by the appearance for the first time of lymphatic gland metastases. It was in this generation also that several examples of compression of the spinal cord occurred. An example of the latter condition will now be described.

Rat 87 (*a*) had been inoculated into the muscles of the back, and the growth had extended toward and invaded the spinal column. Viewed under the microscope the growth was composed in its preserved parts of the large epithelioid cells forming solid alveoli which had penetrated all the soft parts, substituted itself for them and surrounded the vertebral column and ribs (Plate VIII, Fig. 16). The spinal nerves at their emergence from the canal were invaded. The large tumor cells insinuated themselves between the muscle fibers, which were caused to atrophy, and between the nerve sheaths. The bony parts of the vertebræ were not destroyed, excepting the spinous processes, in which no bone remained. The tumor passed to the spinal canal and reached the dura mater, but the spinal cord was not compressed. It is probable that the paralysis was produced through involvement of the spinal nerves roots. Rat 198 (*d*) showed multiple pulmonary nodules, in which the tubular or acinous arrangement of the epithelial cells was well marked. The nodules were often as large as a pea and replaced the pulmonary tissue. The stroma was finely fibrillated and did not conform to the original architecture of the lung. The pleural nodules were particularly interesting. While they possessed a denser and more abundant stroma, they were covered on the free surface with a layer of columnar epithelioid cells proliferating rapidly by mitosis, which on dipping down into clefts and depressions of the tissue gave rise to typical acinous tubules, as viewed in sections. A subcutaneous nodule in the same animal showed similar epithelioid cells proliferating rapidly and forming linear solid strands and not acinous tubules. There was also in this animal an ingrowth of tumor into the vertebral column, which invaded the dura mater but did not infiltrate the spinal cord. The roots of the spinal nerves and the inter-vertebral ganglia were surrounded. This tumor mass showed interesting variations of type. The older and more superficial parts were in a state of coagulative necrosis. Immediately next to them was a mass of solid anastomosing strands of cells pushing into and replacing the muscle fibers, which showed all stages of atrophy. The stroma here developed was highly fibrous. The large tumor cells surrounding the spinal nerves developed in the perineurium and produced a heavy collar of cells about the nerves, so that the main trunks of the nerves were completely degenerated. The type of tumor cells developed within the fibrous stroma and leading into the spinal canal was smaller than elsewhere; the nuclei were darker and the arrange-

ment was radial about the irregularly anastomosing spaces containing the cell masses, so that they were made to resemble in a remarkable way the normal cells lining the narrow spaces. Where the cells could grow more freely in the muscles and fascia beside the vertebral column, the alveoli became more regularly rounded or branching. Here and there a central excavation had taken place among the cells, producing a kind of acinous arrangement, to which the term pseudo-tubular might be applied (Plate IX, Fig. 17). At the outer termination of the muscles, where the growth might be considered as having been free to extend, the cells were large and pale, and were rapidly multiplying by mitosis; they were disposed into columns and made to resemble the form of growth described in the pleural metastases. Rat 411 (*h*) showed some interesting features. There was an extensive tumor growth at the root of the lung involving the great vessels of certain lobes. The vessels were thrombosed and the corresponding lobes of the lungs were necrotic. The tumor passed at times into the interior of the vessels, so that epithelioid cells came to lie between the inner wall and the thrombus in a narrow slit or space, giving rise to tubules containing large multi-nucleated cells. Elsewhere in the lungs there were numerous tumor nodules developing about the bronchi and blood vessels, which penetrated their walls but did not enter the lumena. The tumor cells in these cases might come to lie just between the endothelium of the vessels and the epithelium of the bronchi. In these the alveolar formation was solid. The kidney nodule showed replacement of the parenchyma of the organ by the tumor, the epithelioid cells of which occupied the pre-formed tubules, but the stroma was newly formed.

We shall consider now the examples of lymphatic gland involvement. As stated, the tendency of the tumor at this time was to exhibit the structure of carcinoma. The particular type presented was that of simple carcinoma, in which the alveoli showed great diversity of form, but contained solid masses of epithelioid cells. In the course of the fifth generation, however, this simple form began to show modifications, tending to the acinous arrangement, and although the two types, the solid and acinous, of alveoli were fluctuant, yet the former became more and more common in this and subsequent generations, until it became dominant. We shall pass to the seventh generation before taking up the consideration of the nature of the lymphatic gland metastases.

Rat 404 (*d*) showed, besides the growth in the regional lymphatic glands, metastases in the lungs. The subcutaneous nodule had ulcerated and left a mere capsule of fibrous tissue containing strands of epithelioid cells. It showed no acini. The lungs showed both disseminated miliary and larger nodules, and one lung was quite completely converted into tumor. The main blood vessels leading to this lung were surrounded by tumor and thrombosed, the thrombi being in process of organization. The bronchi had been either surrounded or

invaded by tumor. The pleura also contained nodules. The lungs were partly bound to the chest wall by the tumor growth, and the areolar tissue under the heart and lungs was invaded, but the growth, which extended to the lymph glands in this areolar tissue, had not invaded them. The type of the tumor was the simple alveolar. On the other hand, the lymphatic glands in the region of the superficial ulcerated mass had been invaded, some of them almost entirely replaced by the tumor. The epithelioid cells were pale, some of them presented hypochromatic nuclei, and they formed solid alveoli (Plate IX, Fig. 18). The succeeding animals frequently showed a similar replacement of the regional lymphatic glands, either with solid alveoli or a branching type of the tumor, and in rare instances the tumor passed through the lymphatic glands into the periglandular adipose tissue.

Rat 729 (*d*) of the eighth generation showed the manner in which the lymphatic glands sometimes become infected. The local tumor, of fibrous nature and containing only a few of the epithelioid cells in small groups, approached the gland and became fused with the capsule, after which blunt papillary-like processes, consisting of large epithelioid cells, pushed into the superficial lymphatic tissues, which was the starting point of the invasion.

We return for a moment to Rat 401 (*d*) of the seventh generation, because of a nodule contained within the auricle of the heart and penetrating into the cavity of the auricular appendage. The muscle cells in the region of the tumor were either undergoing atrophy or had entirely disappeared. The tumor itself was an alveolar growth of solid type, possessing a rich fibrous stroma. The ingrowth into the muscle of the heart was accomplished through buds of multinucleated protoplasm, which, enlarging, produced the strands and oval alveoli. The cavity of the appendage contained a fibrinous clot surrounded by hyalin muscle fibres, into which the tumor had penetrated and produced a tubular formation.

At about this time the tumor showed now and then a much softer structure than it had previously done, which can best be illustrated by one or two examples. Rat 808 (*d*) of the eighth generation possessed a tumor the size and shape of an almond, that was composed of cell masses made up of large pale epithelioid cells arranged either in solid alveoli or in tubules, showing lumina. The cortical portion was made up of living cells, while the central part, except for the tissue immediately about the blood vessels, was the seat of a soft necrosis, quite different from the hyalin necrosis usually observed. In this necrosis, which resembled an anæmic infarction, the cell forms were still present, but distorted and discolored and

mingled with fragmented nuclei. This particular form of the tumor became progressively more common, and led to the typical adenoma that constituted the final form in which the tumor occurred. In different examples there was some difference in arrangement of epithelioid cells and in relation of cells and stroma, but the properties were generally preserved. What was particularly impressive in this type of the tumor were the frequent large mitoses in the epithelioid cells.

Rat 743 (*d*) was another example of this type of tumor, which was even more perfectly adenomatous. The mass was several centimeters long, oval in shape, and possessed a fibrous capsule. The cortical layer, still living, was about two millimeters in thickness, while the central part was necrotic and soft, but still showed small islands of living tissue about the capillary and larger blood vessels. There was little or no stroma, and the bulk of the growth consisted of epithelioid cells, proliferating very rapidly and, therefore, showing numerous mitoses. The individual cells were ill-defined, but multinucleated bands and rows of cells had been produced. At the outermost edge of the tumor the epithelioid cells were narrow and often linear, but further inward they were wider, formed oblong masses and frequently showed a peripheral circle about a lumen. There was much curving and bending of the epithelioid cells, so that acini of a variety of shapes were produced. In the islands of tissue left preserved about the vessels in the interior, a tubular, acinous arrangement nearly always was present (Plate X, Fig. 19).

To illustrate the fluctuations which were still going on at this period, Rat 1280 (*i*) of the ninth generation will be used as an example. Besides a nodule in a lymphatic gland, which perforated the capsule and grew into the surrounding areolar tissue, the original nodule was composed of simple alveoli containing pale cells and showing numerous mitoses. The amount of degeneration was not excessive, and no tubules or acini were produced. Two other points may be mentioned: one of the large superficial nerves was surrounded (Plate X, Fig. 20), and a number of mammary gland ducts were included in the growing tumor. This latter condition was by no means uncommon when the implantations were made in the region of the mammary glands of female rats. Frequently

these ducts persisted in the tumor and showed little change. At other times their proper membranes were destroyed and the ducts themselves disintegrated. Rat 2402 (*o*) of the ninth generation showed a lymphatic metastasis consisting of simple alveoli and containing cysts. From the wall of one such cyst an outgrowth of papillomatous character had taken place (Plate XI, Fig. 21). Rat 2668 (*q*) showed a combination of anastomosing branching cell-strands and small solid alveoli filled with pale cells and showing many mitoses. There were lung nodules composed of small solid alveoli of more irregular size than the air vesicles, indicating that they had been newly formed. A new formation of stroma had taken place, and irregular giant cells of the megacaryocytic type were also present (Fig. 7). A considerable rarity was the occurrence of a metastasis in the wall of a vein; and another, a growth in the lumen of a lymphatic vessel (Plate XI, Fig. 22).

Rat 2805 (*l*) of the tenth generation showed a local lesion of the ordinary alveolar type and a growth into the kidney extending from the cortex to the pelvis and invading the renal vein. The tubules had as usual been invaded and obliterated before the glomeruli. The tumor passed to the pelvis and came to lie beneath the pelvic epithelium, which was slowly replaced, but before the replacement was completed the epithelial membrane of the pelvis had undergone a considerable hypertrophy, through which it was increased several times in thickness. Ultimately it became necrotic. The growth of the tumor into the connective tissue of the pelvis caused it to surround the arteries and veins. The former escaped invasion and occlusion. One of the latter was invaded and became thrombosed. Rat 1648 (*a*) of the eleventh generation was interesting as having shown for the first time a lymphatic gland metastasis of the typical adenomatous type, although there were still combined with it solid alveoli, while Rat 2253 (*c*) showed the superficial tumor to be of the soft, pure adenomatous or acinous type. (Plate XI, Fig. 23, Plate XII, Fig. 24, Plate XVI, Photograph 6.)

The succeeding generations were made up of tumors presenting chiefly the adenomatous form, but now and again there still appeared tumors of firmer consistence, containing solid alveoli and fibrous stroma, so that up to the present time the typical adenomatous type

has not become firmly established. Thus Rat 3762 of the twenty-first generation (*d*) showed a nodule the size of a pea in the subcutaneous tissue, composed of small acini which were located chiefly centrally, and small, solid, elongated and oval alveoli making up the larger part and contained within a fibrous stroma. All the cells were pale and undergoing rapid mitosis. The stroma was remarkable for its vascularity. At the extending edge the reticulated tissue contained many small round and spindle cells. Rat 3855 of the twenty-third generation (*c*) presented a subcutaneous nodule, the central part of which was of typical adenomatous, and the cortical part of fibrous and small alveolar structure. This tumor also contained innumerable mitoses. The adjacent striped muscle was being invaded by solid cylinders of the pale cells, which were breaking up into fragments the muscle fibers included within the alveoli or acini. There was no remarkable increase of muscle nuclei, but the morsels of muscular tissue were surrounded by epithelioid cells and were undergoing digestion, although they were not included, properly speaking, within cells. In a similar manner Rat 3983 of the twenty-sixth generation (*a*) showed a local tumor made up of acini and solid alveoli, which was invading the voluntary muscle. The cells composing the tumor at the point of junction with the muscle exhibited a diffuse alveolated growth in which the distinction between epithelioid cells and stroma was indistinctly marked. On the other hand, the muscle was undergoing the same form of fragmentation and solution as has been just described. Rat 3901 of the twenty-fourth generation (*c*) contained a subcutaneous nodule, which, while considerably degenerated, was an excellent example of a branching or papillary adenomatous tumor. The stroma consisted of a delicate fibrous tissue arranged in folds and projections, showing secondary branching, which were everywhere covered with a layer of cells, one or more in thickness, of the epithelioid type, and sometimes of columnar form. At the periphery of the nodule next to the capsule, the branching was less obvious, while acini of the typical form were present. Specimens of this tumor stained by Mallory's phosphotungstic acid method showed extremely well the differentiation between stroma and large cells, and brought out strikingly the branching character of the large papilliferous alveoli. Rat 3917 of

the twenty-fourth generation (*d*) presented another example of the branching papillomatous tumor. In this instance the stroma formed merely a small part of the mass of the tumor, so that by far the greater part was composed of epithelioid cells. It was towards the center of the mass, where the degeneration was advanced, that the papillomatous character was especially pronounced, while in the periphery the branching was more confined, and large solid alveoli, as well as smaller acini, were produced. Towards the center the islands of epithelium were preserved, in the midst of a soft degeneration, but these preserved cells in all cases surrounded the small vessels, the lumina of which were open. Everywhere in the tumor, and even in the islands mentioned, mitosis was going on most actively. Rat 3925, twenty-fourth generation (*e*), was an example of a subcutaneous tumor much degenerated, showing both simple acini and simple solid alveoli, and transitions of one to the other. Rat 3970, twenty-fifth generation, showed a subcutaneous nodule composed partly of acini and partly of solid alveoli, both containing pale cells. The lung of this rat contained a large nodule. This was necrotic with the exception of the extending edge, which passed without sharp demarcation into the adjacent congested lung tissue. The extension was made by means of blunt, solid outgrowths of the main tumor mass into the adjacent air vesicles, and there was an abundant increase in the interstitial tissue of the lung. The diffuse character of the proliferation made it somewhat difficult to determine the tumor nature of the nodule. Rat 4080 of the twenty-eighth generation (*c*) showed a circumscribed pulmonary metastasis composed of pale cells occupying the pulmonary alveoli and completely filling them. There were local areas of degeneration just beginning, with emigration of leucocytes and fragmentation of nuclei, but there was absence of typical acinous arrangement.

INTRA-TESTICULAR INOCULATION AND RETROGRESSION.

In the course of the inoculations several attempts were made to produce intra-testicular growths, with the idea of causing a general lymphatic dissemination of the peritoneum through that means. In all the experiments except one the result was a circumscribed necrosis

of the testicle, with more or less new formation of fibrous tissue, but no tumor growth. In one experiment tumor nodules were produced. This was in the eighth generation. The plan was to make emulsions of the tumor in salt solution and to inject them into the testicle. In the successful experiment two rats developed tumor nodules the size of small shot, just beneath the tunica of the testis and penetrating between the tubules. It was only in the periphery of the nodule that preserved, multiplying tumor cells, lying in a fibrous stroma, were observed. The centers even of these small nodules were necrotic, and consisted partly of degenerated tubular elements. There was no dissemination of the tumors.

Attention has repeatedly been drawn to examples of tumors which showed an unusual amount of fibrous tissue. Fibrous tissue is produced in two ways: (1) It is constantly produced at the edge of the growing tumors, in the course of the formation of the stroma and the capsule. It, however, is also produced in the interior, as a result of degeneration of the epithelioid elements. In growing tumors the central fibrosis comes to be quickly associated with coagulative necrosis of the tumor, which affects first the remains of the epithelioid cells and next the fibrous tissue. Much of this fibrous tissue is hyalin in structure, so that its true character may be more or less masked. (2) The second set of conditions producing fibrous transformation of the tumor is the one which we wish especially to refer to in this place. It occurs in the course of the retrogression of the tumors, and consequently is found in small as well as in larger nodules. In these cases the fibrosis tends to be unassociated with the coagulative necrosis. The fibrillation of the tissue also appears to be preserved and the epithelioid elements to be contained in contracted, narrow, often linear spaces within the tissue. The number of these cells is greatly reduced, being smallest in the central parts where the fibrosis is oldest. By compression they are eventually entirely destroyed through atrophy, in the course of which the nuclei become contracted and pyknotic, and the protoplasm, which takes on a deeper eosin-staining than it does normally, is reduced to a mere rim. It is the peripheral portion which contains the least fibrous tissue and the most epithelioid cells, but there is a striking absence of mitosis. Briefly expressed, retrogression is associated

with an increased fibrosis, a reduced mitosis, and a gradual and ultimately complete shrinkage of the tumor. The removal is not, apparently, through the action of leucocytes. At least, there does not go on any gross destruction by means of these cells. In the end the fibrous tissue is as completely removed as the epithelioid elements, and nothing remains in the local tissues to show the presence, at a previous time, of the tumor.

THE ELEMENTS FROM WHICH THE TUMORS ARE DERIVED.

In order to determine roughly the elements upon which successful implantation of the tumor depended, several methods were employed, the results of which can be summarized briefly.

Emulsions of the tumor prepared in salt solution were filtered through unglazed porcelain, and the filtrate injected into the abdominal cavity and beneath the skin of the rat. In no case did a tumor develop.

Emulsions of the tumor in salt solution were filtered through sterile filter paper and injected into rats in the same manner, but in no case did a tumor develop.

Emulsions of the tumor in salt solution were passed merely through sterile gauze, and the fluid, carrying minute fragments of the tumor, was injected in the same manner into other rats, but never gave rise to tumors. On the other hand, it has been proven that similar small fragments of the tumor, when not injured by the medium in which they are suspended, can give rise to the production of tumors. This has been shown by the successful results of the inoculations of ascitic fluid containing microscopic masses of tumor cells (see page 21).

With a view of excluding, or, indeed, of confirming the presence of microscopic organisms in the tumor, sections of young growing tumors were stained with all the usual aniline dyes in the various ways employed to show bacteria or protozoa, were impregnated with silver nitrate according to Levaditi's method of staining spirochætæ, and were examined in the fresh condition with the ordinary high powers of the microscope and with the special apparatus for dark field illumination, but nothing was ever seen that was suggestive of any other structure than the proper cellular elements of the tumor.

Aerobic and anaerobic cultures were made from young undegenerated and older degenerated tumors, but they never yielded any growth of bacteria.

THE ACTION OF PHOTO-DYNAMIC SUBSTANCES ON THE TUMOR.

Experiments showed that when bits of the tumor of some size were kept immersed for a brief period in physiological salt solution, tumors could still be reared from them, but they also showed that the minute fragments which would pass through the meshes of ordinary gauze were no longer able to yield tumors when exposed to the action of this solution. The failure in the latter experiments was attributed to the poisonous effects of the sodium chloride; and it was proved that Ringer's solution, in which these poisonous effects are balanced by the use of the calcium, was less injurious to the tumor than salt solution alone. A better, because less injurious, fluid for suspending the tumor proved to be rat serum heated to 54° C., but in making the transplantations the fragments of tumor which were introduced were not permitted to come into contact with any extraneous fluid whatever. In this way the best results were obtained.

An effort was made to influence the growth, persistence and transplantability of the tumor through the action of photodynamic agents. We believed at one time that we had secured such an action by means of eosin and Bengal red, but later study of the action of these elements led us to the conclusion that the early results which we had obtained and supposed to be due to the action of the photodynamic anilines was attributable to other causes. We shall give a table illustrating the irregularity of the results of transplantation of a single specimen of the tumor, illustrating particularly the great variation in the number of retrogressions taking place in a series. The method was to expose minute fragments of the tumor to the action of the various solutions given in the table. It was intended that the exposure should be one hour, but the large number of animals to be inoculated required that the exposure extend from one to three hours, since a part of the inoculations were not completed until the expiration of that time. However, the light exposures were all limited to one hour.

	No.	Tumors, Per Cent.	Retrogressions, Per Cent.
Controls	10	10 or 100	3 or 30
NaCl : light	10	10 or 100	3 or 30
NaCl : dark	10	10 or 100	4 or 40
NaCl eosin 1–1000 : light	10	9 or 90	3 or 33
NaCl eosin 1–1000 : dark	10	9 or 90	8 or 88
NaCl Bengal 1–1000 : light	10	10 or 100	10 or 100
NaCl Bengal 1–1000 : dark	9	8 or 88	1 or 12.5
Ringer's : light	10	7 or 70	6 or 85.7
Ringer's : dark	10	9 or 90	2 or 22
Ringer's eosin 1–1000 : light	10	10 or 100	3 or 30
Ringer's eosin 1–1000 : dark	9	9 or 100	3 or 33
Ringer's Bengal 1–1000 : light	9	9 or 100	2 or 22
Ringer's Bengal 1–1000 : dark	10	9 or 90	2 or 22
Serum : light	10	10 or 100	2 or 20
Serum : dark	7	7 or 100	2 or 28
Serum eosin 1–1000 : light	10	10 or 100	5 or 50
Serum eosin 1–1000 : dark	10	10 or 100	4 or 40
Serum Bengal 1–1000 : light	10	9 or 90	1 or 11
Serum Bengal 1–1000 : dark	10	8 or 80	5 or 62

An explanation of the table brings out the fact that the tumor under the influence of the anilines tends to give a larger number of retrogressions than in the absence of the anilines, and also that exposure to sunlight for one hour is possibly to some small extent injurious, but no general deductions can be made from the experiment, and we publish this final table in order to set ourselves right on this point.

MODE OF DEVELOPMENT OF THE TRANSPLANTED TUMOR.

Leo Loeb, and later Bashford, and since then others, have made careful observations on the manner of development of the transplanted fragments of tumors. We have ourselves studied the manner in which the fragments of this tumor undergo development in a new animal. Indeed, we have studied the changes in the transplanted fragments which occur not only in the rat but also in the mouse and the guinea-pig. We owe to Ehrlich the important observation that a tumor fragment introduced into a species from which it was not developed, as from a mouse into a rat, would undergo a limited development in the new species, but would never attain a considerable size or become organically attached to the individual of the new species, or be transplantable from one to another of this new species. On the other hand, such living fragments can be

transferred successfully at some periods from the new to the original species, and then be transplanted further.

We have found in common with Loeb and Bashford that the greater part of the tissue transplanted undergoes necrosis, and that the new tumor is produced from a small number of surviving cells. Our observations were made with the usual fragments transplanted by us and also with a somewhat finer emulsion (made by comminuting the living cortical parts of the tumor by means of a Haaland grinder) deposited beneath the skin in the region of the axilla. A series of rats, mice and guinea-pigs were inoculated on the same day with fragments and emulsion from one source. They were chloroformed at intervals of twenty-four hours; the implanted tumor with the surrounding tissue was removed, preserved and hardened in Zenker's fluid, and sections stained in hematoxylin, eosin and iron-hematoxylin were prepared.

FRAGMENTS IN THE RAT.

The fragments of tissue in the emulsions were several times smaller than the morsels which were introduced by means of the inoculating needle; fewer of them survived, and their development was on the whole much less rapid. The essential process of growth was, however, identical in the two series.

At the end of twenty-four hours considerable portions of the transplanted tissue fragments had become necrotic. The tissue of the fragments, excepting the surviving cells, to be described, presented appearances similar to those which tissue permitted to undergo autolysis at the body temperature outside the body would be expected to show. The interstitial tissue showed few deformed and fragmented nuclei; the fibers were indistinct and more or less fused together; and the epithelioid cells occupying small spaces in the latter tissue were shriveled, with irregular outline, the protoplasm staining deeply in eosin, the nuclei of irregular form and unequal staining powers, and in process of disintegration. The fragments were everywhere, as was to be expected, surrounded by a fibrinous and leucocytic exudate, and a certain number of leucocytes had penetrated within them. Within certain of the fragments a number of

epithelioid cells had survived. These cells might be at the periphery or they might be in the interior of the fragments, sometimes single and sometimes several in a given space. They differed greatly from the degenerating and disintegrating cells, particularly in respect to the behavior of their nuclei, which retained the normal form and distribution of chromatin, although in point of intensity of staining they tended to be hypochromatic. For the most part the cells appeared merely to be survivals, but now and again nuclear changes suggestive of mitosis were met with. However, in view of the fact that the chromatic filaments were somewhat irregular in thickness and arrangement, it cannot be said that those changes actually led to cell multiplication at this early period. It should be mentioned that in some fragments the old blood vessels still remained morphologically unchanged at the end of this period.

At the expiration of forty-eight hours the effects of the transplantation were still more obvious. The stroma and the greater part of the epithelioid cells and the blood vessels in the transplanted morsels had all progressed still further in respect to the disintegrative changes. On the other hand, the surviving epithelioid cells had, either by contrast or actuality, become more sharply demarcated from the surrounding elements. Where they were contained within the interior of the fragments, their number had not greatly increased, although apparently there had already been some increase in number; the nuclei had become richer in chromatin, the surrounding protoplasm clearly outlined, and mitosis had not only become more frequent than in the twenty-four hour period, but the chromatin filaments presented a regular arrangement and uniform thickness suggestive of normal mitosis. At the periphery of some of the fragments the epithelioid cells had clearly multiplied, so that they had come to form a superficial layer, one, two or three cells deep in some places. These cells lay in contact with the inflammatory exudate, and some of them apparently had wandered into the exudate. Mitoses also occurred in them, and even in separated cells surrounded by the exudate. Thus far no organic connections had taken place between the fragments and the surrounding tissue, although in the latter an active proliferation and new formation of capillaries had begun. On the other hand, elongated cells of fibroblastic or angio-

blastic nature had begun to enter the fragments at the periphery, adjoining the host tissues.

After three days the conditions had altered greatly. The fragments had not increased perceptibly in size, but those lying adjacent to the host tissues had become attached through capillary connections with the tissues of the living animal. The capillaries had pushed in various directions and some of considerable size had already formed channels in the interior. The medium in which they lay was apparently nothing else than the degenerated original stroma, and there had been no unmistakable restoration of living interstitial cells. All the cells which had wandered in were in connection apparently with the capillaries. On the other hand, the epithelioid cells had increased greatly, mitosis having gone on rapidly. The cells now formed small groups chiefly, although sometimes single ones were separated from the others. The active changes in the vascularized fragments were greatly in advance of those not already vascularized. Moreover, those fragments or portions of fragments in which the epithelioid cells had not begun to multiply, nor the capillaries to penetrate, had by this time been invaded with large numbers of leucocytes, which were softening and dissolving the necrotic tissue. Finally, in certain fragments there had apparently been beginning multiplication of the epithelioid cells, which had not become vascularized, and which were undergoing a secondary degeneration and death.

At the end of the fourth day the conditions in the vascularized peripheral fragments represented merely progression of the third. In other words, the vascularity having increased, these fragments were now entirely occupied by capillary vessels and proliferated epithelioid cells, while the fragments which were more interior were less advanced in this condition, and might show mitosis of epithelioid cells still going on, although no capillaries were yet visible in the stroma, which was quite completely necrotic. At the same time, occasional islands of epithelioid cells remained alive and were proliferating slowly.

The subsequent progress was rapid. The groups of epithelioid cells continued to increase in number and to expand in size, and coincidentally a fibrillated tissue appeared between them and the

capillaries. At the end of seven days the fragments had become completely vascularized, all of the evidences of acute inflammation had disappeared, the volume of the fragments had increased several fold, and they had formed an indissoluble union with the tissues of the host. At the junction of the two, a small-cell granulation tissue formed a sort of capsule. The positions of the original fragment could still be made out through the remains of a central, paler, partially degenerated, and not completely substituted mass of epithelioid cells and young capillaries, with here and there polynuclear leucocytes and beginning fibrillated tissue. What was particularly striking was the close union of the epithelioid cells and the young connective tissue. This union was so close that alveolation in the proper sense of the term could not be spoken of. In addition, there occurred not infrequently multinucleated protoplasmic masses giving rise to giant cells and large single cells with giant nuclei.

By the ninth day the differentiation between the fibrous stroma and the epithelioid elements had progressed so far that as the connective tissue became more fibrous it left the epithelial cells more sharply demarcated. On the eleventh day this demarcation was still more pronounced, although the condition was not as yet one of definite alveolation. At this time the remains of the original fragment occupying the central portion of the enlarged nodule was still evident. It consisted of a hyalin mass enclosing small groups or single epithelioid cells not in the best state of preservation. On the fourteenth day the nodule was still further increased in size, the connective tissue had become more pronouncedly fibrous, but the epithelioid cells were not so strictly confined as to produce a typical alveolated structure. On the other hand, on the fifteenth day the fragment was quite characteristically alveolated.[5] The connective tissue now formed a definite fibrous stroma enclosing alveoli, chiefly solid but sometimes presenting spaces and, therefore, acinous, of epithelioid cells, which were multiplying rapidly by mitosis. These alveoli were shown, especially in the preparations stained with iron-hematoxalin, to be of irregular size and form and everywhere intersected by the fibrous stroma. In that respect at this period the

[5] This change is one of degree only and would doubtless vary in different experiments.

alveolation was not by any means so regular as was found in some later nodules and as is the rule in simple carcinoma in man.

FRAGMENTS IN THE MOUSE.

The results of the introduction of fragments of the tumor under the skin of the mouse were in many respects, for the early period, similar to those in the rat. The first effect was, of course, a certain amount of inflammatory exudation, but this amount was not greater than that which took place in the rat. The changes in the fragments were also similar, which is to say that the greater part of the tissue composing them degenerated. But what remained alive at the end of twenty-four hours was a certain number of epithelioid cells on the surface or in the interior of the fragment. At the end of the first twenty-four hour period mitosis had not been noticed in the host cells, but the stroma and many of the epithelioid cells were in a state of complete necrosis, and the resistant epithelioid cells had begun to show mitosis. The formation of angioblasts and the organic connection with the mouse of the fragments also occurred on the third day, at which time there had been a great proliferation by mitosis of the epithelioid cells, particularly those lying immediately next to the tissues of the mouse, which were united to the fragments, although the mitosis was not limited to those cells. The increase in the epithelioid cells on the fifth day was considerable, and they formed groups within the homogeneous residue of the stroma, which was now in process of invasion by capillaries. On the seventh day the new vessels had penetrated deeply into the fragment; there was a striking hyperemia, and the epithelioid cells were still in process of multiplication. On the eleventh day the healing process was still more advanced, but remains of the epithelioid cells, which were, however, inactive, were still present. On the fifteenth day the vascularization was still more advanced, and had reached almost to the center of the fragments, which were occupied by masses of leucocytes undergoing fragmentation, but the specimens showed that multiplication by mitosis was still going on actively in the epithelioid cells, especially in the vicinity of the tissues of the host. The absence, therefore,

of active mitosis in the two previous periods was the result not of the exhaustion of the tissue but of accidental circumstances in connection with the transplanted fragments. Although the latest period in which active mitosis was discovered in the fragments transplanted to the mouse was fifteen days, the large number of them contained in one series of specimens of that period indicates than they might still be found under favorable circumstances even at a later period.

We have transferred fragments which have remained in the mouse for different periods back to the rat and have succeeded in rearing these fragments as late as eight days after their implantation in the mouse.

FRAGMENTS IN THE GUINEA-PIG.

Fragments transplanted to the guinea-pig aroused a somewhat greater inflammatory reaction and exudation than in the other animals mentioned. The changes which took place in the transplanted fragments did not differ essentially from those already described. While the great mass of the tissue died and showed the changes of autolysis, groups of epithelioid cells in the interior or near the surface survived. At the end of the first twenty-four hour period these surviving cells were still quiescent, while at the end of forty-eight hours they had changed significantly: they had become sharply demarcated from the rest of the tissue, were more numerous than they had been in the previous twenty-four hours, and were showing active mitotic division. Indeed, this activity was more marked than it had been under similar circumstances, either in the rat or the mouse. The multiplication was taking place in the interior of the fragments, as well as on the surface. At the latter situation the new cells had already formed a layer several deep. There was still activity, but it was less in these cells at the end of the third day, at which time there were fewer organic connections with the host than had been noted in the other animals, and many fewer new blood vessels had formed. On the seventh day the vascularization was well advanced, but not equally about all the fragments. About some the exudation was very great and had

penetrated into the interior. Karyokinesis was, however, still going on in the epithelioid cells, although not actively, and particularly at the periphery of the fragments. The epithelioid cells in the interior were reduced in numbers, and were degenerating. On the ninth day the fragments had been greatly reduced in size and had become much more disintegrated, so that the demarcation of the epithelioid cells was uncertain.

SUMMARY.

The rat tumor described is to be regarded probably as an embryoma located in the seminal vesicle, from whose histological elements it was partially derived.

Originally the tumor was composed apparently of a simple tissue of imperfectly organized structure, but containing certain glandular elements derived in part from the seminal vesicle and in part from other included epithelial organs.

The included glandular elements had already undergone in the original tumor a carcinomatous development, although the carcinomatous moiety was not disseminated throughout the mass, but was confined to portions near its peripheral boundary, adjacent to the wall of the vesicle.

In the course of transplantation the simple and imperfectly organized structure of the tumor became substituted by a more complex and perfectly organized tissue, which bore resemblance to simple alveolar carcinoma.

The tumor at this time not only developed in the subcutaneous and intra-muscular tissue and the abdominal cavity, according as the grafts were made, but it gave rise to metastases through the blood current in the lungs and kidneys.

In the fifth generation the simple carcinomatous structure began to be replaced by a tissue resembling adeno-carcinoma, and coincidentally metastases appeared in the superficial and rarely in the deeper, distant lymphatic glands.

The adeno-carcinomatous form of structure became progressively more established in the succeeding generations, in process of which the stroma of the tumor changed to a delicate fibrillated tissue supporting the acini, and differing materially from the rich fibrous stroma that usually supported the simple alveoli.

The tumor had not become established as an adeno-carcinoma of the pure type by the twenty-eighth generation, at which time simple alveoli still appeared, but the stroma of the tumors bearing the latter structure was relatively delicate, and the consistence of the tumors relatively soft, in contradistinction to the earlier type, which was much firmer.

The degenerating tumors of the earlier type showed much fibrous tissue of a hyalin quality, while those of the later type showed coagulative necrosis of a soft quality.

The metastases partook more of the nature of simple carcinoma than of adeno-carcinoma, even in the later type. The tumor cells composing the metastases tended to be substituted for the epithelial cells of the lungs and kidneys and the lymphoid cells of the lymphatic glands, and merely to occupy the already prepared stroma of these organs. Hence the structure of the organs in the metastases was not greatly disturbed. When a metastasis appeared in the pleural membrane, in which fibrous tissue was readily produced, the acinous type of tumor tended to appear, and in the course of the invasion of the cardiac muscle and other solid tissues, acini also tended to be produced.

The tumor presented a high degree of invasive power, and its growth was not interrupted by muscle, fascia, bone or cartilage.

The dilated esophageal extremity of the stomach tended to become invaded. When the tumor reached the mucous membrane, ulcers developed, and implantation to the opposite surface of the mucosa sometimes occurred.

The retrogression of the growing tumors was accomplished through atrophy and degeneration of the epithelioid cells contained in the tumor and an increasing degree of fibrosis of the stroma. Ultimately all parts of the tumor disappeared completely. Nothing was discovered to indicate that the metastases ever underwent changes of the nature of those leading to retrogression of the superficial nodules.

Although the metastases were such as were produced chiefly through the blood current, it was most rare to find masses of tumor

cells in the blood vessels, unless they had penetrated them secondarily from without.[6]

The manner of development of the fragments of the transplanted tumor showed the large epithelioid cells to be the proper elements of the tumor. All other tissue elements succumbed, and the reproduction of the tumor depended upon the proliferation by mitosis of these cells, the stroma for which was supplied by the tissues of the new host.

There was no essential difference in the manner of survival of grafts introduced into rats, mice and guinea-pigs, except that in the last two species the process of multiplication of the epithelioid cells came to an end in a few days, in spite of the vascularization of the transplanted fragments, which at first promoted proliferation.

The number and location of fragments surviving in the rats depended in part on the rapidity and degree with which the vascularization took place. The first stages of proliferation did not depend upon the vascularization, but unless new vessels had entered the fragments by the third day, the epithelioid cells already produced tended to degenerate rather than to proliferate further.

A highly glandular organ such as the testicle proved not to be very suitable for the tumor implantations.

Salt solution proved to be injurious to the tumor tissue, especially when it was in a state of fine division. The tumor was acted on injuriously by sunlight, and this injurious action appeared to be somewhat intensified by the presence of certain photo-dynamic anilines.

Filtered tumor emulsions and even strained tumor emulsions in salt solution did not produce new tumors upon inoculation, while small aggregates of the tumor cells, such as occur in ascitic fluid, did set up the formation of new tumors.

No microörganisms were recognized in the tumor, and no bacterial cultures were obtained from non-ulcerated tumors.

[6] We are indebted to Miss Menten for a very careful study of large numbers of serial sections of the lungs of rats, in which metastases were present, and in which no visible metastases could be seen. She succeeded in a single instance in finding a small mass of tumor cells within a blood vessel, unassociated with a developed metastasis, and this was in a lung already the seat of metastatic nodules.

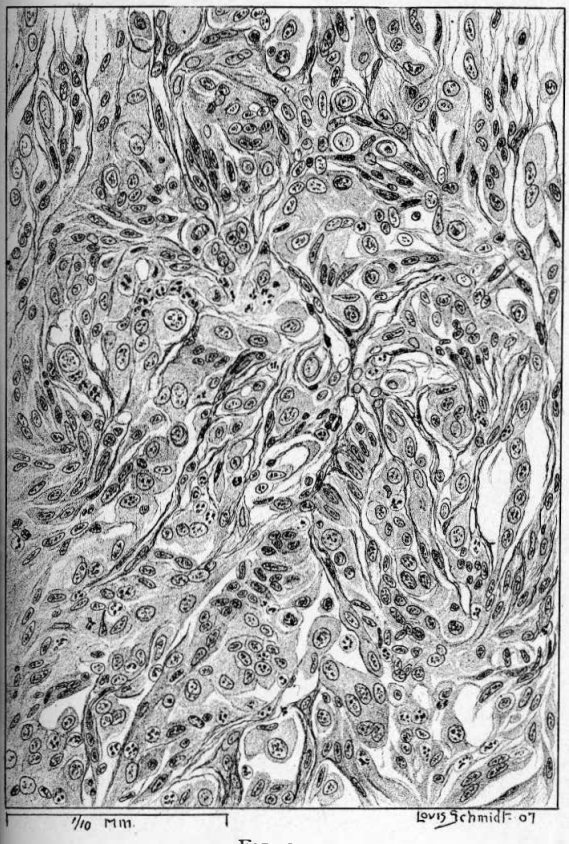


FIG. 1.

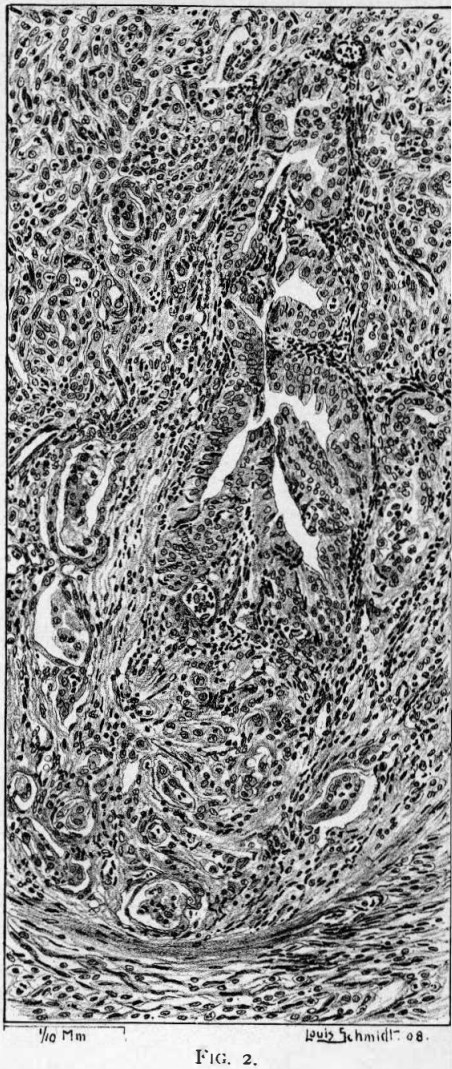
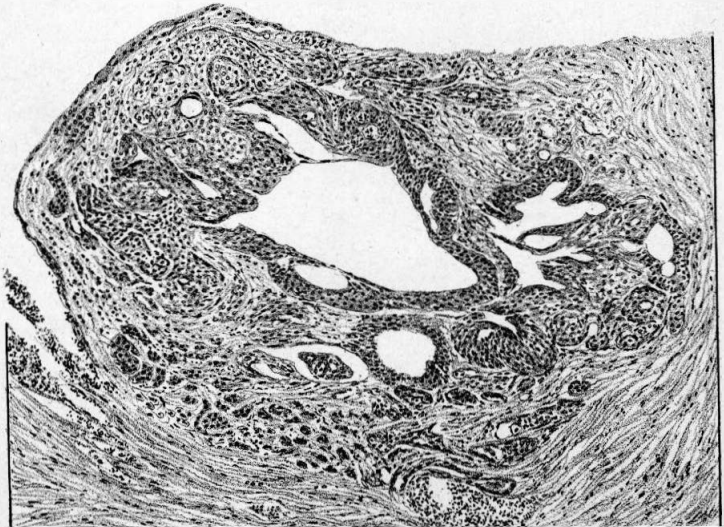
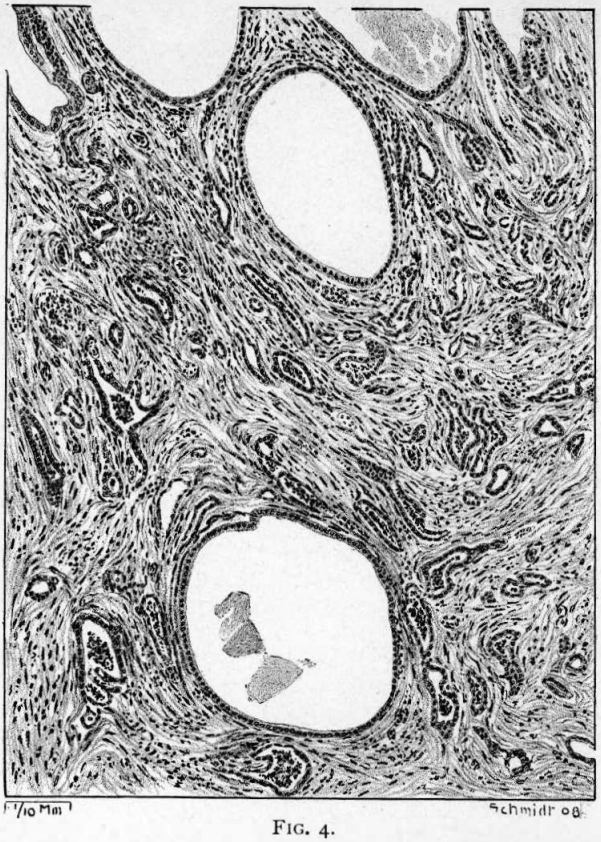


FIG. 2.





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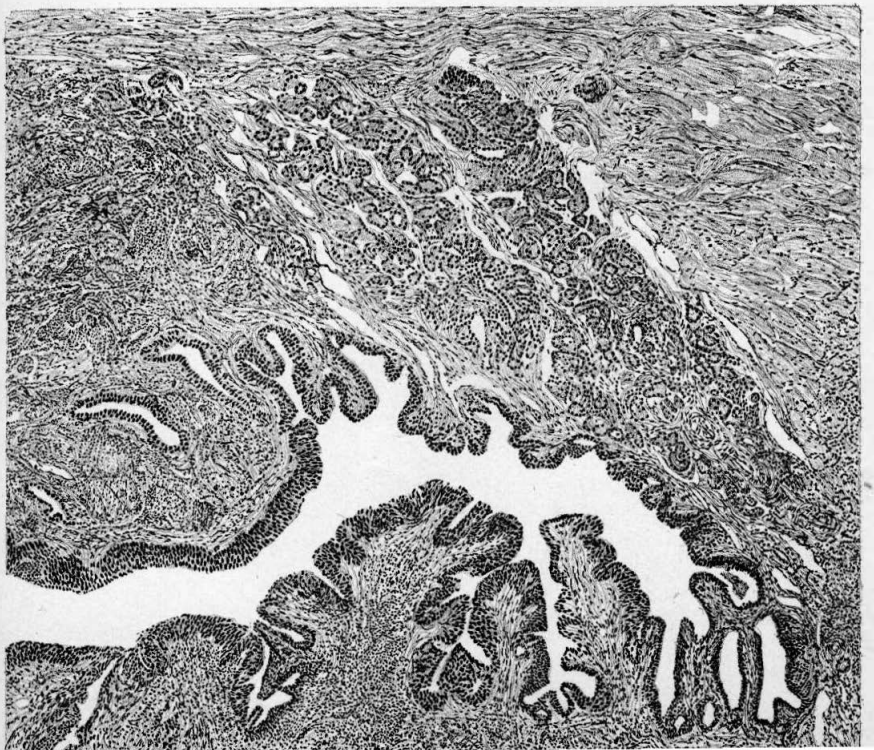


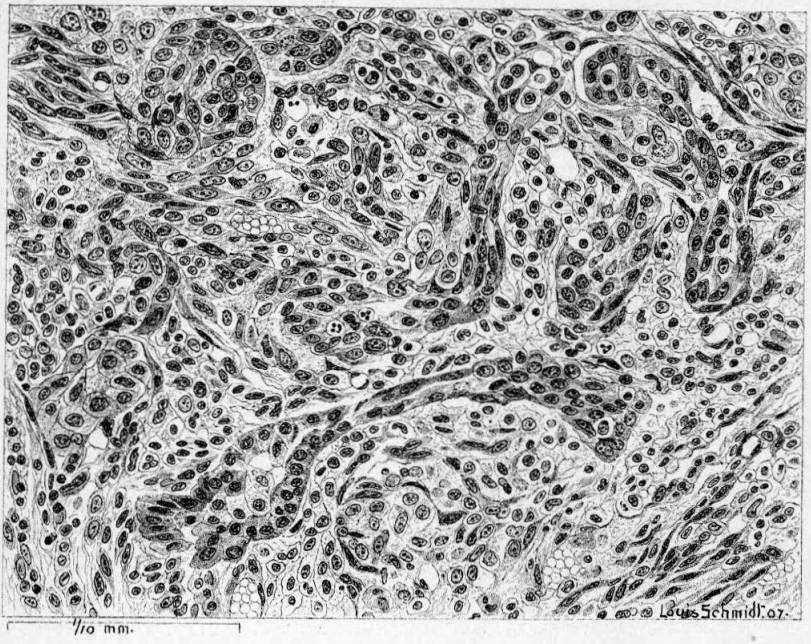
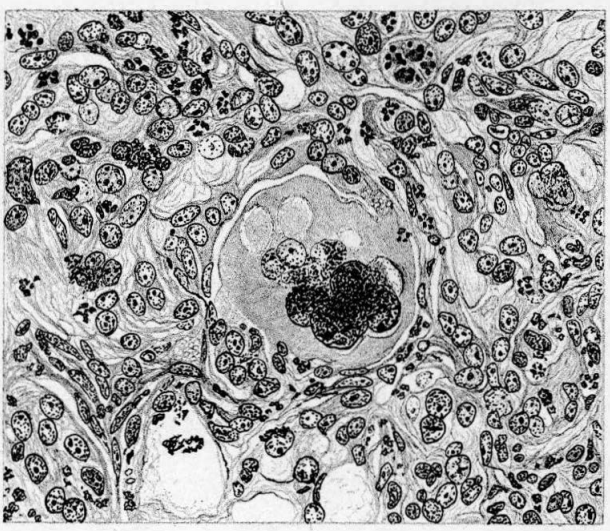


FIG. 6.



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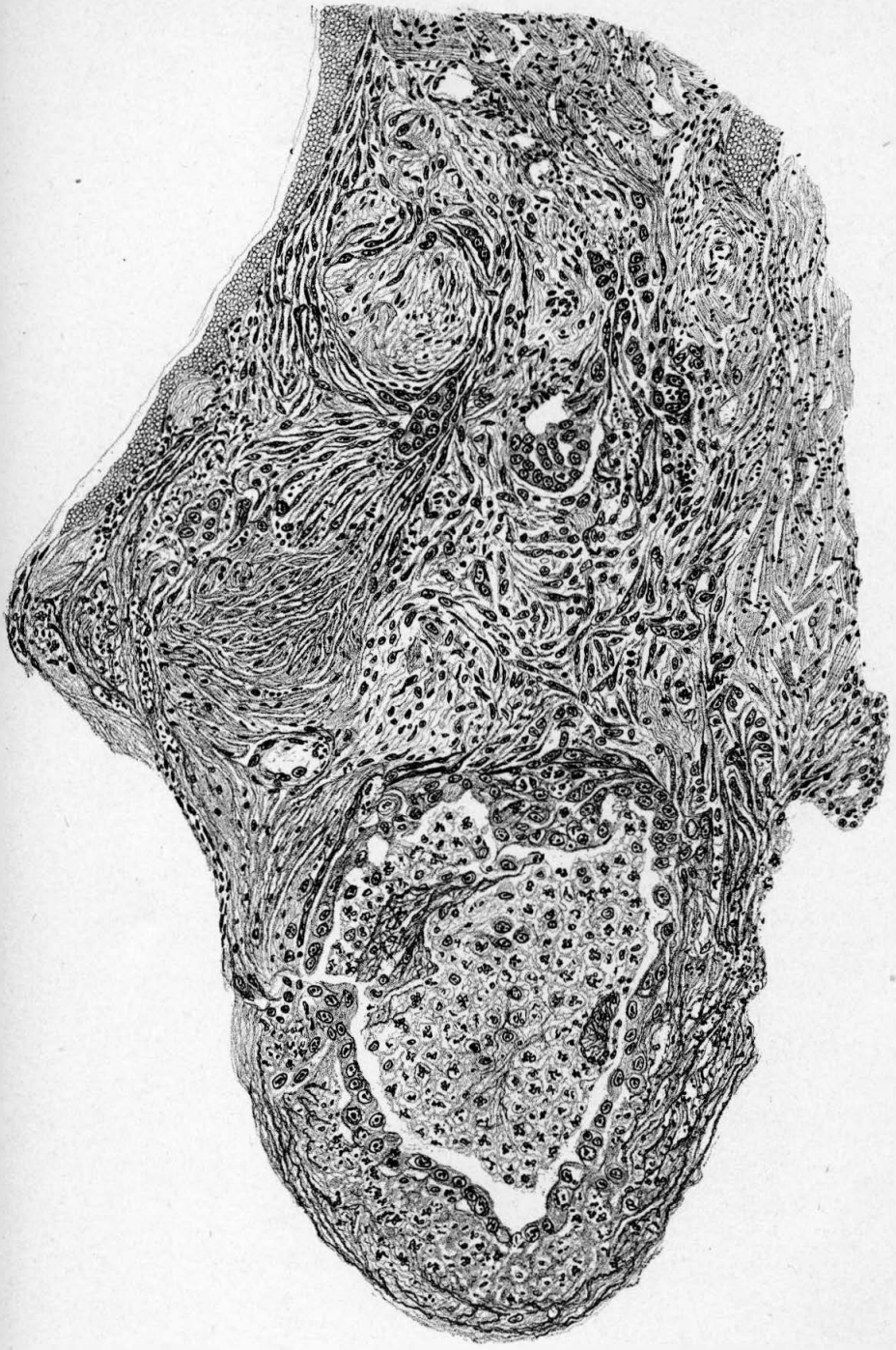


FIG. 8.

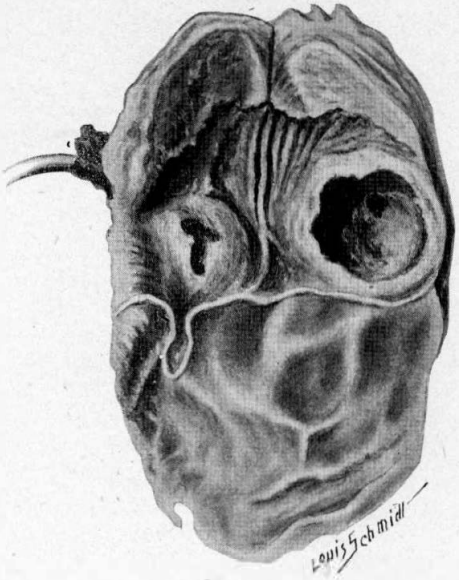


FIG. 9.

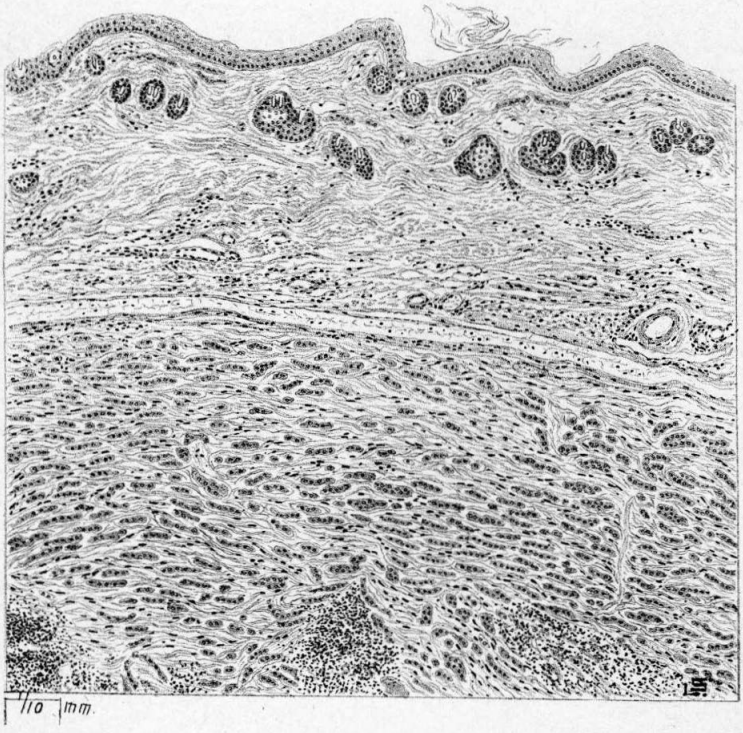


FIG. 10.

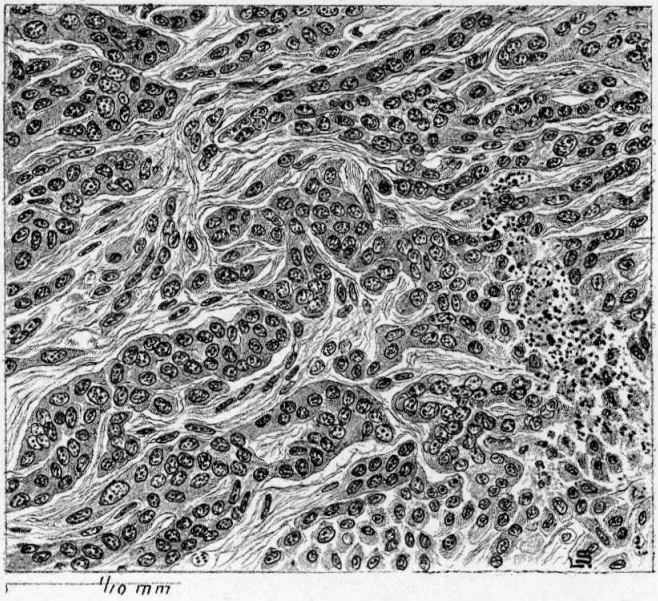


FIG. 11.

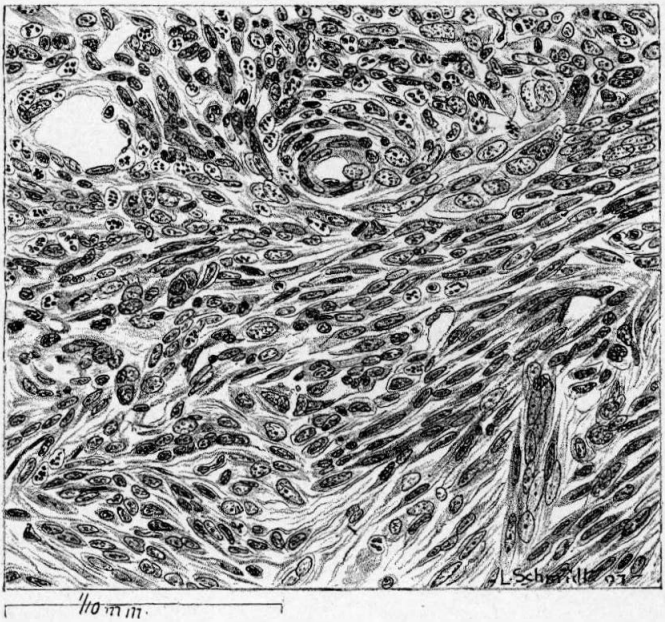


FIG. 12.

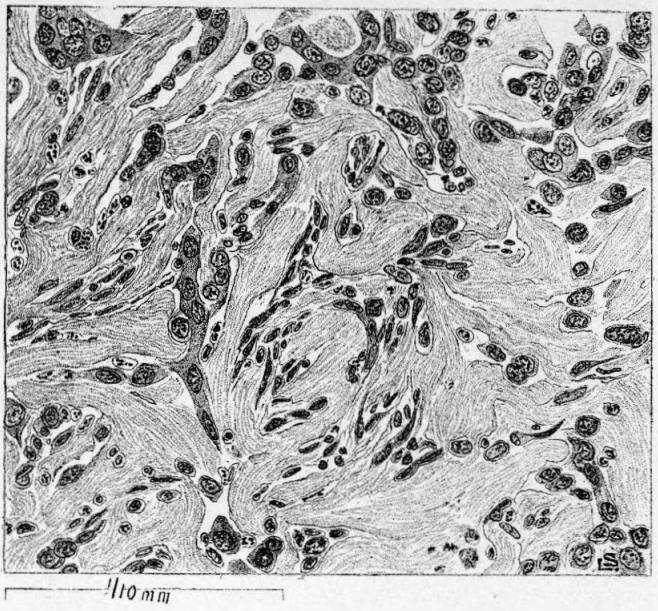
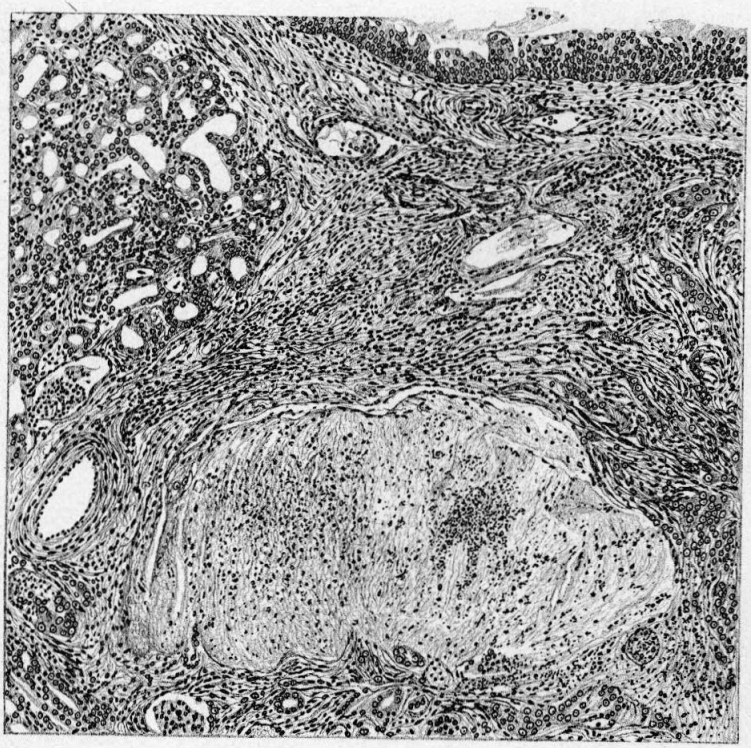


FIG. 13.



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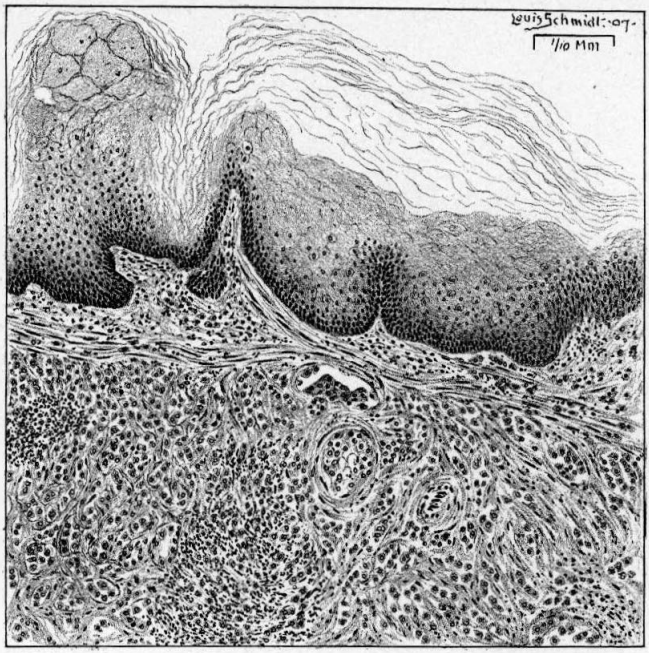


FIG. 15.

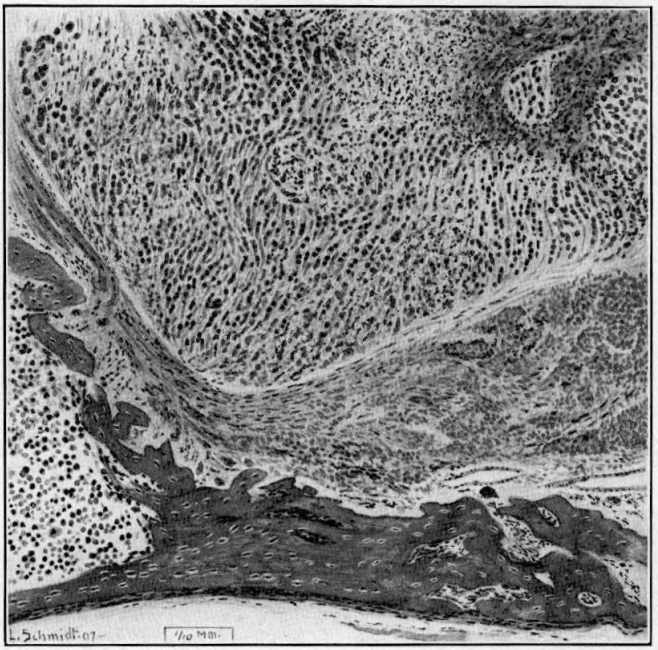


FIG. 16.

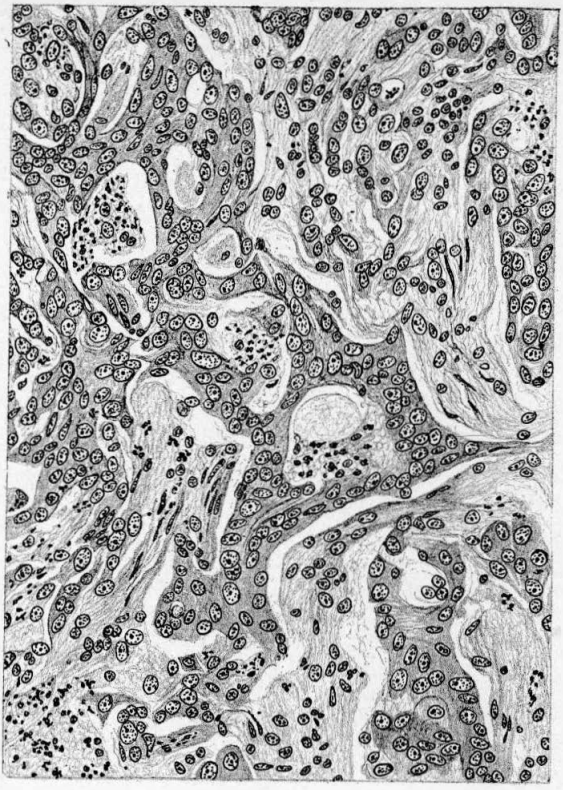
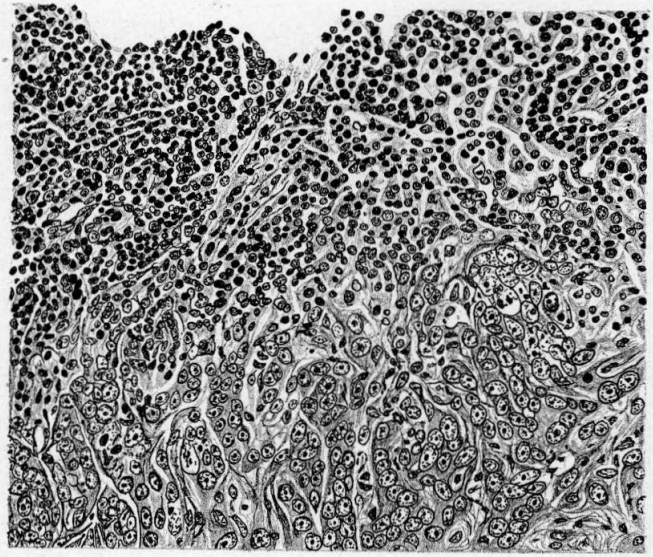


FIG. 17.



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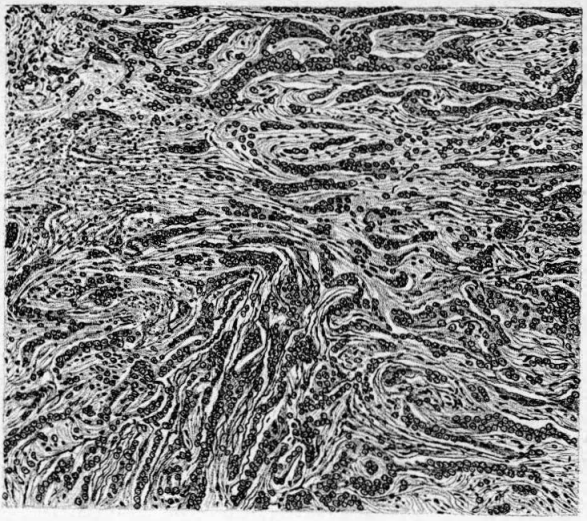


FIG. 19.

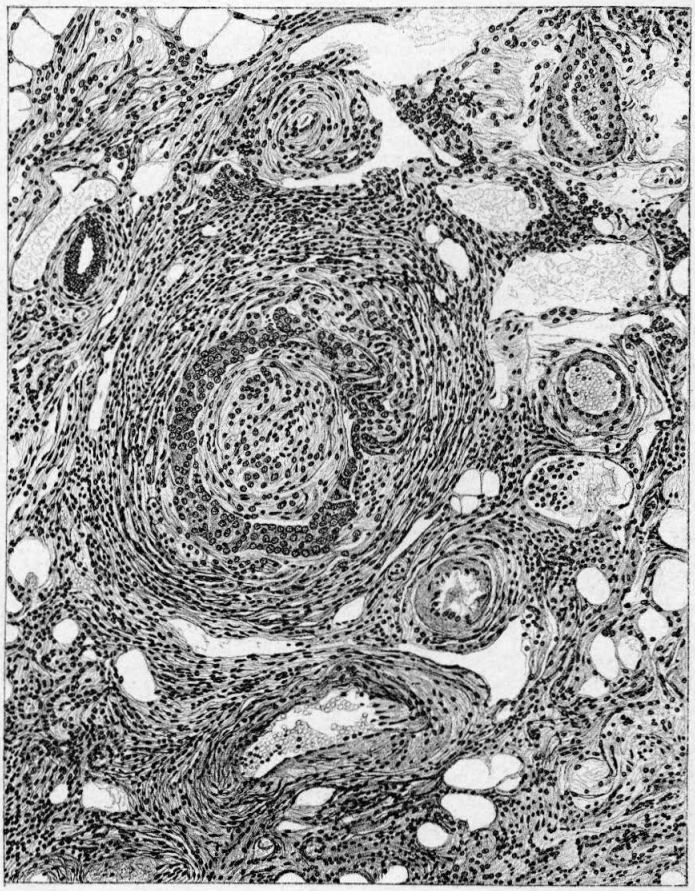
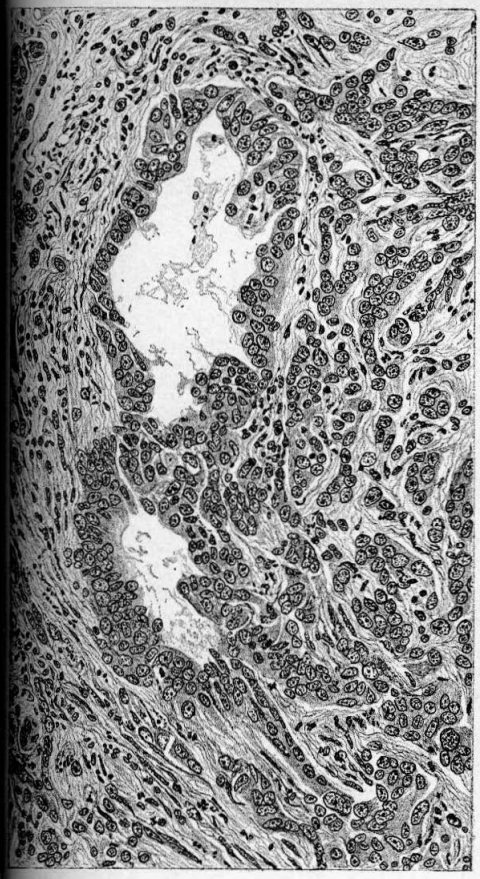


FIG. 20.



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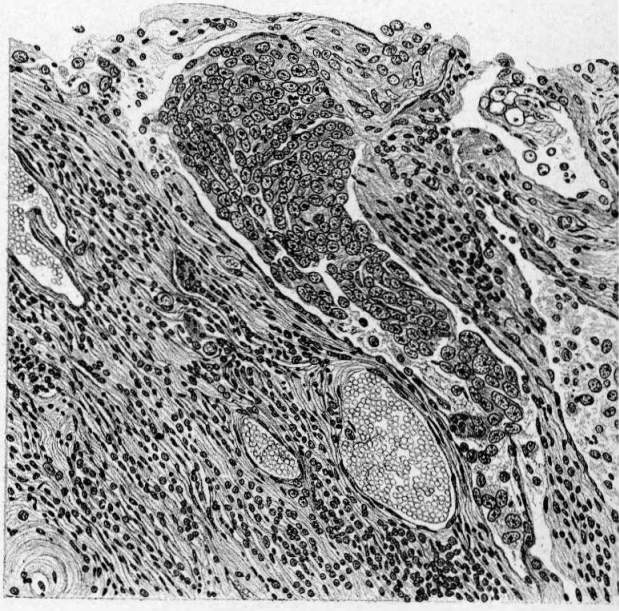

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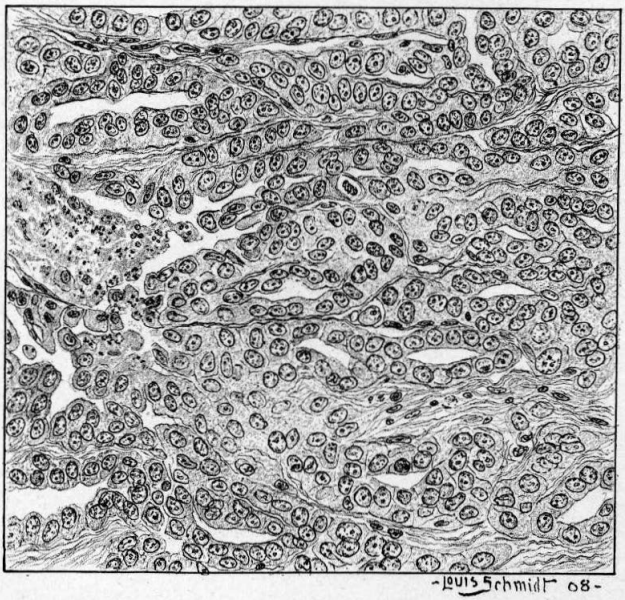

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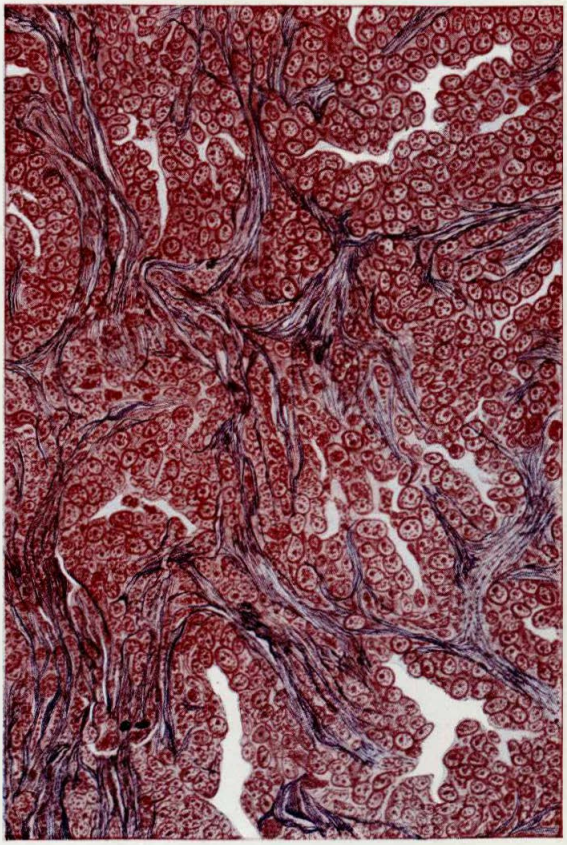
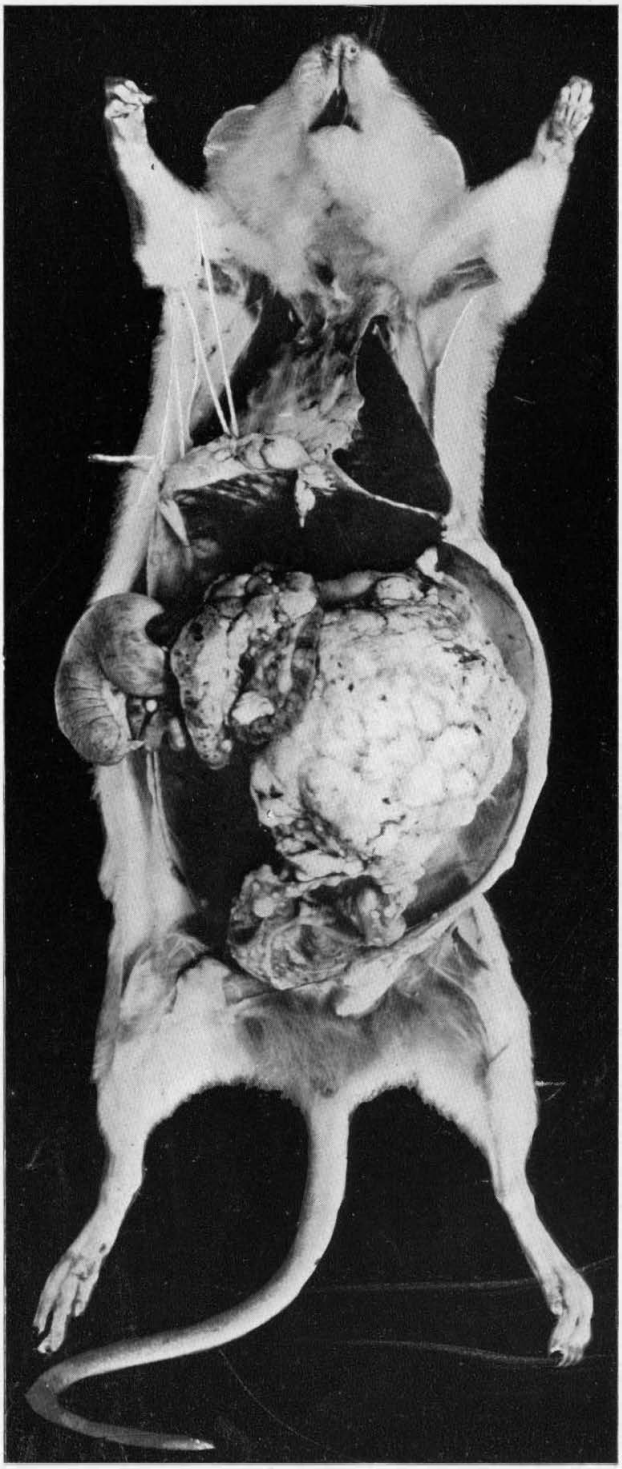
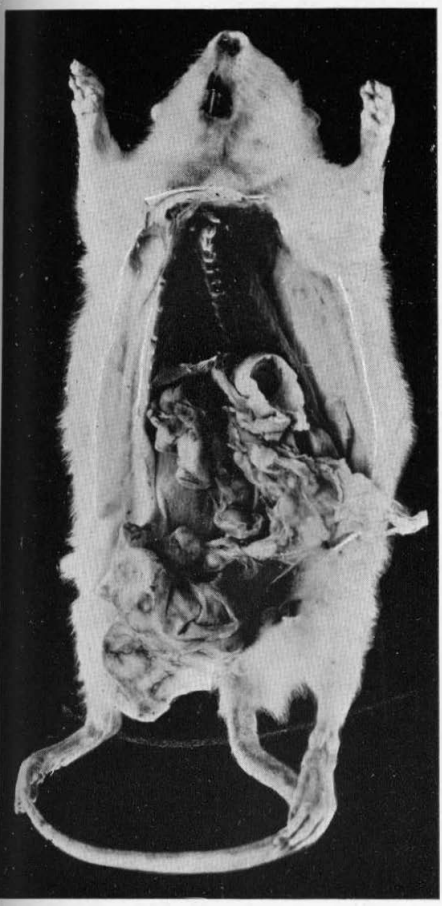


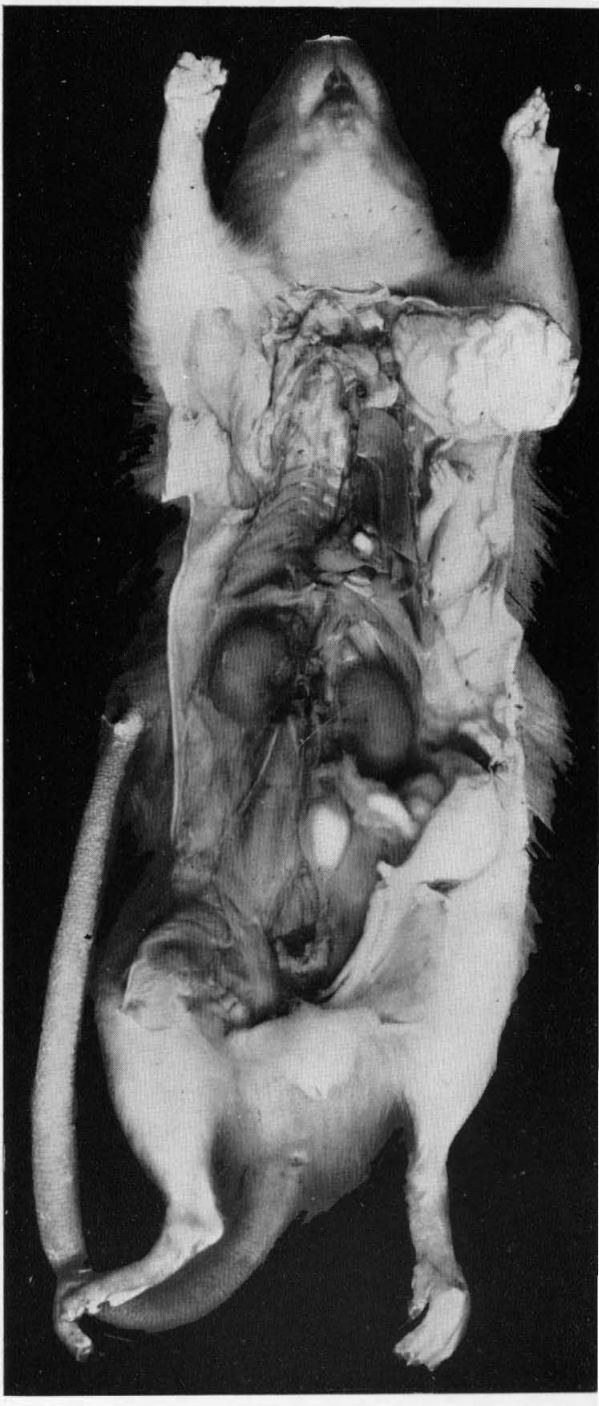
FIG. 24.



PHOTOGRAPH 1.



PHOTOGRAPH 2



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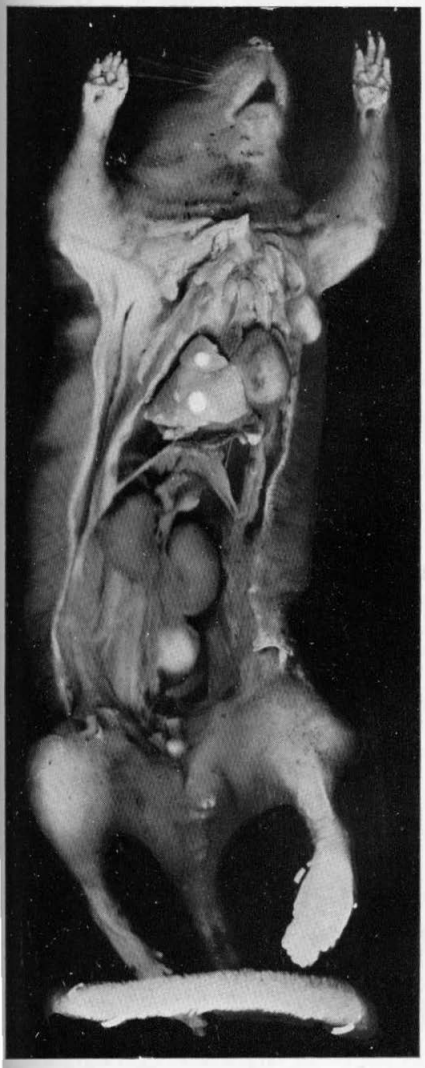

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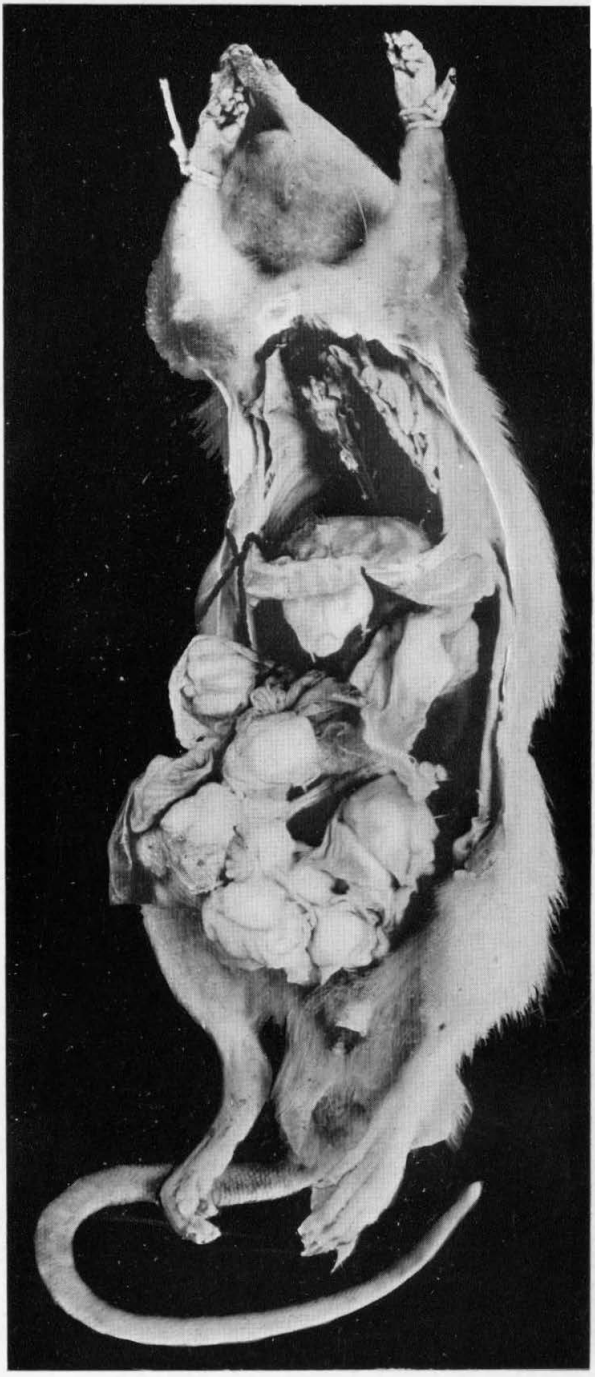

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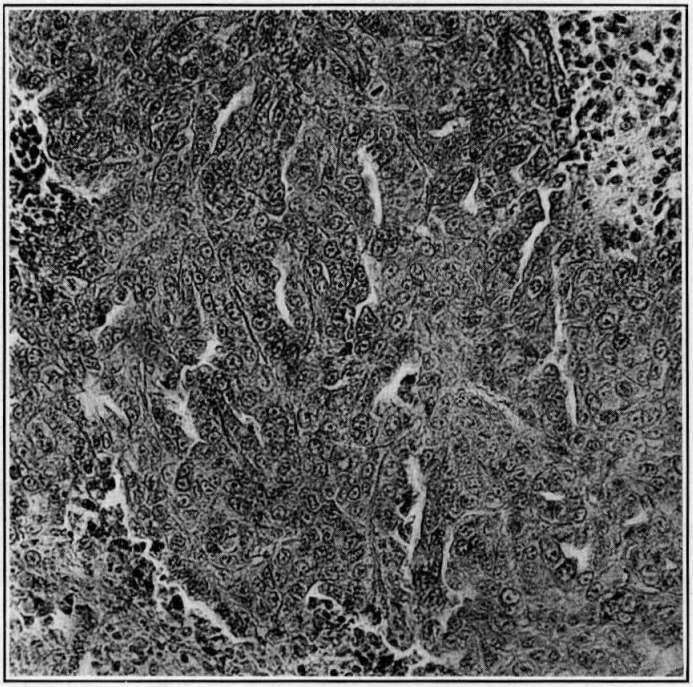

PHOTOGRAPH 6.

EXPLANATION OF PLATES.

PLATE I.

FIG. 1. No. 3 eye-piece; 6 objective. Showing the relation between the spindle and polymorphous cells occurring over considerable areas of the tumor.

FIG. 2. No. 3 eye-piece; 6 objective. Glandular tubule included in the tumor undergoing a typical proliferation or being dissected by ingrowth of the tumor.

FIG. 3. No. 3 eye-piece; 3 objective. Dilated glandular tubule enclosed in hyalin connective tissue adjacent to alveoli enclosing epithelial cells, probably derived from the tubule.

PLATE II.

FIG. 4. No. 3 eye-piece; 3 objective. Junction of the seminal vesicle, showing dilated glandular spaces, with the tumor, and typical carcinomatous alveoli possessing lumena.

FIG. 5. No. 3 eye-piece. 3 objective. Branching tubular formation or duct adjacent to a glandular mass possessing structures of a serous or mucous gland.

PLATE III.

FIG. 6. No. 3 eye-piece; 6 objective. Irregular carcinomatous alveoli embedded in the stroma, containing spindle and other cells resembling the epithelium-like polymorphous cells of the alveoli.

FIG. 7. No. 3 eye-piece; 6 objective. Pulmonary tumor nodule of alveolar structure, showing a mononucleated giant cell of the megacaryocytic type.

PLATE IV.

FIG. 8. No. 3 eye-piece; 6 objective. Invasion of the heart wall and coronory sinus by the tumor.

PLATE V.

FIG. 9. No. 3 eye-piece; 6 objective. Stomach opened to show the ulcerated tumor masses in the esophogeal segment. The elevated, undulating white line shows the demarcation between the esophogeal and gastric mucosa.

FIG. 10. No. 3 eye-piece; 3 objective. Subcutaneous tumor nodule showing the relation of the young alveoli to the stroma. A definite demarcation separates the tumor from the cutis.

PLATE VI.

FIG. 11. No. 3 eye-piece; 6 objective. Showing the usual structure of the tumor in the stage of carcinoma simplex.

FIG. 12. No. 3 eye-piece; 6 objective. From the edge of a recent rapidly growing graft showing the original development of spindle cells and small included masses of polymorphous cells which have not yet arranged themselves into definite alveoli.

PLATE VII.

FIG. 13. No. 3 eye-piece; 6 objective. Hyalin transformation of the tumor in the course of retrogression showing the persistence of small groups and masses of the polymorphous cells within the hyalin connective tissue.

FIG. 14. No. 3 eye-piece; 3 objective. Invasion of the splenic vein by the tumor which presents a typical tubular alveolar structure. The vein is occluded by a thrombus which is itself becoming invaded with tumor acini.

PLATE VIII.

FIG. 15. No. 3 eye-piece; 3 objective. The invasion of the submucous and subepithelial layer of the esophageal segment of the stomach by the tumor which shows distinct alveolation. Beginning degeneration of the superficial epithelial cells.

FIG. 16. No. 3 eye-piece; 3 objective. Invasion of the vertebral column. The alveolar tumor in the section is adjacent to the cartilages and bony structures.

PLATE IX.

FIG. 17. No. 3 eye-piece; 6 objective. Pseudo-acinar excavation of the alveoli.

FIG. 18. No. 3 eye-piece; 6 objective. Invasion of the regional lymphatic glands by the alveolated tumor. The stroma which is slight is furnished by the gland.

PLATE X.

FIG. 19. No. 3 eye-piece; 3 objective. Atypical carcinoma simplex in which the alveoli are linear and bent in several directions.

FIG. 20. No. 3 eye-piece; 3 objective. Invasion of the large peripheral nerve by the tumor.

PLATE XI.

FIG. 21. No. 3 eye-piece; 6 objective. Lymphatic metastasis undergoing cystic degeneration and showing papillary outgrowths in the wall.

FIG. 22. No. 3 eye-piece; 6 objective. Invasion of a lymphatic vessel by the polymorphonuclear cells of the tumor.

FIG. 23. No. 3 eye-piece; 6 objective. Typical soft adeno-carcinomatous form of the tumor.

PLATE XII.

FIG. 24. No. 3 eye-piece; 6 objective. The same stained with Mallory's aniline-blue to show the relation between the stroma and epithelial cells.

PLATE XIII.

PHOTOGRAPH 1. (Rat. 47, p. 23.) Large, omental nodule, multiple, small omental and mesenteric nodules, and nodules in the diaphragm, all developing from a single fragment introduced into the peritoneal cavity.

PLATE XIV.

PHOTOGRAPH 2. (Rat 46, p. 24.) The tumor growing about and into the cardia of the stomach where a cup-shaped ulcer is produced.

PHOTOGRAPH 3. (Rat 231, p. 26.) A large tumor metastasis in the axillary gland and smaller metastasis in the retroperitoneal and other lymphatic glands, and a growth into the pleural cavity.

PLATE XV.

PHOTOGRAPH 4. (Rat 231, p. 26.) Metastasis in the axillary and retroperitoneal glands, and in the lungs.

PHOTOGRAPH 5. (Rat 424, p. 27.) The development of multiple nodules from an intraperitoneal injection of ascitic fluid containing microscopic tumor fragments.

PLATE XVI.

PHOTOGRAPH 6. (Rat 2253, p. 42.) The histological appearance of the typical, soft, pure, adenomatous and acinous growth.

THE BIOLOGY OF A MIXED TUMOR OF THE RAT.

By J. W. JOBLING, M.D.

(From the Laboratories of the Rockefeller Institute for Medical Research, New York.)

The tumor, of which the pathological anatomy has just been fully described, has been studied under a number of artificial conditions with a view of establishing certain facts regarding its biology. The more extensive biological studies of tumors in the lower animals made up to the present time have been carried out on mice, and to a much less extent on dogs and rats. Indeed, there does not exist in the literature such an exhaustive study of a tumor in other animals comparable to many studies which have been carried out in connection with the transplantable tumors of mice. It is, therefore, of some interest to ascertain to what extent the general facts established for tumors of mice are especially true for the transplantable tumors of other species of animals, so that in the description to follow, along with certain observations applying peculiarly to this tumor, others will be described which are based on previous experiments of a similar nature made upon mice.

GROWTH AND RETROGRESSION.

The phenomena of growth of the tumor were studied under a number of artificial conditions. The first of these which we are to consider refers to the transplantation generations, and particularly to the percentages of successful implantations and the percentages of retrogressing tumors in the several series. We will discuss these points in relation to the first twenty-five transplantation generations.

Beginning with the third generation, the number of rats inoculated for each one ranged from 13 to 125. The lowest per cent. of successful inoculations was observed in the third generation, since only four animals of the thirteen inoculated developed tumors, and of these four, two finally disappeared by retrogression. The age of

the tumor used for the inoculations was 132 days, and as no careful selection was made of the fragments transplanted, this factor doubtless played a part in the result. The tumor employed for inoculation in the fourth generation was forty-eight days old and measured one centimeter in diameter. Of the 35 rats inoculated, 24, or 68.5 per cent., developed tumors, of which 4, or 16.6 per cent., later underwent retrogression. From this time on the tumors chosen for use in inoculating the other rats were about one centimeter in diameter, although they were of very different ages. The youngest tumor employed was 23 days old, and the oldest, 71. In the seventh generation the number of successful inoculations reached 96.6 per cent. That is, of 30 rats inoculated, 29 developed tumors, of which 6, or 20.7 per cent., subsequently disappeared. From this time on, in spite of certain fluctuations, the percentage of successful inoculations remained high, being usually 90 per cent. or over, and falling once only, namely, in the twenty-third generation, to 68.4 per cent. In the twentieth generation all of the 29 rats inoculated developed tumors.

Along with the development of increased power of growth in the inoculated rats, there went a diminished tendency to spontaneous absorption of the tumors. Until the twelfth generation the number of retrogressions was high and reached sometimes 52.7 per cent. in the tenth generation, and 32 per cent. in the twelfth. After this, the percentage of retrogressions tended to remain below 10 per cent.; once it fell to 2 per cent., and once, namely, in the twenty-second generation, it rose as high as 23.6 per cent.

That the tumor is a slowly growing one is readily seen from the length of time the inoculated animals survived the implantations and the relatively small size which the tumors attained at the time of the death of the rats. The tumor causes death probably in one of three ways: first, through ulceration of the skin and secondary infection; second, through invasion of vital parts; and third, through metastasis. In respect to the degree of local development, the tumor stands far behind the Jensen and Lewin rat tumors, which we have also had the opportunity of studying. After the implantation of fragments the earliest evidences of increase in size were obtained on the seventh to the tenth day. From that period the growth is pro-

gressive and quite uniform, but not rapid, and the tumors reach sometimes a considerable size, measuring not infrequently three centimeters in their greatest diameter.

ACTIVE IMMUNITY.

It is now established, chiefly as a result of the accurate studies of Gaylord and Clowes,[1] Jensen, Ehrlich,[2] Bashford,[3] and still others, that mice which first developed transplantation tumors, subsequently undergoing absorption possess a high degree of refractoriness, comparable perhaps to a state of active immunity to subsequent inoculations of similar or even diverse tumor fragments. We observed in conformity with this established fact that the rats, which, having developed tumors, subsequently lost them through absorption, showed a considerable degree of refractoriness upon reinoculation with the same kind of tumor fragments. An experiment was then made to determine whether this state of resistance or immunity, so-called, was a fixed or enduring quality or whether it was subject to quantitative changes produced through lapse of time. The following tabulation bears on this question:

TABLE I.

Influence of Time Period on Reinoculability of "Immune" Rats.

Elapsed period since disappearance.	Number reinoculated.	Number developing tumors.	Number in which tumors disappeared.
First 30 days	15	1	1
30 to 60 days	16	5	0
60 to 90 days	26	4	0
90 to 150 days	4	0	
Totals	61	10	1
Control	80	69	21

Table I shows, first, that the state of refractoriness left by the retrogression of growing tumors is not perfect, but is of high degree; and it further indicates that this degree is greatest immediately after the disappearance of the tumors, and becomes reduced subsequently

[1] Gaylord and Clowes, *Med. News*, 1905, lxxxvii, 698.
[2] Ehrlich, *Arb. a. d. k. Inst. f. exper. Therapie*, 1906, No. 1, 77.
[3] Bashford, *Proc. of the Royal Soc.*, Series B, 1907, lxxix, 164.

by mere lapse of time. Within a period of 90 days after the retrogression of the tumor in 61 rats, 10, or 16.5 per cent., proved reinoculable with a tumor which yielded in the control animals 86.2 per cent. of tumors. Moreover, of the 10 tumors developing in the refractory rats, only one was afterwards absorbed, and that was in a rat coming within the first thirty-day period since the original absorption. Among the control rats, on the other hand, the retrogressions were considerably higher, and equalled 31.8 per cent.

It is significant that on a second attempt to implant successfully tumor grafts in 13 rats that had resisted a first reinoculation, from 30 to 150 days previously, none were successful. The control rats of this series gave 75 per cent. of successful implantations.

NATURAL REFRACTORINESS.

It has been repeatedly observed by all who have studied transplantable tumors that animals which resist implantation of tumor grafts are not wholly immune, but are merely refractory, and can often be successfully inoculated with a similar tumor of higher virulence, or, as is sometimes stated, with greater capacity for growth. We were interested not only in confirming this result with the rat tumor, but also in ascertaining to what extent the effect of the first unsuccessful implantation tended to add to the natural refractory state of the animals. Having ascertained that mere lapse of time was attended by diminution of this refractory state in some rats (Table I), a test similar to that employed in them was now applied to the rats which resisted a first inoculation. The result is shown in Table II.

TABLE II.

Influence of Time Period on Inoculability of Negative Rats.

Elapsed period since unsuccessful inoculation.	Number reinoculated.	Number and per cent. developing tumors.	Number in which tumors disappeared.
First 30 days	17	2 (11.7)	0
30 to 60 days	60	31 (51.6)	12
60 to 90 days	62	33 (53.2)	3
90 to 120 days	47	27 (57.4)	3
120 to 150 days	14	5 (35.7)	1
Totals	200	98 (49)	19
Controls	80	69 (86.2)	21

But before discussing the results, another tabulation (Table III) will be given, since it expresses the effect of a third implantation of tumor fragments in rats which had resisted two previous inoculations.

TABLE III.

Influence of Time Period on Inoculability of Negative Rats.

Elapsed period since second negative inoculation.	Number reinoculated.	Number and per cent. developing tumors.	Number in which tumors disappeared.
First 30 days	18	2 (11.1)	1
30 to 60 days	17	3 (17.6)	0
Total	35	5 (14.2)	1
Control	20	15 (75)	6

These tabulations indicate clearly that failure in respect to the tumor implantations depends upon factors which are not controlled entirely by the animal subjected to the inoculation, or even by the fragment of tumor implanted, but represents the joint result of the action of both sets of conditions on each other, namely, those inherent in the animal and those inherent in the tumor. The virulence or the capacity for growth of the tumor used to inoculate the negative animals at the time of the first inoculation did not exceed the average of the tumors employed for implantation at this period of the study, and yet 49 per cent. of successful inoculations were secured. And while the tumor used to inoculate the negative animals at the time of the second inoculations showed among the controls a lower virulence or a lessened capacity for growth than the tumor used for the first reinoculation, yet a further 14.2 per cent. of successful implantations were secured.

The case is not so clear as regards the natural refractory state's being increased by a first unsuccessful inoculation. Table II is suggestive of such an effect as regards the small number of successful reinoculations in the first thirty-day period following the unsuccessful implantation, as compared with the greater number of successful inoculations at later periods. But the figures given are indications merely, and do not serve to establish the point.

SECONDARY AND TERTIARY IMPLANTATIONS.

Ehrlich[4] has pointed out that in the case of actively growing mouse tumors, secondary implantations undertaken at a time at which the first tumor is growing rapidly usually fail to grow. Without discussing the theoretical views which he has offered to explain this observation, we wish to report the results of secondary and tertiary implantations carried out with this slow growing rat tumor. As has already been pointed out, this tumor exhibits in a high degree the property of metastasising in nearby and distant parts of the body, from which it is concluded that the effect on the organism of a growing tumor was not such as to interfere with or inhibit the growth of a secondary tumor. Table IV exhibits the results of the reinoculations, and serves to bring out several points of considerable interest.

TABLE IV.

Reinoculation of Rats with Growing Tumors.

Age of primary tumor at time of reinoculation.	Average size of primary tumor in cm.	Number reinoculated.	Number of primary tumors continuing to grow after reinoculation.	Number with growing tumors developing secondary tumors.	Number of primary tumors not growing or retrogressing.	Number with stationary or retrogressing tumors developing secondary tumors.	Number of secondary tumors retrogressing.
First 30 days	0.7	63	36	36	27	1	2
30 to 60 days	1.1	53	43	23	10	0	0
60 to 90 days	1.3	83	78	62	5	0	0
90 to 120 days	1.2	15	11	9	4	0	0
120 to 150 days	2.5	5	5	2	0	0	0
		Number of rats inoculated.	Number developing tumors.	Number in which tumors disappeared.			
Control		80	69	21			

It shows, in the first place, that a growing tumor does not prevent the successful implantation of a second tumor of the same kind, but it also indicates that the secondary implantations are likely to yield a smaller number of growing tumors, the susceptibility of the rats inoculated having been established, than the primary inoculation. The influence of the time element during which the first tumor has

[4] Ehrlich, *Arb. a. d. k. Inst. f. exper. Therapie*, 1906, No. 1, 77.

been growing in the body appears to be of some importance in determining the result, but the factor that exercises a definite and determining influence is not the original susceptibility of the animals to inoculation, but the state of the primary tumor with reference to its later history. So long as the primary tumor is itself expanding the secondary inoculations are readily accomplished, but when the primary tumor has become stationary in size, or has begun to undergo retrogression, the secondary implantation usually fails. In only one instance among 46 cases did a rat in which the primary tumor was being absorbed develop a secondary tumor. In view of the fact that rats showing retrogressing tumors do not develop secondary tumors, while those with growing tumors tend to develop them, it would be expected that few or none of the secondary tumors should later suffer retrogression. Table IV shows that in only two rats was there a disappearance of the secondary growths.

The influence of the period of growth of the primary tumor on the result of the secondary implantations is shown by the fact that in the first thirty-day period all the rats in which the primary tumor continued to grow developed secondary tumors, and that some rats with growing tumors in the later periods did not develop secondary tumors.[5]

Still another test of the reinoculability of rats with growing tumors was carried out with 29 animals in which two tumors were growing at the time of the third inoculation. This implantation was made during the period of from 30 to 60 days of the duration of the two growing tumors, with the result that of the 29 rats inoculated 23 developed tertiary tumors. It follows from these observations that with the rat tumor with which these tests are made reinoculation is possible in all or nearly all the animals, provided the tumors which originally developed from the first implantation are still growing, and have not either become stationary, or begun to undergo retrogression.

[5] Recently Gay has attempted to show that rats inoculated with this tumor are subject to reinoculation only after the period of metastasis has been reached, and that at an earlier period the result of a secondary inoculation is to cause rather absorption of the original tumor than the successful implantation of the second. These statements are in direct conflict with the observations represented by Table IV.

PROMOTING INFLUENCE OF TUMOR EMULSION.

In the course of experiments performed with a number of organic and other substances to be related, the influence on the growth of the tumors of an emulsion of the tumor substance itself was studied. Previous experiments had shown that fine emulsions of the tumor

TABLE V.

Effect on Tumor Growth of Heated and Unheated Tumor Emulsion.

Mode of treatment.	Treated with emulsion.			Control.	
	Number inoculated.	Number and per cent. developing tumors.	Number and per cent. in which tumors disappeared.	Per cent. developing tumors.	Per cent. in which tumors disappeared.
Unheated emulsion one day and tumor next day	8	7 (87.5)	3 (42.7)	100	70
Heated emulsion one day and tumor next day	10	10 (100)	5 (50)	20	50
Tumor one day and heated emulsion next day	9	9 (100)	6 (66)	100	70
Unheated emulsion subcutaneously and tumor 10 days after	10	6 (66)	6 (100)	80	75
Unheated emulsion intraperitoneally and tumor 10 days after	10	7 (70)	6 (85)	80	75
Heated emulsion subcutaneously and tumor 10 days after	28	28 (100)	4 (14)	82.5	36
Heated emulsion twice at 10-day intervals and tumor 10 days after second injection	10	10 (100)	1 (10)	70	56.2
Heated emulsion three times at 10-day intervals and tumor 10 days after third injection	10	10 (100)	0	87	83.6
Heated emulsion and tumor 20 days after	10	10 (100)	2 (20)	70	56.2
Heated emulsion and tumor 30 days after	10	10 (100)	4 (40)	87.5	83.6
Heated emulsion twice at 10-day intervals and tumor 20 days after second injection	10	8 (80)	0	87.5	83.6
Heated emulsion three times at 10-day intervals and tumor 30 days after third injection	9	9 (100)	1 (11)	90	55.5

in salt solution did not give rise to tumor formation, so that there was little or no risk of having tumors develop from the injected emulsion. The emulsion which was made of a uniform milky appearance was divided into two portions, one being subjected to

heat in a water-bath, at a temperature of 55° C. for thirty minutes before the injection, and the other not being further treated. A standard suspension of lecithin was used as control. Injections of one cubic centimeter of the emulsion were made into the peritoneal cavity and the tumor material used was of the type of growth not definitely adeno-carcinomatous. The experiments have not been repeated since the tumor assumed the latter form. At a later period, and, therefore, subsequent to the injection of the emulsion, tumor fragments were inoculated beneath the skin, and the results observed, as recorded in Table V.

As the table shows, the effects of the injections of the emulsion are considerable. They will now be taken up separately for consideration.

The effects of the unheated and of the heated emulsions begin to be displayed as early as twenty-four hours after their injection, and in about the same degree. Thus the number of positive inoculations is the same in both series, and the number of retrogressions is also about the same. As compared with the control series, the effect of the unheated emulsion is seen in the smaller number of disappearing tumors, and of the heated emulsion in an increasing number of positive implantations. It chanced that the tumor chosen for the experiments with the heated emulsion gave in the controls a low percentage of positive implantations, but as the same tumor was inoculated into the animals previously having had injections of the heated emulsion, the contrast is very marked. On the other hand, the injection of the heated emulsion on the day following the implantation of the tumor morsels is followed by little or no special influence on the number of retrogressing tumors, since they were about the same as in the control series.

The next set of experiments yielded much more striking results. Unheated emulsion was injected subcutaneously and intra-peritoneally, and heated emulsion subcutaneously ten days before the tumor implantations. The rats which had received the unheated emulsion behaved very much as did the controls, since the number of positive inoculations and of retrogressions was about average, while the rats receiving the heated emulsion gave 100 per cent. of positive implantations, and a small number, 14 per cent., of

retrogressions, as against 82.5 per cent. of positive implantations and 36 per cent. of retrogressions in the control series. It appears, then, that rats treated ten days in advance with a heated tumor emulsion show increased susceptibility to the tumor implantations and a diminished tendency to tumor retrogressions, as compared with untreated rats or rats treated with unheated emulsion. Indeed, the unheated emulsion proved not to exhibit any predisposing effect whatever on the growth or the persistence of the tumors.

Another series of experiments was performed with the heated emulsion. The experiments were so arranged that the injections were made two or three times at ten day intervals, and the tumor implantations were conducted at ten, twenty, and thirty day intervals after the second and third injections. These experiments will show the promoting influence of the heated emulsion on the growth and endurance of the tumors. We possess no knowledge of the nature of the constituent of the heated emulsion upon which depends the effect described, or of the mechanism of the promoting influence. In view of facts already established for mice tumors, that blood corpuscles and several kinds of tissues of the same animal species exercise, when injected in advance of the tumor implantations, a restraining influence on the tumor growth, the absence of such a restraining influence following the development of the unheated emulsion of tumor and the development of the promoting qualities following the injection of the heated emulsion are points worthy of special attention. It remains to be ascertained whether this effect is peculiar or limited to this rat tumor, or to a small number of transplantable tumors, or whether it is applicable equally or in some degree to transplantable tumors in general. We are engaged at present in elucidating this point.

TABLE VI.

Summary of Effect on Growth and Persistence of Tumor of Heated Emulsion.

Series.	Total number of rats treated.	Number and per cent. developing tumors.	Number and per cent. in which tumors disappeared.
Control	28	26 (92.8)	19 (73)
Unheated emulsion	28	20 (74.2)	15 (75)
Heated emulsion	106	104 (98.1)	23 (22)

We have summarized in Table VI the effects of the heated emulsion on the growth and persistence of the tumor.

Thus it will be seen that among 28 control rats 26 developed tumors, equalling 92.8 per cent., of which 19, or 73 per cent., later underwent retrogression. Among 28 rats treated with unheated emulsion, 20, or 74.2 per cent., developed tumors, of which 15, or 75 per cent., underwent retrogression. These figures are to be contrasted with the next, in which of 106 rats treated with the heated emulsion, 104, or 98.1 per cent., developed tumors, of which 23, or 22 per cent., later were absorbed.

The fact should be mentioned that when a very large number of animals were to be inoculated a large tumor was always selected, so as to insure uniform results. As the large tumors are more advanced in degree than the small ones, they tend to give a lower percentage of successful implantations and a higher percentage of retrogressions. In the present case these points emphasize the effects of the heated emulsion.

EFFECT OF HEATED EMULSION ON IMMUNE RATS.

As has been already pointed out, rats which have recovered spontaneously from the tumors possess an increased degree of resistance to reimplantation of tumor fragments. In view of the power of the heated emulsion to overcome the natural resistance to the implantation of the tumor, it became desirable to ascertain whether this induced or increased resistance could also be set aside by the emulsion. The experiments made to test this point were carried out on two small series of rats which had recovered from tumors that had developed in animals previously receiving the heated emulsion.

In the first series of eight rats, the tumors had entirely disappeared for a period of from 30 to 90 days, and they received no new injection of the heated emulsion. Three of the eight rats developed tumors from the new implantations, of which one tumor subsequently disappeared. The second series of nine rats had been free of tumor for a period of from 30 to 60 days. The heated emulsion was again injected ten days prior to the second tumor implantations. Four of these developed tumors, of which two subsequently disappeared. The control rats for these series gave 100 per cent. of

successful inoculations and no retrogressions, indicating that the tumor used for implantation was of maximum virulence. There is, therefore, no evidence, so far as this experiment goes, of any influence of the emulsion in promoting successful implantation in these rats.

The experiment was now extended to rats which had not been previously injected with the heated emulsion, but which had recovered spontaneously from tumors. They received the heated emulsion in the usual way, and afterwards implantation of tumor fragments. Two series of control animals were employed: one consisting of animals not previously inoculated, to establish the virulence of the tumor, and the other "immune" rats, so-called, in which no emulsion had been introduced. Table VII summarizes this experiment, from which it will be seen that of ten rats in which tumors had disappeared, from 30 to 120 days previously, one only developed a tumor, and this subsequently disappeared; whereas the ten control animals, which had not been previously inoculated, all developed

TABLE VII.

Effect of Heated Emulsion on Immune Rats. No Emulsion (control.)

Time period since disappearance of tumor.	Number inoculated.	Number developing tumors.	Number in which tumors disappeared.
30 to 120 days	10	1	1
Control	10	10	0
Emulsion on Same Day as Tumor.			
30 to 90 days	10	3	0
Emulsion Ten Days Before Tumor.			
30 to 120 days	10	5	0

tumors, of which none disappeared. These results are to be compared with those obtained in animals in which the injection of the emulsion and the implantation of the tumor were made on the same day, and the emulsion injected one day and the tumor implanted ten days subsequently, as are shown in the lower part of the same table.

Thus, of ten rats which had recovered from 30 to 90 days previously, and which received the emulsion and the tumor on the same

day, three developed tumors; whereas of the ten which had recovered from 30 to 120 days previously, and which received the tumor ten days after the heated emulsion, five developed tumors, in both cases there being no retrogressions.

The experiments are quite suggestive and indicate that the heated emulsion can overcome in some degree the induced resistance or immunity produced by the absorption of the tumor, and also that while this influence exerted by the emulsion is greater in rats which had not previously received the emulsion, it may still be exerted upon animals possessing the increased resistance given by recovery from the tumor following the emulsion. Moreover, the effect begins to be apparent on the same day as the injection of the emulsion, although it is more pronounced ten days afterwards.

EFFECTS OF THE HEATED EMULSION ON RATS WITH DISAPPEARING TUMORS.

Rats with growing tumors may be inoculated a second and a third time with tumor grafts. Rats recovering spontaneously from tumors acquire an increased resistance to tumor implantation. Hence, recovery from the tumor is attended by a rise in resistance in the organism associated with the absorption of the tumor, or to local conditions that affect the nutrition of the growth. The interesting question, therefore, arises as to whether this state of increased resistance which prevails during the retrogression is general or local, and whether it is capable of preventing the development of a secondary graft and can be overcome by the promoting influence on the tumor growth exercised by the heated emulsion. The incidental question was also put, namely, whether a secondary graft in an animal with a disappearing tumor can affect that tumor in such a way as to bring about a renewal of its growth. Table VIII supplies the answer to these questions as far as they have been worked out.

By a system of measurement we were able to ascertain fairly precisely the period at which growth ceased in the tumors and retrogression set in. In this experiment particular attention was paid to these two points. The results seem to indicate that the heated emulsion may overcome in some degree the condition of the organism

that operates to bring about tumor absorption. This indication, which we wish to state guardedly, is based on the observation that of the ten rats with retrogressing tumors receiving the emulsion, in only two did the tumors progress to complete disappearance, which is in contrast to the usual observation. Generally speaking,

TABLE VIII.

Secondary Inoculation of Rats with Disappearing Tumors. No Treatment.

Time period since retrogression began.	Number of rats in series.	Number from which the tumor disappeared.	Number of these rats developing new tumors.	Number in which tumors renewed growth.	Number of these rats developing new tumors.	Number in which primary tumors remained stationary.	Number of these rats developing tumors.
30 to 90 days	10	5	none	3	3	2	2
Control	10	All developed tumors					
Emulsion Ten Days Before Tumor.							
30 to 90 days	10	3	1	4	4	2	1
Control	10	All developed tumors					

when retrogression has once begun, it proceeds to complete disappearance, provided the animals survive the necessary period. Moreover, while in the control series no new implantations developed in animals in which the primary tumors disappeared, yet one of three such animals of the series receiving the emulsion developed a secondary tumor.

A further result of this experiment was the successful implantation of new tumor grafts upon rats with retrogressing tumors. In view of this result, it may be considered as doubtful whether the state of the organism as a whole influences at all times the local tumors in respect to growth and recession, and whether local activities, and particularly the nutritive conditions, may not well play at times the determining part. It has been observed by Velich, Loeb[6] and others that the excision of a part of a stationary or retrogressing tumor nodule may be followed by renewed activity, which is explicable most readily on the supposition that the limitation of growth is affected by local conditions. But a somewhat more significant point developed by this experiment is the awakening, as it were, of

[6] Loeb, *Arch. f. Anat., Physiol. u. wissensch. Med.*, 1902, clxvii, 175.

growth in the receding tumors by means of a secondary inoculation. Thus in seven of twenty rats showing receding tumors, a new growth began, following and apparently as the result of the reinoculation of the secondary graft.

EFFECTS OF ORGANIC SUBSTANCES ON TUMOR GROWTH.

The question which next arises concerns the element of the tumor emulsion upon which its promoting influence depends. We have already stated that we have not been able to determine this substance. We have, however, endeavored to ascertain whether the substance resides in the fluid serum or lymph contained within the emulsified tumor, or within the cells themselves. We have approached this question indirectly by testing the effect of the blood serum of the rat itself. We have, moreover, had in mind in the planning of these experiments the statement of Gaylord and Clowes,[7] that the blood serum of mice which have recovered spontaneously from implanted tumors exercises an immunizing or restraining effect on the development of implanted grafts of mouse tumors, and these effects are not exhibited by the serum of normal rats. It is true that Michaelis,[8] Ehrlich[9] and others have not confirmed this experiment, but since it lay in our way, we have not failed to make certain observations to cover this point.

For the purpose of this experiment the blood serum from several different series of rats was employed. Thus, the effects of the serum obtained from normal rats was compared with those of the serum obtained from rats which had failed to develop tumors on inoculation, and, hence, were designated "negative"; from rats which had recovered spontaneously from growing tumors, and designated "immune"; and finally, from rats possessing growing tumors at the time the serum was collected, and designated "positive." This series of experiments was controlled with bouillon, Ringer's solution, normal horse serum, and heated and unheated emulsion of the tumor itself. The procedures were in different experiments somewhat varied, so that this serum and the other sub-

[7] Gaylord and Clowes, *Johns Hopkins Hosp. Bull.*, 1905, xvi, 130.
[8] Michaelis, *Zeit. f. Krebsforsch.*, 1906, iv, 1.
[9] Ehrlich, *Arb. a. d. k. Inst. f. exper. Therapie*, 1906, No. 1, 77.

stances were given sometimes before and sometimes after the tumor implantations, as is indicated in the tables.

Table IX summarizes the experiment made with these substances which were given on the day preceding that on which the tumor implantations were made.

TABLE IX.

Effect on Tumor Implantations of Blood Serum, etc., Given Preceding Day.

Substance injected.	Number of rats inoculated with tumor.	Number and per cent. developing tumors.	Number and per cent. in which tumors disappeared.
None—control	10	10 (100)	3 (30)
Bouillon—control	10	5 (50)	4 (80)
Normal rat serum	10	8 (80)	7 (87.5)
" Positive " rat serum	5	5 (100)	3 (60)
" Negative " rat serum	6	5 (83)	2 (40)
" Immune " rat serum	4	4 (100)	1 (25)

The summary is instructive, since it shows, apparently, that the bouillon exercises a certain restraining effect, and that next to the bouillon the normal rat serum is the most influential substance. On the other hand, the serum from the so-called " positive " rats did not restrain the original taking of the grafts, while of the series of five animals employed three subsequently suffered retrogression of the tumors, which is a much higher percentage than the retrogressions suffered by the control series. The serum from the rats designated " negative " and " immune " affected the original implantations and retrogressions practically not at all as compared with the control series.

TABLE X.

Effect on Tumor Implantations of Blood Serum, etc., Given Following Day.

Substance injected.	Number of rats inoculated with tumor.	Number and per cent. developing tumors.	Number and per cent. in which tumors disappeared
None—Control	10	2 (20)	1 (50)
Bouillon—Control	10	3 (30)	2 (66)
Horse serum—Control	10	2 (20)	1 (50)
Ringer's solution—Control	10	6 (60)	5 (83)
Normal rat serum	10	6 (60)	5 (83)
" Positive " rat serum	4[10]	3 (75)	3 (100)
" Negative " rat serum	10	4 (40)	2 (50)
" Immune " rat serum	10	4 (40)	3 (75)
Heated emulsion	10	10 (100)	5 (50)

[10] Six of the ten rats inoculated died soon after the inoculation.

Table X exhibits the effects of the injections of the various substances carried out on the day following the tumor implantations.

It chanced that in this experiment the implanted tumor was of poor growing property. In some respects this was an advantage, inasmuch as it allowed for the play and exhibition of influences that are not so easily detectable where the power of growth is maximum. It is clear from the experiment that the rat sera tend rather to promote than to inhibit the success of the implantations, but what is particularly striking in this experiment is the promoting influence on the original tumor graft of the heated emulsion.

Table XI exhibits the influence of these substances when given ten days before the tumor implantation.

TABLE XI.

Effect on Tumor Implantation of Blood Serum, etc., Given Ten Days Before.

Substance injected.	Number of rats inoculated with tumor.	Number and per cent. developing tumors.	Per cent. in which tumors disappeared.
None—Control	10	8 (80)	75
Bouillon—Control	10	7 (70)	71
Horse serum—Control	10	6 (60)	33
Ringer's solution—Control	10	7 (70)	71
Normal rat serum	10	9 (90)	33
"Positive" rat serum	10	9 (90)	33
"Negative" rat serum	10	8 (80)	62
"Immune" rat serum	10	9 (90)	33
Unheated emulsion, subcutaneously	10	6 (60)	100
Unheated emulsion, intraperitoneally	10	7 (70)	85.6
Heated emulsion intraperitoneally	9	9 (100)	22

It supports the previous observations in indicating that the nature of the rat serum plays no part in determining the result of the implantations, and that of all the substances employed the heated emulsion alone produces a marked and undeniable influence on the growth and persistence of the grafts.

The several tables bring out in spite of certain irregularities the important data sought by the experiments. It is perhaps possible to inhibit in some degree tumor development by injecting into the body a short time before the tumor fragments are implanted certain relatively indifferent fluids, of which bouillon seems to be as active as any. On the other hand, the injection at any period, as regards the tumor implantations, of the serum of the blood of the rat,

whether obtained from normal animals, animals carrying tumors, failing to develop tumors, or recovering from them, entirely fails to produce any effect either in the direction of restraining or of promoting growth. It is, therefore, safe to conclude that the property affording resistance or relative immunity to certain rats to the primary inoculation, or developed through spontaneous recovery from tumors once successfully implanted, does not reside in the serum of the blood of these animals.

EFFECT OF BLOOD CORPUSCLES AND OTHER TISSUES ON TUMOR GROWTH.

Bashford[11] observed that the injection of the blood of the mouse exercised a restraining influence on the development of mouse tumors. Schoene[12] observed that the injections of suspensions of mouse embryos produced a similar effect, and Michaelis[13] and Borrel[14] observed that the injections of suspensions of liver and spleen acted apparently in the same manner. The activity of the blood in this respect was shown by Bashford[11] to depend on the corpuscles, and not to reside in the serum, and it may be assumed that in the other cases mentioned it is also the cellular elements and not the fluids upon which the activities depend. The experiments to be related refer to the effects of blood corpuscles separated from

TABLE XII.

Effect on Tumor Growth of Blood Corpuscles Given on Preceding Day.

Substance injected.	Number of rats inoculated with tumor.	Number and per cent. developing tumors.	Number and per cent. in which tumors disappeared
None—control	10	10 (100)	7 (70)
Bouillon—control	10	6 (60)	3 (50)
Ringer's solution—control	10	9 (90)	3 (33)
Normal rat serum	9	8 (88)	1 (12.5)
Washed rat corpuscles	10	9 (90)	4 (66.6)
Unheated emulsion	8	7 (87.5)	3 (42.8)
Heated emulsion	9	9 (100)	6 (66.6)

[11] Bashford, *Annual Report of the Imperial Cancer Research Fund*, 1906, iv, 5.

[12] Schoene, *Münchener med. Woch.*, 1907, liii, 2517.

[13] Michaelis, *Zeit. f. Krebsforsch.*, 1906, iv, 1; *Deut. med. Woch.*, 1907, xxxiii, 1826.

[14] Borrel, *Bull. de l'Inst. Pasteur*, 1907, v, 605.

the serum, washed, suspended in salt solution, and injected into the body, on the tumor implantations. The blood corpuscles and the substances used for controls were injected on the day preceding the implantation of the tumor, or ten or twenty days preceding that operation.

Table XII exhibits the effects produced on the tumor growth by an injection of corpuscles made on the day preceding the tumor grafts.

At the end of this brief period no effect has been produced, since the number of successful inoculations is about the same as those of the controls, which indeed is maximum, while the number of retrogressions is about the equivalent of the controls, indicating that no marked inhibition of growth through the development of increased resistance has been produced. On the other hand, the effect of the injections of blood corpuscles ten days before the tumor implantation is unmistakable, since, as Table XIII shows, not only is the number of successful inoculations greatly reduced, as compared with the control, but all of the tumors which originally developed underwent subsequent retrogression and disappeared.

TABLE XIII.

Effect of Blood Corpuscles Given Ten Days Before Tumor Implantation.

Substance injected.	Number of rats inoculated with tumor.	Number and per cent. developing tumors.	Number and per cent. in which tumors disappeared.
None—control	10	10 (100)	0
Normal rat serum	10	8 (80)	4 (50)
Washed corpuscles	10	4 (40)	4 (100)

The effect of the blood corpuscles is still present as late as twenty days after their injection, as can be seen by reference to Table XIV.

TABLE XIV.

Effect of Blood Corpuscles Given Twenty Days Before Tumor Implantation.

Subtance injected.	Number of rats inoculated with tumor.	Number and per cent. developing tumors.	Number and per cent. in which tumors disappeared.
None—control	10	10 (100)	3 (33)
Normal rat serum	10	10 (100)	2 (20)
Washed corpuscles	10	7 (70)	5 (71.4)

Apparently the effect is disappearing, since the number of successful implantations has not only increased over those of the ten-day period, although still smaller than the controls, but the number of retrogressions has also diminished.

The effects, therefore, of the injection of washed corpuscles of the rat into the peritoneal cavity on the development of grafts of this rat tumor placed beneath the skin is shown by these experiments to be considerable. The effect is not developed within twenty-four hours of the injection of the corpuscles, at which time such inhibition as has been afforded by bouillon, for example, is at its height. But at the expiration of ten days the inhibitory effect of the washed corpuscles is very great, and this effect is still appreciable at the expiration of twenty days, but how much longer it persists we have not undertaken to ascertain. This is in conformity with Bashford's observations on mice, who found that the refractory condition produced by the injection was not so marked four days after the injection as at the expiration of ten days, and that it persisted for at least three weeks.

The final experiment of this series was made with emulsions of several organs in a manner similar to that employed with the blood corpuscles and also with the emulsions of the tumor. These suspensions were injected into the peritoneal cavity, and ten days later the implantations of the tumor fragments were made beneath the skin. Table XV summarizes the results of this experiment.

TABLE XV.

Effect on Tumor Growth of Organ Emulsions Given Ten Days Before.

Substance injected.	Number of rats inoculated with tumor.	Number and per cent. developing tumors.	Number and per cent. in which tumors disappeared.
None—control	10	8 (80)	7 (75)
Unheated liver	10	6 (60)	3 (50)
Heated liver	10	4 (40)	1 (25)
Unheated muscle	10	8 (80)	3 (37)
Heated muscle	10	8 (80)	1 (10)
Unheated spleen	10	8 (80)	3 (37)
Heated spleen	10	5 (50)	2 (40)
Unheated kidney	10	8 (80)	3 (37)
Heated kidney	10	8 (80)	4 (50)
Unheated testicle	10	8 (80)	2 (25)
Heated testicle	10	8 (80)	2 (25)
Unheated tumor	10	5 (50)	4 (80)
Heated tumor	10	10 (100)	8 (80)

It is obvious from examination of the table that emulsions of the organs produce no remarkable change in the organism of the rats, through which the development of the tumor grafts is seriously inhibited. There are, indeed, four places in the table which indicate that inhibition of some degree had taken place. These are in connection with the heated and unheated liver, the heated spleen, and the unheated tumor emulsion. The greatest degree of inhibition observed was in connection with the heated liver, but as compared with the action of the blood corpuscles it may be said that the organs containing much blood tend to be more inhibitory than those which contain little blood, and no action would appear to be exerted by the cells peculiar to the organs. Lastly, as far as the results obtained from the heated organic emulsions can be compared with those obtained from the heated tumor emulsions, they may be said to be of the reverse character.

EXPERIMENTS ON THE INFLUENCE OF RADIUM BROMIDE ON A CARCINOMATOUS TUMOR OF THE RAT.

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INTRODUCTION.

A considerable number of observations have already been published regarding the use of radiation by means of radium bromide in the treatment of malignant tumors. One of the earliest investigators of the subject was Exner,[1] who employed it in the treatment of epithelioma and sarcoma, and who was the first to report on the microscopic examination of tumors so treated. He noted particularly the overgrowth of connective tissue which preceded the disappearance of the tumor cells, and which corresponded in amount more or less with the reduction in size of the growth. The observations which followed Exner's publication were not always so favorable. The greater number of reports emphasized the beneficial effects of radium upon rodent ulcer, which unless it extends over a wide surface is said readily to improve and to disappear completely under the influence of the radiations. Those who have reported successful results are Lehmann,[2] Foreau de Courmelles,[3]

[1] Exner, Ueber die Behandlung von Oesophaguskarzinom mit Radium-strahlen, *Wiener klin. Woch.*, 1904, xvii, 96; Ueber die Art der Rückbildung von Karzinommetastasen unter der Einwirkung der Radiumstrahlen, *ibid.*, p. 181; Ueber die bisherigen Dauerresultate nach Radiumbehandlung von Karzinomen, *Deutsche Zeit. f. Chirurgie*, 1904, lxxv, 379.

[2] Lehmann, Essai sur l'action thérapeutique du Radium, *Arch. gén. de méd.*, 1906, i, 1301.

[3] Foreau de Courmelles, Un peu d'histoire de la thérapeutique du radium, *Actualités médicales*, 1904, xvi, 84.

Czerney,[4] Braunstein,[5] McLeod,[6] Sichel,[7] McIntyre,[8] Davidson,[9] Scholz,[10] Schwarz,[11] Abbe,[12] and others. There is difference of opinion as regards its merits in the treatment of epitheliomata proper. Undoubtedly a considerable number of growths thus treated begin to improve almost immediately, and gradually entirely disappear, while others, apparently similar, continue on the contrary to extend in spite of the treatment. Thus Lehmann, Abbe, Czerney and Scholz report cures in many cases, and Foreau de Courmelles speaks of improvement through its use, while others report either negative or very transitory beneficial effects. The results with the more malignant forms of tumors have been almost uniformly unpromising. In the case of carcinoma of the esophagus producing constriction, it has been possible through the use of a specially constructed apparatus to produce amelioration of some of the most distressing symptoms which result from the obstruction (Exner and Einhorn),[13] but in no case was a cure effected. Abbe states that he observed carcinomata to disappear under the influence of radium, but he also states that there was a marked tendency towards recurrence.

The results obtained through the employment of radium in transplantable mouse tumors have been more gratifying. Apolant[14]

[4] Czerney, *Zeit. f. Krebsforsch.*, 1907, v, 27.

[5] Braunstein, Ueber die Wirkung der Radium-emanation auf bösartige Tumoren, *Therapie d. Gegenwart*, 1904, xlv, 412.

[6] McLeod, Further Observations on the Therapeutic Value of Radium, *Brit. Med. Jour.*, 1904, i, 1366.

[7] Sichel, *Brit. Med. Jour.*, 1904, i, 182.

[8] McIntyre, Radium and Its Therapeutic Effects, *Brit. Med. Jour.*, 1903, ii, 1524.

[9] Davidson, Radium Bromide, *Brit. Med. Jour.*, 1904, i, 181.

[10] Scholz, Ueber die physiologische Wirkung der Radium-strahlen und ihre therapeutische Verwendung, *Deutsche med. Woch.*, 1904, xxx, 94.

[11] Schwarz, Ueber die Wirkung der Radium-strahlen, *Pflügers Archiv*, 1903, c, 532.

[12] Abbe, The Subtle Power of Radium, *Med. Record*, 1904, lxvi, 321; The Specific Action of Radium as a Unique Force in Therapeutics, ibid., 1907, lxxii, 583. Radium in Surgery, *Jour. of the American Med. Assn.*, 1906, xlvii, 183.

[13] Einhorn, Radium Treatment of Cancer of the Esophagus, *Jour. of the American Med. Assn.*, 1905, xiv, 8; Radium Receptacles for the Stomach, Esophagus, and Intestines, *Med. Record*, 1904, lxv, 399.

[14] Apolant, Ueber die Einwirkung der Radium-strahlen auf das Karzinom der Maüse, *Deutsche med. Woch.*, 1904, xxx, 454; Ueber die Rückbildung der Maüsekarzinome unter dem Einfluss der Radium-strahlen, *Deutsche med. Woch.*, 1904, xxx, 1126.

caused the inoculated carcinoma in mice to undergo retrogression in all the animals exposed to the radiations, and a large percentage subsequently entirely disappeared. He states as a result of the microscopical examination of tumors exposed to radium that no relation existed between the amount of radiation and the degree of change induced, and he advanced the theory that the primary action of the rays was on the tumor cells, and that there resulted from this a secondary stimulus which led to an overgrowth of the connective tissue that was characteristic of the disappearing tumors. Bashford,[15] who used the Jensen tumor, corroborated Apolant's results, but he attributed the injurious action of the radium to a variety of factors.

EXPERIMENTAL OBSERVATIONS.

The radium used in the experiments to be related was kindly supplied by Mr. Hugo Lieber, of New York, and later by Dr. Robert Abbe. The specimens supplied by Mr. Lieber were contained in hard rubber capsules covered with a mica plate, and in the form of celluloid coatings. Of the former there were three grades: (1) 10 milligrams of so-called 1,000,000 activity; (2) 25 milligrams of so-called 10,000 activity; and (3) 25 milligrams of so-called 1,000 activity. The celluloid disks were coated with gelatin containing radium of 10,000 and 25,000 so-called activities.

These latter coatings are said to possess the advantage of yielding the maximal action of all the rays emitted, since they can be brought into immediate contact with the skin without any intervening substance which may absorb the less penetrating rays. As far as could be ascertained with the apparatus at hand, and through the use of the electroscope, no loss in activity took place from the coatings after they had been in contact with the underlying tissues, although the tissues themselves were proven to have become radio-active. The coatings were bound by means of plaster strips to the skin covering the tumors, and the capsules were fitted into small felt pads possessing circular openings and were also bound in the same manner

[15] Bashford, Action of Radium on Transplanted Mouse Tumors and Its Relation to the Spontaneous Arrest of Their Growth, *Scientific Report of the Imperial Cancer Research Fund*, 1905, No. 2, Part II, p. 56.

to the skin. The period of radiation ranged from five minutes to six hours, and there was also considerable variation in frequency of treatment.

The rat tumors subjected to radiation belonged to the transplantation generations between the ninth and the seventeenth, inclusive, and corresponded to the stage of simple carcinoma and adeno-carcinoma. The method was to select a number of inoculated animals for radiation, and to retain a similar number of animals in a corresponding condition, as controls. The size of the tumors at the beginning of the radiation varied from 0.5 to 2 centimeters in diameter.

It is noteworthy that the effects of the first radiations were very severe, not on the tumor so much as on the general condition of the animals. That is to say, all the rats subjected to radiation in the first few days of the experiment died. This result was attributed to the fact that the radium had been lying undisturbed in a leaden box for a period of some months, and there had, therefore, been an accumulation of rays and possibly the alpha rays especially, which proved injurious in the manner indicated. A similarly unfortunate result followed the first application of the celluloid coatings, which had also remained unused for several months. Later on, after the treatment had been inaugurated, no such deleterious effects were encountered.

In all but three of the animals submitted to radiation with the Lieber specimens the nodules continued to grow. Complete disappearance occurred in three instances after radiation with the 1,000,000 specimen. These animals showed on subsequent post-mortem examination normal organs. At first sight it might appear that the disappearance of these tumors was referable to the radiation, but in view of the considerable tendency to spontaneous retrogression exhibited by this tumor this assumption is not wholly justified. Indeed, the decision in this case would have to be made, not from the number of retrogressions occurring, but rather from the number of tumors which continued to extend. Viewed in this light, the inevitable decision would be that the radiations had not produced the disappearance of the tumors.

There is one cause of temporary diminution in the size of the

tumors subjected to radiation that should be mentioned. When ulceration takes place there occurs frequently an apparent diminution in the size of the nodules, but this is produced through the loss of tissue from sloughing and subsequent collapse of the peripheral thin shell remaining. The ulceration tends to occur earlier in the radiated than in the non-radiated nodules, probably because of the injury to the skin produced by the radiation. Now the tumors which have undergone this early ulceration, far from being retarded in their growth, continue to extend actively at the borders and ultimately to produce metastases and to cause death.

A series of experiments was made on animals which had received the tumor inoculations on the two sides of the body. The purpose of the experiments was to ascertain if the radium when applied to the tumor on one side would exert any influence on the growth of the tumor on the opposite side of the body, or if the structure of the tumors on the two sides would exhibit any marked differences. The results of the experiments indicated that no special influence was exerted by the radiation either on the tumor immediately treated or on the one on the opposite side. The relative rates of growth showed nothing that the controls did not also show, and the microscopical characters of the two sets of tumors were also in agreement.

At the conclusion of the first series of experiments, which were quite negative in result, we secured through the kindness of Dr. Abbe the use of a preparation of radium much stronger than any which we had previously employed. This specimen consisted of a hard rubber capsule as described, containing ten milligrams of 1,800,000 radio-active radium bromide. This constitutes what Dr. Abbe calls a standard cell. Through its use and his aid by the use of his photographic method we were able to standardize the various preparations loaned us by Mr. Lieber. Thus the specimen containing ten milligrams of 1,000,000 radium was shown to be $\frac{1}{6}$ of the standard strength, and the twenty-five milligrams of 10,000, $\frac{1}{25}$ of of the standard strength. The coatings of 10,000 and 25,000 radioactivity were respectively about $\frac{1}{500}$ and $\frac{1}{300}$ of the standard cell. To obtain the theoretical effects of the standard cell it would therefore be necessary to continue the radiations with the weaker

preparations as many times longer as they are weaker than the standard cell.

Twenty-four rats possessing tumors were subjected to radiation with Dr. Abbe's standard cell, of which nineteen were radiated for one hour, two for two hours, one for three hours, one for one hour and twenty-five minutes, and one for one hour and twelve minutes. The size of the tumors in this series of rats at the time of the radiation ranged from 0.4 by 0.6 centimeters to 1.0 by 1.6 centimeters.

The first noticeable effect of the radiations was the loss of hair over the radiated area, which was accompanied by a severe dermatitis, followed from ten days to two weeks later by ulceration in several nodules. The area of necrosis and original ulceration corresponded exactly to the dimensions of the overlying exposed surface of the radium. The ulcers, once formed, extended afterwards. In six of these twenty-four animals the tumors underwent shrinkage, and in four of these six, in which the tumors were much smaller, they completely disappeared. To some of the animals which survived and in which retrogression did not take place, a second radiation of one hour was given a month later, but no influence was exerted on the progress of the tumors. The results of this experiment must be interpreted in the manner of the last, that is to say, the retrogressions were not more numerous than often occur spontaneously, and the fact that so many of the tumors continued to extend in spite of the radiations indicates that this tumor when developing beneath the skin is not subject to marked inhibition of growth through the radiation.

In order to represent the experiments which were carried out, and the results obtained, the number of animals in each experiment, as well as the nature of the experiment, will be stated.

Nine rats were treated with the radium coatings. The radiations were repeated weekly during the life of the animals. In no case did the tumors disappear, and in every case but one in which there was survival beyond the first few days following the first radiation there was increase in size. In one instance a partial shrinkage of the tumor took place, but complete disappearance never occurred.

Nineteen rats were submitted to radiation with the capsule of

10,000 radium bromide. The number of exposures varied from one to twenty-four. In two animals only did the tumors disappear. In all the others they continued to extend. Hence no effect on the development of the tumor was exerted by these radiations.

Eighteen rats were submitted to radiation with the 1,000,000 specimen of radium bromide. The radiations ranged from one to ninety in number, and from a few minutes to several hours in duration. In one instance only was there complete disappearance of the tumor, and in four instances was there practical arrest of the growth.

Twenty-four rats were submitted to radiation with the 1,800,000 specimen of radium bromide. In four rats the tumors completely disappeared and in two others there was arrest of development. In the others the tumors extended until the death of the animals.

Six rats in which tumors were growing on both sides of the body were submitted to radiation of the tumor on one side. The number of radiations varied from two to fifteen. The radium employed consisted of the 10,000 and 1,000,000 capsules. No appreciable healing effects were produced.

The almost uniformly negative results obtained with the radium in the treatment of the rats led to the testing of the radium preparations on certain mouse tumors. The Jensen tumor, which had previously been studied in this respect by Bashford, was first chosen. Eight mice, for which a number of control animals were kept, were submitted to radiation with the several Lieber preparations, for varying periods of time. In six of these eight the tumors completely disappeared in from two weeks to two months. The two tumors which had not completely disappeared were found on microscopical examination to be completely necrotic. No living cells were found in the tumor nodules proper. The next experiments with mice were carried out with animals in which the Ehrlich sarcoma was developing. The Abbe standard cell was employed, and the radiation continued for one hour. No appreciable effect was exerted upon this tumor, which continued to grow in all the animals as it did in the controls. On subjecting these tumors to microscopical examination, no differences in structure as compared with the unradiated specimens could be discovered.

CONCLUSIONS.

The carcinoma of the rat with which we have dealt, when developing in the form of nodules beneath the skin and near the surface, is not subject to inhibition of growth and such injury as tends to produce retrogression through the influence of radium emanations. White rats are, on the other hand, highly susceptible to the injurious effects of certain of the radium emanations, which may produce death within a short time following the exposure. The local injurious effects of the emanations, when producing no general disturbance in the condition of the animals, suffice often to cause falling out of the hair, the development of a severe dermatitis and ulceration of the skin and the tumors. The extension of the tumors at the edges of the ulcer is not materially restrained.

The Jensen mouse tumor has been found by us as by others to be readily subject to the injurious effects of the radium emanations, which act upon it in such a manner as to lead to its disappearance without causing appreciable injury to the general health of the mice in which the tumor is growing. On the other hand, the Ehrlich spindle-cell sarcoma of mice is not subject to the inhibiting influence of the radium emanations, but continues to grow without apparent diminution of energy, in a manner similar to the control animals not submitted to radiation.

SPONTANEOUS TUMORS OF THE MOUSE.

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PLATES XVII–XXVIII.

Since Hanau[1] first showed, with a carcinoma of the rat, the possibility of successful transplantation of a malignant tumor from one animal to another of the same species, numerous observations have been made along similar lines.[2] Morau[3] showed that the mouse was subject to transplantation of carcinomatous tumors, and Loeb[4] demonstrated that other carcinoma and sarcoma could be successfully transplanted in rats. Jensen,[5] and later Loeb, carried out careful histological examinations of fragments of transplantable mouse tumors, removed at varying periods following inoculation, and ascertained that the new development of the tissue took place entirely from the transplanted epithelial cells and not from the tissue of the host. Bashford[6] has not only confirmed these observations, but he has shown conclusively that the stroma of the tumors is the element that is derived from the host. In recent years an immense activity has developed in connection with the experimental study of transplantable tumors, and many facts of fundamental biological importance have been settled in the course of this experimental investigation. In the following paper no special reference will be

[1] Hanau, A., *Fortschr. d. Med.*, 1889, vii, 321.

[2] It is historically of interest to allude to the unsuccessful attempt of Joseph Leidy to transplant a human mammary carcinoma into the frog, reported by him to the Academy of Natural Sciences of Philadelphia in 1851 (*Proc.*, v, p. 201). Four pieces of the tumor, each half an inch long by one-eighth of an inch broad and thick, were inserted beneath the integument of the back of a large frog.

[3] Morau, H., *Arch. de méd. exper. et d'anat. path.*, 1894, vi, 677.

[4] Loeb, L., *Jour. of Med. Research*, 1901, vi, 28.

[5] Jensen, C., *Cent. f. Bakt., Orig.*, 1903, xxxiv, 28, 122.

[6] Bashford, E., *Scientific Report of the Imperial Cancer Research Fund*, 1905, No. 2, 24.

made to the general lines of investigation being followed, but there will be described, as briefly as possible, the histological structure of and the results obtained from the transplantations into mice of a considerable number of tumors developing spontaneously in that class of animals. During the past three years there have come into our hands twenty-six mice showing spontaneous tumors. These twenty-six animals showed, according to conclusions based upon my study, forty-one primary tumors. In one mouse there was present what appeared to be either a general hyperplasia of lymphoid tissue, or else many metastases from a large superficial lympho-sarcoma, but, of course, this animal was taken as representing one tumor formation. However, ten of the mice, or 38.4 per cent. of the entire number, showed more than one tumor each. Three of these showed three tumors each, and seven, two tumors each.

If we compare our observations with others reported in the literature, we shall find that they agree generally with previous observations, although differing considerably in detail. Thus Apolant[7] reported that among 221 mice showing spontaneous tumors he found 276 primary tumors; that is, 12 per cent. of the mice mentioned possessed more than one tumor. Tyzzer[8] has also reported multiple primary tumors in mice, and Murray[9] mentions that of 119 mice with spontaneous tumors 142 tumors in all were detected. In other words, in Murray's series 15 per cent. of the animals possessed more than one tumor. Reporting further, he states that fourteen mice showed two tumors each; three, three tumors each; and in one animal the entire inguinal mamma had been transformed into a group of nodules. In six of the animals of our series[10] there were present large subcutaneous tumors, and in six, what were taken to be primary tumors of the lung. One mouse showed a subcutaneous sarcoma, a cyst-adenoma of the ovary, and an adenomatous tumor of the lung; another mouse showed a superficial

[7] Apolant, A., *Arb. a. d. k. Inst. f. exper. Therapie,* Frankfurt, 1906, No. 1, 11.

[8] Tyzzer, E., *Fourth Report of the Caroline Brewer Croft Fund Cancer Commission,* 1907, 27.

[9] Murray, *Scientific Report of the Imperial Cancer Research Fund,* 1908, No. 3, 69.

[10] Jobling, *Proc. of the Soc. for Exper. Biol. and Med.,* 1908, vi, 10.

sarcoma together with cyst-adenomata of both ovaries; and, finally, two mice each presented two subcutaneous tumors of different types.[11]

TRANSPLANTATION.

The voluminous literature which has already accumulated on the transplantation of mouse tumors contains many statements of successful and unsuccessful transplantation, but there are available relatively a small series of observations which enable us to determine the percentage of transplantable tumors. Ehrlich[12] reports upon 230 tumors, of which 94 were inoculated into other mice, and 11 per cent. of these proved to be transplantable. Tyzzer[13] reports four carcinomata of mice, two of which were successfully transplanted. Bashford[14] reports that 15 out of 32 sporadic tumors transplanted gave negative results. Of our series of 41 tumors, 26 were inoculated into other mice, and of these 20, or 77 per cent., developed. From these tumors 1,128 mice were inoculated, of which 855 survived more than two weeks. Of these, 104, or 12.1 per cent., developed tumors. Of these 104 grafts which began to grow, 36, or 34 per cent., later underwent retrogression. The number of tumors developing in the first generations varied from 2 to 46 per cent. and the period of incubation of the first tumors ranged from 30 to 180 days. In Ehrlich's series 1,504 mice were inoculated with material from 94 primary tumors, 41 of which, or 2.7 per cent., developed tumors. The percentage of tumors developing in the first generation of our different implantations ranged from 2 to 50 per cent. Bashford[15] transplanted 32 spontaneous tumors of the

[11] Tyzzer in a later article (*Boston Med. and Surg. Jour.*, 1909, clxi, 103) says: "Different types of tumors frequently occur in a single animal. Of the 49 animals of this series 11 presented primary tumors of two types, and 1 animal, primary tumors of four types. In the last there were a hypernephroma, a lymphosarcoma, a papillary cyst-adenoma of the lung, and an adeno-carcinoma of the ovary."

[12] Ehrlich, P., *Arb. a. d. k. Inst. f. exper. Therapie*, 1906, No. 1, 77.

[13] Tyzzer, E., *Fourth Report of the Caroline Brewer Croft Fund Cancer Commission*, 1907, 27.

[14] Bashford, *Scientific Report of the Imperial Cancer Research Fund*, 1908, No. 3, 313.

[15] Bashford, *Scientific Report of the Imperial Cancer Research Fund*, 1905, No. 2, Part II, 19 and 30.

mamma into 2,278 mice, which survived a sufficient time to permit of a final estimation, and 72 tumors developed. That is, one inoculation in 31.1 mice was successful, yielding 3.2 per cent. of successes.

LOCALITY.

In describing the location of the spontaneous tumors of 221 mice, Apolant[16] states that 25 per cent. were situated on the side of the abdomen, 16 per cent. in the region of the vulva, and 15 per cent. between the sternum and the submaxillary glands. Tyzzer[17] reports 20 cases, in which 5, including one lympho-sarcoma, were located in the subcutaneous tissues of the abdomen, 12 were primary lung tumors, one was a lympho-sarcoma of the thorax, and two were adenomata of the kidneys.[18] Murray[19] does not describe accurately the frequency with which the tumors were found in different parts of the body, but judging from the diagram prepared by him and showing the positions of 142 primary tumors, the great majority developed on the side of the thorax and in the inguinal regions.

In our series[20] of 41 primary tumors, 9 appeared in the lungs, 3 in the ovaries, and 29 in the subcutaneous tissues. Of the last, 4 were located in the neck, 9 on the side of the thorax, 14 in the inguinal region, and 2 in the region of the vulva. This series does not include the mouse in which there were multiple growths composed of lymphoid tissue.

[16] Apolant, A., *Arb. a. d. k. Inst. f. exper. Therapie,* 1906, No. 1, 11.

[17] Tyzzer, E., *Fourth Report of the Caroline Brewer Croft Fund Cancer Commission,* 1907, 27.

[18] In a later paper (*op. cit.*) Tyzzer says: "I have now studied a series of 62 primary tumors in mice, and of these, 37, or about 60 per cent., originated in the lung. The next most frequent type of tumor in my series is the lympho-sarcoma, of which there were 10. Only 8 of the 62 tumors were situated externally and 6 of these were epithelial tumors. There occurred 4 tumors of the kidney, of which 2 were undoubtedly hypernephroma. In 2 mice ovarian tumors occurred and in 1 mouse, a sarcoma."

[19] Murray, *Scientific Report of the Imperial Cancer Research Fund,* 1908, No. 3, 69.

[20] Jobling, *Proc. of the Soc. for Exper. Biol. and Med.,* 1908, vi, 10.

METASTASIS.

Hanau[21] in his original article reported the existence of metatases in the lymphatic glands in the neighborhoood of the cancer of the rat. Similar metastases, either appearing with the primary tumor, or as a result of transplantations, have been described by Loeb,[22] Borrel,[23] Haaland,[24] Apolant,[25] Flexner and Jobling,[26] Michaelis,[27] and Tyzzer.[28] With few exceptions the metastases have been confined to the lungs. Apolant reports that of his series of 221 mice suffering from spontaneous tumors, metastases in the lungs were found five times, or in 2.2 per cent. of the animals. Murray[29] reports that of 68 mice with spontaneous tumors, 27 presented lung metastases, and 3, lymphatic gland metastases. In 26 mice of our series lung metastases were found in 5, or in about 20 per cent. of the animals, and no metastases were discovered in any other organ of the body.

MAMMARY TUMOR.

Of the 41 tumors, 29 developed in positions of the body corresponding with the distribution of the mammary glands, and these, with the exception of 5, all presented well defined adenomatous structure. We have concluded in conformity with the present view of different workers that these tumors originated from the mammary gland. Attempts have been made to classify these tumors according to types. Michaelis[30] distinguishes three different types, and Haaland,[31] four; but probably the most satisfactory classification at present is that offered by Apolant.[32]

[21] Hanau, A., *Fortschr. d. Med.*, 1889, vii, 321.
[22] Loeb, L., *American Jour. of the Med. Sciences*, 1903, cxxv, 243.
[23] Borrel, *Ann. de l'Inst. Pasteur*, 1903, xvii, 81.
[24] Haaland, *Ann. de l'Inst. Pasteur*, 1905, xix, 165.
[25] Apolant, *Arb. a. d. k. Inst. f. exper. Therapie*, 1906, No. 1, 11.
[26] Flexner and Jobling, *Jour. of the American Med. Assn.*, 1907, xlviii, 420.
[27] Michaelis, *Zeit. f. Krebsforsch.*, 1907, v, 189.
[28] Tyzzer, E., *Fourth Report of the Caroline Brewer Croft Fund Cancer Commission*, 1907, 27.
[29] Murray, *Scientific Report of the Imperial Cancer Research Fund*, 1908, No. 3, 69.
[30] Michaelis, *Zeit. f. Krebsforsch.*, 1906, iv, 1.
[31] Haaland, M., *Ann. de l'Inst. Pasteur*, 1905, xix, 165.
[32] Apolant, H., *Arb. a. d. k. Inst. f. exper. Therapie*, 1906, No. 1, 11.

He begins by referring to the difficulty of classifying these mouse tumors, and speaks of the similarity of their histological structure to thyroid tumors of man. He distinguishes two main groups, adenoma and carcinoma, and further divides the adenoma into simple adenoma, cyst-adenoma, hemorrhagic cyst-adenoma and papillary cyst-adenoma. The carcinoma he divides further into simple alveolar carcinoma, hemorrhagic cyst-carcinoma, and papillary carcinoma. We have followed Apolant's classification in describing the twenty-five tumors of our series, composed of epithelial cells and stroma, and arising in the mammary region. These twenty-five tumors were composed of ten hemorrhagic cyst-adenomata, twelve adeno-carcinomata, two cyst-adenomata, and one alveolar carcinoma. Before proceeding to a somewhat more detailed description, it should be stated that these tumors are not strictly homogeneous, but that almost every one shows heterogeneous areas of a type differing more or less from the one under which it was classified, so the most that can be said is that the dominant structure has been chosen for the purpose of classification.

ADENO-CARCINOMA.

By far the greater number of the tumors of mice thus far reported belong to this class. It is interesting to recall that Hanau's[33] original study was of a squamous cell carcinoma of the vulva of the rat, but the tumors of mice and rats described since that time have been with few exceptions adeno-carcinomata of the mammary gland. Of the twelve spontaneous tumors of this class coming into my hands, ten were used for transplantation, of which nine were successfully transplanted into other mice. Of 464 mice inoculated with these tumors, 407 survived two weeks or longer, of which 57, or 14 per cent., developed tumors. The average period of incubation for this series was 63 days. Among the ten animals whose tumors were transplanted, four showed lung metastases and four also exhibited primary tumors of the lung.

These tumors were, with one exception, in which there was ulceration of the skin, freely movable and apparently encapsulated. The tumors in two of the series showed areas of keratinization.

[33] Hanau, *Fortschr. d. Med.*, 1889, vii, 321.

The keratin transformation of the epithelial cells of these tumors has long been known. It was present in Hanau's original carcinoma, and has been described by Borrel and Haaland,[34] Erdheim,[35] Haaland,[36] Bashford[37] and Lewin.[38] In Lewin's case, as in Hanau's, the condition was present in tumors of the rat, and in all the others, in tumors of mice. In Lewin's case the keratinization did not appear until the third generation, when it became diffuse. In only one of the two cases observed by us did the transplanted fragments develop, and it is interesting to record that the keratinization did not appear in the subsequent tumors.

Generally speaking, these tumors are solid in structure and grayish-white in color. They are composed of microscopical lobules filled with closely packed, regular acini, which often contain a material of homogeneous structure staining deeply in eosin. That these tumors are to a certain extent invasive is shown by the presence of striated muscle fibers in the main tumor. The stroma separating the lobules tends to be dense and thick, although cellular in places, while that separating the acini is delicate and sometimes difficult to make out. The acini are usually lined with a single layer of cells, cubical in shape, and possessing nuclei rich in chromatin. All the tumors of this class show in addition to the acinous arrangement solid nests of cells. These cells are no longer cubical, but irregularly shaped, and possess a large vesicular nucleus, which is less rich in chromatin. These cells also invade the acini, which they do not quite fill. The two kinds of cells are readily differentiated by their staining characters.

DESCRIPTION OF THE INDIVIDUAL TUMORS.

1. The first of this series was in an old, white, female mouse (Original iv). The tumor occupied the left inguinal region, measured three and one-half centimeters in diameter and had invaded the lumbar and abdominal muscles. The skin was involved

[34] Borrel and Haaland, *Compt. rend. Soc. de biol.*, 1905, lviii, 14; Haaland, *Ann. de l'Inst. Pasteur*, 1905, xix, 165.

[35] Erdheim, *Zeit. f. Krebsforsch.*, 1906, iv, 33.

[36] Haaland, *Norsk Mag. for Lægesvidensk.*, 1907, v, 105.

[37] Bashford, Murray and Haaland, *Berliner klin. Woch.*, 1907, xliv, 1194.

[38] Lewin, *Berliner klin. Woch.*, 1907, xliv, 1602.

and ulcerated. On section there were small cysts and hemorrhagic foci. The right lung presented a nodule the size of a pin head. The structure was characteristic, being chiefly adenomatous (Plate XVII, Fig. 1), but showing some solid nests of cells and numerous mitotic figures. As was to be expected, small hemorrhages were also present. The lung nodule consisted of a metastasis composed mainly of a solid growth of epithelial cells, with, occasionally, the appearance of acini. It was situated just beneath the pleura and showed numerous mitotic figures. The stroma was delicate.

From the original tumor 50 mice were inoculated, of which 3, or 6 per cent., developed tumors. Of these 3, a growth was first noted in two on the nintieth and in one on the one hundred and fiftieth day after implantation. The transplantation tumors were made up of solid growth of tumor cells (Plate XVII, Fig. 2), between which the stroma was slight and cellular, and sometimes edematous. There were numerous mitotic figures, many vessels, and frequent hemorrhages. The necrotic areas were frequent.

In the succeeding generations the tumor preserved its histological characters, but in the sixth generation the necrosis and hemorrhages became more numerous, and acini, similar to those in the original tumor, became common (Plate XVIII, Fig. 3). The successful transplantations of this tumor never exceeded 40 per cent., and the incubation period averaged ultimately about fifteen days.

2. An old, white, female mouse (Original vi) showed a tumor measuring one and one-half centimeters situated in the left inguinal region. The skin over the tumor was tense and purple, but free. The tumor was movable. On section there were a few hemorrhagic areas in an otherwise grayish-white tissue. The right lung showed a tumor nodule.

The inguinal tumor was of glandular type, consisting of tubules, some of which were dilated to form cysts, and there were associated solid areas of cells (Plate XVIII, Fig. 4). The hemorrhagic cysts were apparently of secondary origin, and caused by hemorrhage into necrotic foci. The lungs showed several small metastases confined about the blood vessels and composed of solid nests of epithelial cells. In one instance there was observed tubular formation within a small blood vessel. In one place a growth was observed in an

artery, from which it extended into branches, and in another place several large arteries were occluded by the growth. There were numerous mitotic figures present.

59 mice were inoculated, of which 3, or 5 per cent., developed tumors. The average period of incubation was 120 days. Under the microscope the secondary tumors resembled one another, but differed greatly from the original tumor. The adenomatous structure so striking in the original had been succeeded by solid growths of cells in the secondary tumors (Plate XIX, Fig. 5). Mitotic figures were numerous, and hemorrhages were common in the stroma and between the tumor cells. With material from the first transplantation generation, 143 mice were inoculated, of which 8, or 5.6 per cent., developed tumors. This tumor was carried to the 7th transplantation generation, and the number of successes never exceeded 50 per cent. The microscopical structure of the transplantation generations remained essentially fixed, and consisted chiefly of solid nests of cells with here and there acini and a small amount of stroma. The only striking variation was a somewhat greater development of the cellular and edematous stroma. Hemorrhages appeared not infrequently.

In the second generation one of the tumors presented an appearance somewhat suggestive of a mixture of sarcoma and carcinoma (Plate XIX, Fig. 6). The epithelial cells formed lobules, the central portions being usually necrotic. The lobules were separated from one another by thick bands of very cellular stroma. Large blood vessels were present, the walls of which, in many instances, were composed only of a layer of endothelial cells. It was at first thought that we were dealing here with a transition of the carcinoma into a sarcoma, but the daughter tumors which developed gave no further evidences of this structure.

3. An old, white, female mouse (Original vii) showed a large semi-fluctuating tumor in the right inguinal region. The skin was tense and purple in color, but free. On section the tumor was composed simply of two large cysts filled with a dark brownish fluid. The tissue at the margins was of a grayish color and friable. Under the microscope the preserved tissue showed a structure similar to No. 2.

From this tumor 18 mice were inoculated, of which 3, or 16.6 per cent., developed tumors. The incubation period for two of the three was 45 days, and for the other it was 70 days. Under the microscope these tumors were seen to be composed of closely packed acini, and small hemorrhages were observed. For the second transplantation generation, 61 mice were employed, and 9, or 14.7 per cent., developed tumors. The shortest incubation period was 30 days, and the longest was 150. For the third generation, 56 mice were inoculated and 4, or 7.1 per cent., developed tumors. Of these 4, 3 subsequently were completely absorbed. The histological structure of the transplantation generations was similar to that of the original tumor. The acinous arrangement recurred, but cysts were also present. Mitotic figures were numerous.

4. A very old, white, female mouse (Original xii) presented a tumor measuring one and one-half centimeters in the right inguinal region. The tumor was freely movable. In consistence it was quite firm. The left lung presented at the lower margin a grayish-white wedge-shaped tumor measuring three millimeters in diameter. In the right axilla was a nodule measuring 0.5 centimeter. The original tumor showed under the microscope a somewhat compound structure. While the main portion was composed of solid growths of epithelial cells, there were some acini and small dilated cysts. Where the glandular type occurred, the stroma was fibrous, while in the more solid portions it was delicate. There were some hemorrhages, and mitotic figures were numerous. The lung nodule proved to be a primary cyst-adenoma and will be described elsewhere.

The nodule in the right axilla proved to be composed of the closely packed tubules separated from one another by delicate stroma. The nuclei of the small cells were dense and mitotic figures were not found. No remnants of lymphoid tissue were discovered, and it is probable that this represented an independent tumor formation.

50 mice, 30 white and 20 colored, were inoculated, of which 33 survived two weeks or longer, and one developed tumor, which, however, subsequently underwent absorption. It was detected for the first time 30 days after the inoculation.

5. An old, white, female mouse (Original xiii) showed a tumor measuring one and one-half centimeters in the left inguinal region, which was freely movable. The consistence was firm and the cut surface grayish-white in color. Under the microscope this tumor was almost of pure glandular type (Plate XX, Fig. 7). Microscopical examination of the lungs showed a primary cyst-adenoma which will be described elsewhere.

In the first generation, 80 mice were inoculated and 67 survived. 8 of these, or 11.9 per cent., developed tumors, two of which subsequently underwent absorption. The average incubation period was 70 days. In the second generation, 20 mice were inoculated, and 6, or 33 per cent., developed tumors. The incubation period was 30 days. The structure of the transplantation tumors was similar to the original. In the third generation, 20 mice were inoculated, of which 6, or 33 per cent., developed tumors, and the incubation period was 25 days. In this generation the histological type had altered, so that some of the tumors corresponded to the original, and others were composed of solid lobules with necrotic centers and heavy stroma (Plate XX, Fig. 8). In both, mitotic figures were numerous.

6. A japanese waltzing mouse (Original xv) presented a tumor situated in the front of the neck, almost in the median line, freely movable, and measuring one centimeter in diameter. Under the microscope this tumor was made up of numerous acini and a few cysts. The cells forming the more solid portions had through pressure become flattened and spindle-shaped. The stroma was slight, and mitotic figures were numerous. Two small metastases containing acini, but less uniform in structure than the original tumor, were present in the lungs.

40 mice, consisting of 30 white and 10 waltzing mice, were inoculated. None of the white mice developed tumors. Seven of the dancing mice survived longer than two weeks, of which three, or 42.8 per cent., developed tumors. These three died while the tumors were still small, and no further transplantations were attempted. The transplantation tumors resembled histologically the original.

7. A white, female mouse (Original xx), showed, in addition to

two adeno-carcinomata of the type we are describing, other primary tumors, namely, two hemorrhagic cyst-adenomata of the neck and inguinal region, and a primary cyst-adenoma of the lung. One of the adeno-carcinomata, situated on the lower part of the abdominal wall, measured two centimeters, and the other, which was situated in the left inguinal region, measured seven millimeters. These tumors presented a distinctly glandular character, being composed of acini similar to the others of the series. Muscle fibres had in some places been included in the growing tumors.

40 mice were inoculated, of which 28 survived, and 12, or 42.8 per cent., developed tumors. The incubation period ranged from 30 to 60 days. The tumors presented microscopically two types: first, similar to the original; and second, solid cell nests devoid of acini. 40 mice were inoculated in the second generation, and yielded 16, or 40 per cent. of tumors, the average incubation period being 30 days.

8. An adult, light brown, female mouse (Original xxiii) showed a tumor measuring one by one and one-half centimeters, in the right inguinal region, surrounded by subcutaneous fat. It was solid in consistence. In microscopical structure the tumor was of the glandular type. It contained small cysts, and the stroma was fairly well developed. Mitotic figures were numerous.

26 white (?) mice were inoculated, of which 2, or 7.6 per cent., developed tumors, one appearing at the end of 60, the other at the end of 120 days. Except for more numerous hemorrhages, they resembled the original. In the second generation, 20 mice were inoculated, of which 3, or 15 per cent., developed tumors. The structure had now altered: there were fewer acini, a general solid growth of cells, and numerous hemorrhages. Mitotic figures were numerous.

9. A white, female mouse, apparently very old (Original xxiv) showed a tumor measuring 1.3 centimeters on the right side of the thorax, just below the axilla. The structure was solid and lobulated and there was involvement and ulceration of the skin. Under the microscope the growth was more solid in type than the other tumors of this series, but a certain amount of acinous formation could be made out. Keratinized foci (Plate XXI, Fig. 9) were num-

erous, the keratinized cells sometimes appearing at the margin of a necrotic area, sometimes surrounded by necrotic material, and sometimes occurring in the preserved parts of the tumor.

50 mice were inoculated with this tumor, but no secondary tumors developed.

10. A yellow, female mouse (Original xxv) presented a tumor measuring one-half centimeter in diameter, situated on the right side of the neck and freely movable. The tumor was friable, and showed minute hemorrhages. There were numerous nodules in the lungs. The microscopical structure was acinous and similar to others in this series, and there were numerous small cysts present containing a serous exudate and sometimes blood. Within some of the cysts there were keratinized areas, showing typical "pearls" (Plate XXI, Fig. 10). Some solid areas of cells also occurred. The lung metastases were numerous and were confined to the blood vessels, which, in many instances, were occluded by them. In other cases the lung tissue was replaced. When the growth occurred in the blood vessels it was usually solid, and mitotic figures were numerous. When the growth in the vessel did not fill it, the outer surface was sometimes covered by endothelium, and in one instance a second independent growth of tumor was observed to have taken place between the old mass, which was covered with endothelium, and the vessel wall. The central portions of some of the large intravascular growths had become necrotic and formed cavities. The extra-vascular growths showed acini and some solid nests and numerous mitotic figures (Plate XXII, Fig. 11).

There was a larger, wedge-shaped nodule in the lung, which proved to be a primary cyst-adenoma, and which will be described elsewhere (Plate XXII, Fig. 12).

Of 50 mice inoculated, 23, or 46 per cent., developed tumors. Of 33 mice inoculated in the second generation, 18, or 54.5 per cent., developed tumors. In structure the secondary tumors resembled the original, except that keratinization was not observed.

HEMORRHAGIC CYST-ADENOMA.

A somewhat special interest is attached to this class of tumors because Ehrlich[89] employed them in an extensive series of experi-

[89] Ehrlich, *Arb. a. d. k. Inst. f. exper. Therapie,* 1906, No. 1, 77.

ments to induce immunity. Having discovered that they were transplanted with greater difficulty than other mouse tumors, and that animals which had successfully resisted their implantation presented an increased resistance to the inoculation of other tumors readily transplantable to normal mice, he employed them for producing this refractory or immune state. On the other hand, some investigators have succeeded in transplanting this type of hemorrhagic tumor. Hertwig and Poll[40] report a successful instance, and Tyzzer[41] reports a successful instance, so far as the first generation is concerned, while Gierke[42] reported that of 47 such tumors, 38 were transplanted successfully by him. He gives the details of his experiments with 35 separate tumors. Of these 35, 29 were transplantable, and of the 29, 15 became extinct as follows: 8 in the first generation, 5 in the second, and 2 in the third. In only one instance was he successful in carrying a tumor of this class into the fourth transplantation generation. Gierke states that of 2,851 mice which survived for a sufficient time, 187, or 6.5 per cent., developed tumors. The highest original result ever attained was 33 per cent., and the highest subsequent one was 70 per cent. of the tumor fragments implanted.

The tumors of this class can frequently be detected from their gross appearances, since the skin covering them is usually tense and purplish in color, and the section of the tumor shows large cysts containing bloody contents and little original tumor tissue. They are as a rule incapsulated. Under the microscope the more solid portions are composed of regular acini, but everywhere there are cysts of variable size, produced through dilatation of the acini and through necrosis of the tumor. The cells which line the acini are usually cubical and rich in chromatin, while those lining the cysts are usually flattened. The stroma is usually delicate in character, but may be thicker and denser, or edematous. Large hemorrhagic foci are scattered through the sections, as well as large thin-walled vessels. The walls of the vessels are so thin at times that they

[40] Hertwig and Poll, *Abhand. d. k. preuss. Akad. d. Wissensch,* Berlin, 1907, i, 1.

[41] Tyzzer, E., *Fourth Report of the Caroline Brewer Croft Fund Cancer Commission,* 1907, 27.

[42] Gierke, E., *Scientific Report of the Imperial Cancer Research Fund,* 1908, No. 3, 115.

are made out with great difficulty, and thus it becomes uncertain sometimes whether the blood is contained within the vessels, or within a cyst. The mitotic figures are usually infrequent.

In our series there were ten tumors belonging to this class, from which 365 mice were inoculated. However, as 61 died within the first two weeks, only 304 survived long enough to permit the development of tumors. Of these, 21, or 6.9 per cent., developed tumors and later seven of the tumors became absorbed. Of these ten tumors, seven, or 70 per cent., gave transplantation tumors in the first generation. Of these seven, two failed to develop tumors in the second generation, and one in the third, while the remaining four were transplanted successfully through successive generations. In one of the ten mice a lung metastasis was present, although in three others primary lung tumors occurred.

1. The tumor measured one and one-half centimeters in diameter, and appeared in the right inguinal region of a female mouse (Original i) kindly presented to us by Dr. F. C. Wood of New York. The skin covering the tumor was tense, partially free of hair, and purplish in color. The tumor was fluctuating on palpation, and on incision, a large amount of dark fluid escaped from a smooth-walled cyst on the inner wall of which papilliferous masses were attached.

The solid portion of the tumor was suspended in normal salt solution, after being cut into minute fragments, and 15 mice were inoculated under the skin. None developed tumors. Under the microscope the tumor was found to be composed mainly of cysts in the tissue, between which there were regular acini. Hemorrhages of different durations had taken place into the cysts. A small number of mitotic figures were seen, and occasionally solid masses of cells.

2. A brown, female mouse, 15 months old (Original ii), showed a tumor on the left side of the body, just below the axilla, which measured 1.75 centimeters. The tumor was grayish-white in color, lobulated and presented on section many small hemorrhagic cystic areas. On the opposite side of the body in the same general position a nodule 4 by 6 millimeters occurred. It was loosely attached

to the skin, and is described under the cyst-adenomata. Under the microscope the larger tumor was composed of closely packed tubular acini, lined by a single layer of epithelial cells. ' Sometimes the epithelium was arranged in several layers, or entirely filled the spaces. Hemorrhages were scattered through the tissue, being contained within cyst-like spaces. The blood vessels presented very thin walls and appeared often as sinusoids. One of the mammary glands adjacent to the nodule showed much dilated tubules, filled with a glandular eosin-staining detritus.

41 mice, of which 19 were brown and 22 white, were inoculated with fragments which had not come into contact with saline solution, and none of the 19 brown mice developed tumors, while 21 of the 22 white mice developed them. One of these was first noticed on the 150th and the other on the 180th day after inoculation. From the former, 20 white mice were inoculated, one of which died in a few days, and of the remaining 19, 3, or 15.7 per cent., developed tumors, one of which subsequently underwent absorption. The incubation period in this series ranged from 60 to 90 days.

Microscopical examination showed the transplantation tumors to be more solid in character than the original, to contain hemorrhages and to show quite numerous mitotic figures. Some acini were present, and the epithelial cells in the two kinds of structures resembled the original. Microscopical examination of the tumors of the second transplantation generation showed marked changes as compared with the original. There were still present numerous acini and cysts, but the latter were produced by a disintegration of necrotic cells. The tumors were also more lobulated, and there were ingrowths from the sides of the lobules of a somewhat variable character. However, the larger part of the tumor consisted of a solid growth of cells. The stroma was cellular and well developed in places, but the blood vessels were still large and thin-walled. Hemorrhages were not infrequent, but not so common as in the original tumor. Mitotic figures were not infrequent.

In the third generation, forty mice were inoculated, yielding one slowly growing tumor. In the fourth generation, twenty were inoculated yielding seven tumors, or 35 per cent. Subsequently, six

of these seven were absorbed. Further transplantations were not attempted.

3. White, female, apparently old mouse (Original iii) showed a tumor on the left side, just below the axilla and extending in front almost to the median line. The skin was tense and purplish in color. On removal the tumor was dark brown, and on section exuded a dark fluid. It measured three by one and one-half centimeters. Under the microscope it proved to be composed of lobules consisting of closely packed acini. The centres of the lobules usually were cystic, and the cysts contained an eosin-staining, homogeneous substance and blood. Some solid nests of cells existed, and mitotic figures were numerous. Muscle fibres were contained in the upper pole of the tumor.

59 mice were inoculated, of which 4, or 6.7 per cent., developed tumors. The first growth was noticed on the 120th day, and the later growths on the 150th and 180th days. All but small parts of three of the tumors were excised, and used to inoculate 34 mice. The fragments of tumor left behind underwent subsequent absorption. Of the 34 mice of this series, one developed a tumor at the end of 60 days. As the tumor was growing very slowly, an incision was made in it with the idea that it might be stimulated to more active growth, but after the operation there was complete absorption. The microscopical examination of the first transplanted tumors showed cystic growths in which the cysts were filled with blood (Plate XXIII, Fig. 13). The general structure was acinous. The microscopical examination of a fragment removed from the second generation transplantation showed a papillomatous growth with cysts and some hemorrhages (Plate XXIII, Fig. 14).

4. White, female, evidently old mouse (Original viii). The tumor had been partially eaten away by another mouse in the same cage. It was on the left side and partially surrounded the vagina. In color it was dark brown, and contained several cysts in which there was a dark brown fluid. Microscopical examination showed the tumor to contain a large amount of dense stroma, and to be divided into small lobules, which themselves were made up of acini. Hemorrhages were common in and between the latter.

30 mice were inoculated with fragments of this tumor, but no secondary tumors developed.

5. White, female mouse (Original ix) showed a tumor measuring two centimeters on the right side just below the axilla. The skin was tense and purplish in color. The external portion of the tumor was composed of a large cyst and there were smaller cysts in other portions. Under the microscope the tumor was found to be composed almost entirely of cysts which in places were being replaced by granulation tissue. The sections removed from the deeper portions alone showed traces of the original tumor. Many hemorrhages were present, and the blood vessels were numerous.

40 white mice were inoculated from this tumor, of which 25 died in less than two weeks. Of the 15 surviving, one, or 6.6 per cent., developed a tumor, and the incubation period was 60 days. This mouse died during the hot summer months, and decomposition set in so rapidly that no further attempts were made to transplant it to other mice. Microscopical examination showed the transplanted tumor to be like the original.

6. White, female and apparently old mouse (Original x) showed a tumor measuring two centimeters in the left inguinal region. The skin over it was tense and purplish in color. On section, the tumor was composed almost entirely of cysts containing a dark fluid (Plate XXIV, Fig. 15.). The cyst walls were thin, and there was little of the solid tumor tissue left except at the margins. A small nodule was present just beneath the pleura in the left lung, which proved to be a primary cyst-adenoma. In microscopical appearances this tumor was similar to others of this class, and cysts and hemorrhages were everywhere present. There were numerous mitotic figures.

30 mice were inoculated, of which 9 died within a few days. Of the 21 surviving, 2, or 9.5 per cent., developed tumors. The incubation period was 60 days. One of the two was ultimately absorbed, and the remaining mouse with tumor died before it became large enough to be used for transplantation.

7. A brown, female and apparently old mouse (Original xi) showed a tumor at the right axillary region, measuring two by one and one-half centimeters. It was composed of a large cyst externally, and was filled with a dark, thick brownish fluid. The deeper portion was grayish-white in color and firm in consistence. There

were numerous lung metastases. Excepting for the thickness and density of the stroma, this tumor was very similar to others previously described. In some places there was a tendency towards development of papillary outgrowths in the walls of the lobules. The lobules for the most part were filled with closely packed acini, and the stroma contained many large phagocytic cells filled with blood pigment. Mitotic figures were not numerous. The lungs were thickly studded with metastases, confined chiefly to the blood vessels. Few of the larger vessels had escaped invasion. The growths in the lung resembled the original, even to the formation of cysts and the presence of hemorrhages. This animal died during one night and when it was examined the next morning there was already beginning decomposition. 40 mice, of which 10 were white and 30 light brown, were inoculated, and no tumors developed.

8. An adult, white, female mouse (Original xvi) presented a tumor in the right inguinal region, measuring two and one-half centimeters. The skin was attached and ulcerated. The tumor was gray and contained numerous hemorrhages. A single small cyst was observed. The left lung contained a small primary tumor. Under the microscope the superficial tumor was distinctly hemorrhagic in type, but less homogeneous in structure than the others of this class. The arrangement of the epithelium was highly varied. Sometimes it was glandular and formed large cysts; at other times there were lobules filled with a solid growth. Necrosis was common, and hemorrhages frequent, and the blood vessels were large and possessed thin walls. In some places the acini had become greatly dilated, and into their lumina larger cells with vesicular nuclei had penetrated.

From this tumor 40 white mice were inoculated, of which 12 died in a few days. Of the 28 surviving, 6, or 21 per cent., developed tumors; the incubation period was 20 days. Three of these tumors subsequently underwent absorption. The microscopical structure of the secondary tumors agreed with the original.

Of 37 mice inoculated from the first transplantation generation, 13, or 35 per cent., developed tumors, of which 8 subsequently underwent absorption. The incubation period was about 25 days.

The glandular or acinous structure present in the original tumor could be made out hardly at all in the tumors of this generation. The stroma had become well developed, and subdivided the tumor into small lobules, which were filled with cells showing no definite arrangement. A striking feature was the strong development of the stroma. Necrosis of the central portion of the lobule was common.

20 mice were inoculated for the third generation, of which 5, or 25 per cent., developed tumors. Three of these subsequently underwent absorption. The incubation period was about 25 days. The structure remained about the same as the previous generation. 28 mice were inoculated in the fourth generation, of which 11, or 39.2 per cent., developed tumors. Eight of these subsequently were absorbed. Microscopical appearances of this generation were similar to the original tumor.

9. Dark, gray, female mouse (Original xix), among the stock animals of the institute, showed a tumor 1.3 centimeters in diameter, situated beneath the skin of the lower portion of the abdomen, to the left of the middle line. It had slightly invaded the muscles of the thigh. The surface was irregular. The tissue was hemorrhagic and there were numerous cysts. In appearance it was found to be composed of numerous cysts and necrotic areas containing hemorrhages, and areas of epithelial cells showing little definite arrangement. Mitotic figures were common. The blood vessels were greatly dilated.

From this tumor 20 mice, 10 of the color of the original, and 10 white, were inoculated. Of the 10 white mice inoculated, 2 died in a few days, and of the 8 surviving, one developed a tumor after 45 days, which later became absorbed. One of the ten colored mice developed a tumor after 120 days which was used for further inoculation. Under the microscope this tumor was less adenomatous in structure than the original. It showed hemorrhages and necrotic areas. From this tumor 15 mice (colored?) were inoculated, of which 2, or 13.3 per cent., developed tumors. In one the incubation period was 160 days and in the other, 90. The structure remained about the same as in the preceding generation. Further transplantation was not undertaken with this tumor.

10. White, apparently old, female mouse (Original xx) showed on neck anterior to the right of the median line, a tumor two and one-half centimeters in diameter. The surface was gray and mottled with dark brown, the latter portions being cystic. Under the microscope this tumor was adenomatous in structure, consisting of acini intermingled with solid masses of epithelial cells. Areas of necrosis and hemorrhage occurred, the former being more frequent. The acini were frequently dilated into cysts, some of which contained a homogeneous eosin-staining material, and others, blood. There were numerous mitotic bodies. The blood vessels were widely dilated.

50 mice were inoculated, of which 5, or 10 per cent., developed tumors. One of these later underwent absorption. The incubation period was 30 days. The structure of these tumors differed from the original in that the stroma was far better developed and was very cellular (Plate XXIV, Fig. 16). Large hemorrhagic areas occurred and the acinous arrangement had almost completely disappeared. There were large lobules composed of central cavities containing cells, and hemorrhages were common.

40 mice were inoculated from this generation, of which 3, or 1.5 per cent., developed tumors, all of which underwent absorption before they reached a size large enough to be used for further transplantation.

CYST-ADENOMA.

Of this class there were two tumors, and since they occurred in mice in which the other classes of tumors were present, and which were of larger size, they were not submitted to transplantation.

1. The first of these, which occurred in Original ii, was situated on the right side of the thorax and measured .6 centimeter in its greatest diameter. In form it was flat and freely movable. The tumor with which it was associated was a hemorrhagic cystadenoma.

2. The second of these tumors was in Original v. It was situated on the left side of the thorax, was flat, and measured 0.5 centimeter in its greatest diameter. The tumor with which this was associated belonged to the class termed by Apolant solid carcinoma.

In histological structure these growths were very similar to one another. They were made up of cysts developed from dilated acini, and lined by a single layer of cells, some of which were flattened. The cysts, however, frequently contained a homogeneous, eosin-staining material. No mitotic figures were discovered. The stroma was delicate in character.

The possibility, of course, exists that these second tumors of smaller size were metastases. The type, however, of the smaller tumors was wholly different from the larger ones, and approached more nearly the typical adenomatous form than did the others. Since each was distinctly incapsulated, and there were no mitotic figures discovered, and no evidences available of growths having developed in the lymphatic glands, they were regarded as independent tumors.

ALVEOLAR CARCINOMA.

According to Apolant, in this type of tumor, no acini, strictly speaking, are present, but the tumor consists of lobules containing alveoli closely packed with epithelial cells. One such tumor came under observation among our series. It occurred in a white female mouse (Original v), was situated in the left inguinal region and measured 2 by 2 centimeters. Neither the skin nor the abdominal muscles were involved in it. Its central portion was necrotic, consisting of a cheesy mass, while the cut surface presented a granular appearance. Its external contours were irregularly lobulated, and nutrient blood vessels radiated over its surface. Near the left axilla there was a flat nodular growth measuring 0.5 centimeter, and presenting the same appearances as the large tumor.

Microscopically this tumor consisted of lobules filled with closely packed epithelial cells, many of which through pressure had become flattened and spindle-shaped. These solid masses of cells bore a certain resemblance to spindle-cell sarcoma. Where the lobules were large, the central portions were necrotic. Mitotic figures were numerous. It should be stated that at one point where the stroma was unusually well developed a few acini occurred, and that the second flat nodules presented the characters of cyst-adenoma.

49 mice were inoculated from the alveolar tumor, of which 10,

or 20 per cent., developed tumors. The average incubation period was 70 days. This tumor is now in the sixth generation, and the highest percentage of implantations thus far obtained has been 59 per cent. The structure of the transplanted tumors is similar to that of the original.

MOLLUSCOIDAL TUMOR.

A tumor differing greatly from all others which have come to our attention will now be described. It resembles closely a tumor described by Haaland,[48] and, owing to a certain resemblance to the lesions of *molluscum contagiosum,* he termed it "tumeur molluscoide." He describes the tumor as being composed of lobules with rounded bases at the periphery which radiate from a common centre in the manner of the spokes of a wheel. The lobules are broader at the periphery, and the central portions of them show frequently the presence of keratin. The tumor which came to our attention agrees closely with this one, although the degree of keratinization was somewhat less. According to Haaland's view, the tumor developed from hair follicles, and we are of the opinion that our tumor took its origin in the same structures. He inoculated twelve mice, but in no case did he get a secondary growth.

This tumor occurred in a full grown, brown, female mouse (Original xxii). The growth was situated in the right inguinal region, and measured 2 by 3 centimeters. It had become attached to the skin, and ulceration was beginning. The tumor also penetrated the abdominal muscles and projected slightly into the abdominal cavity. It was grayish-white in color, rather firm, and showed one hemorrhagic area. There were no cysts. In the upper portion of the lower lobe of the left lung a nodule measuring one millimeter in diameter was present.

At the margin of the tumor, where it invaded the neighboring tissues, the cells were arranged in columns, consisting of solid growths of epithelial cells (Plate XXV, Fig. 17). Where these columns were cut transversely, they presented somewhat the appearance of tubules. The general tendency of the tumor was to grow in such columns. Tubular formations were also present, some of which

[48] Haaland, *Ann. de l'Inst. Pasteur,* 1905, xix, 163.

branched and appeared to end blindly in solid masses of cells. These tubules presented several layers of cells. Mitotic figures were very common. Throughout the section irregular masses of kerato-hyalin occurred (Plate XXV, Fig. 18). They usually occupied the central portions of the solid lobules which were found both in the periphery and central portions of the tumor. As a rule, these areas were more abundant in the solid strands themselves, but they also occurred in the tubular formations, the lumen of some of which were filled with it. The nodule in the lung proved to be a primary cyst-adenoma.

50 mice were inoculated with the large tumor, but none developed secondary tumors.

PAPILLARY CYST-ADENOMA OF THE LUNG.

The class of tumors to which the primary lung tumors to be recorded belong was first described by Livingood,[44] who found it accidentally in the lung of a mouse dying of an experimental bacterial infection. Much later Haaland[45] reported five instances of this tumor, but the most complete description in the literature is that of Tyzzer,[46] who described a series of twelve such tumors.[47] It is noteworthy that in Tyzzer's series of cases, six of the animals presented tumors in other parts of the body, or in other words, were the subject of multiple primary tumors. Moreover, the circumstance should be mentioned that in the examples described by Haaland the mice had previously been inoculated with tumor fragments, so that he has considered the possibility of their origin from the inoculated material. Murray[48] has recently reported finding this type of lung tumor, but he does not state with what frequency.

Our observations are limited to nine primary tumors of the lung

[44] Livingood, L. E., *Johns Hopkins Hosp. Bull.*, 1896, vii, 177.

[45] Haaland, *Ann. de l'Inst. Pasteur*, 1905, xix, 165.

[46] Tyzzer, E., *Fourth Report of the Caroline Brewer Croft Fund Cancer Commission*, 1907, 27.

[47] In a later report (*op. cit.*) Tyzzer states that he has met with 37 mice showing primary lung tumors. In one family of 25 mice, four examples of primary adeno-carcinoma of the lung were found; and in another family of 100 mice, springing from a mouse with an adenoma of the lung, 15 mice developed tumors.

[48] Murray, *Scientific Report of the Imperial Cancer Research Fund*, 1908, No. 3, 69.

of this type, one animal of this series presenting two such tumors. These tumors were distributed as follows: two of them occurred in mice in which there were hemorrhagic cyst-adenomata, four in mice with adeno-carcinomata, one in a mouse with multiple superficial tumors, and one in a mouse in which the superficial tumor was a spindle-cell sarcoma. It was this last animal which showed two primary lung tumors, one in each lung. With one exception, the tumors were all too small to be used for purposes of transplantation.

1. The first of these tumors which we came across was in Original Mouse xii. It was located in the lower margin of the left lung, was grayish-white in color, and measured three millimeters in diameter. It projected slightly above the surface of the pleura, and was sharply outlined. Its microscopical appearance consisted of irregularly projecting folds of connective tissue containing cavities which were lined by columnar epithelial cells, the outlines of which were sharp (Plate XXVI, Fig. 19). A small bronchus, the lumen of which was almost occluded by the growth, approached the periphery of the tumor. The epithelial cells of the tumor and the bronchus were almost indistinguishable. The central part had begun to degenerate and contained a greater amount of fibrous tissue. The cavities of the tumor contained large cuboidal cells, undergoing degeneration. Mitotic figures were not discovered. The pulmonary tissue immediately adjacent to the tumor was collapsed. The epithelial cells covering the papillary outgrowths of connective tissue formed a single layer, and the stroma was small in amount.

Serial sections showed the growth to be wedge-shaped, and the apex to consist of two or three papillary outgrowths from the wall of a bronchiole. From the front of the organ the growth extended along a bronchiole into the larger bronchi and the surrounding tissue.

2. The second tumor of this type occurred in Original Mouse x. The nodule measured 0.5 millimeter and was located in the upper lobe of the left lung, projecting slightly above the surface of the organ, and being sharply outlined. Under the microscope this tumor proved to be more solid than No. 1. It also invaded the pulmonary alveoli to a larger extent, which it appropriated to supply its framework. The individual cells were round and the nuclei were rich in chromatin. Mitotic figures were not discovered. In the spaces

between the papillary outgrowths numerous rather large cuboidal cells were present.

3. The third tumor was found in Original Mouse xiii. It was not seen at the original examination of the animal, and was detected subsequently in the study of sections of the lungs. The tumor was located in the central portion of the upper lobe of the right lung. In structure it resembled lung tumor No. 1, except for the epithelial cells, which were cubical in shape (Plate XXVI, Fig. 20). The general structure of the tumor was also more compact, since the spaces between the papillary projections were smaller. Serial sections showed the tumor to be conical in shape, and the apex of the cone to lie in close relation with and apparently to spring from a terminal bronchiole (Plate XXVII, Fig. 21).

4. This tumor was found in Original Mouse xvi. On the anterior surface of the lower lobe of the left lung, a grayish-white mass one millimeter in diameter existed. It projected above the pleura and was sharply outlined. Under the microscope this mass was found to be composed of papillary outgrowths covered by a single layer of epithelial cells of cubical form. The cells were smaller than in the preceding tumors of this type. A special feature of this tumor was the presence of a homogeneous material separating the papillary projections in the more solid portions, and filling the small cystic spaces. Mitotic figures were not discovered. There was compression of the surrounding lung tissue.

5. The next tumor of this class was found in Original Mouse xx. It was situated in the upper lobe of the right lung and measured one and one-half centimeters in diameter. Its structure was very similar to those already described. The central portion was fibrous, because of degeneration of that part of the tumor. This fibrous tissue contained clefts in which there had been fatty acid crystals, and the clefts were surrounded by foreign body giant cells. The surrounding lung tissue was collapsed.

6. The next tumor of this type occurred in Original Mouse xxii. It measured one millimeter in size and occurred in the upper portion of the lower lobe of the left lung. It resembled in structure the previous tumors described.

7. The seventh of the series occurred in Original Mouse xxv. In

this animal the lung showed several nodules, two of which were situated near the apex of the right lung, and measured about two millimeters in diameter. One of these only was found to be a primary pulmonary tumor, the others being metastases of the superficial adeno-carcinoma. The primary tumor corresponded to the general tumors of this type and differed greatly from the metastatic nodules.

8. In Original Mouse xxvi two primary lung tumors were present. One was found in the upper lobe of the right lung and the other in the upper lobe of the left lung. They measured about one millimeter in diameter. The superficial tumor with which they were associated was a spindle-cell sarcoma. In structure they were quite similar to others of this class (Fig. 12).

SARCOMA.

The literature does not contain, as far as I have been able to see, records of the successful transplantation of primary sarcoma of the mouse into other mice, although sarcomata of rats have been in several instances transplanted successfully. Ehrlich and Apolant[49] have reported the finding of two mixed tumors composed of sarcomatous and carcinomatous elements in the first generation, and Murray[50] alludes to an instance of a spindle-cell sarcoma found by Jensen in a mouse sent to him by Bashford.[51] In the latter instance the growth surrounded one of the kidneys and although it was transplanted into a large number of mice, it gave rise to no implantation tumors. In the instances mentioned as having been reported by Ehrlich and Apolant, they regard the tumors as having been original carcinomata, which were being transformed during the original generation into sarcomata. Ehrlich and Apolant,[52] Loeb,[53] Liepman[54]

[49] Ehrlich and Apolant, *Berliner klin. Woch.*, 1907, xliv, 1397.

[50] Murray, *Scientific Report of the Imperial Cancer Research Fund*, 1908, No. 3, 73.

[51] Bashford and Murray, in a later article (*Proc. of the Royal Med. and Chir. Soc. of London*, Series B, 1909, lxxi, 311), mention a melanoma or melandric sarcoma of the ear of a mouse, but do not allude to transplantation.

[52] Ehrlich and Apolant, *Berliner klin. Woch.*, 1905, xlii, 871.

[53] Loeb, *Med. Bull.*, 1906, xix, 113.

[54] Liepman, *Münchener med. Woch.*, 1907, liv, 1345.

and Bashford[65] all report examples of the transformation of carcinoma of the mouse into spindle-cell sarcoma. In all of these instances excepting one, namely, that of Loeb, the changes occurred in relatively late generations, whereas the example reported by Loeb is the first instance in which the change began in the first generation. I have observed two instances of what appeared to be primary sarcomatous superficial tumors of the mouse. In both instances tissue was examined from all parts of the tumors in order to exclude as far as might be possible a mixed carcinoma and sarcoma in the first generation. No evidence of epithelium or of cancerous tissue was detected.

1. The first of these tumors was found in a white, adult, female mouse (Original xviii). It was located on the left side immediately inferior to the foreleg, and extended over the lower ribs. It measured three centimeters in diameter. The skin was invaded, and there was an ulcer measuring half a centimeter in diameter which was superficial and covered with a scab. The tumor was grayish-white in color, firm in consistence and had extended into the muscles about the ribs and projected into the pleural cavity as a flat mass with a concave surface. On section it presented central necrotic masses. There were no metastases, but both ovaries were enlarged, firm, oblong in shape and about eight times their normal size.

Under the microscope this tumor proved to be a spindle cell sarcoma, possessing highly invasive properties and insinuating itself between the voluntary muscle fibers (Plate XXVII, Fig. 22). The ribs were surrounded by tumor cells, but were not invaded themselves. The enlarged ovaries were the seats of papillary cyst-adenomata, which will be described in another place.

60 mice were inoculated from the superficial tumor, of which 45 survived two weeks or longer. In one instance a tumor developed, which was detected on about the 70th day. The tumor grew slowly and a portion of it was removed for examination with a view of stimulating the growth of the remainder. The mouse succumbed soon after the operation, before the growth had reached a size large enough for further transplantation.

2. This tumor occurred in Original Mouse xxvi. It was situated

[65] Bashford, *Berliner klin. Woch.*, 1907, xliv, 1238.

on the left side of the chest just below the fore leg, measured three centimeters in diameter, was flattened and had invaded the skin. A small ulcer had appeared which was also attached to the chest wall, although it had not penetrated to the pleura. It was grayish-white in color and rather firm in consistence. Tissues immediately surrounding the ulcer were hemorrhagic, while around the main tumor they were edematous. A small nodule measuring one millimeter in diameter was found in the lower portion of the upper lobe of the right lung. The corresponding portion of the left lung showed a similar nodule. These were primary tumors which have been described elsewhere. Both ovaries were enlarged to about five times the normal size.

The microscope showed this tumor to be also a spindle cell sarcoma which had invaded the adjacent muscle fibers. Many of these, or remains of them, were contained in the mass (Plate XXVIII, Fig. 23). A large number of eosinophilic cells were scattered through the tumor. The left ovary was the seat of a cyst adenoma to be described elsewhere.

40 white mice were inoculated from the superficial tumor, of which 14, or 35 per cent., developed tumors. The average incubation period was 30 days. The tumors presented the same histological character as the original, and are now growing in the second generation.

LYMPHO-SARCOMA AND HODGKIN'S DISEASE.

The first tumors of this class were described by Haaland.[56] He described in all six examples of mice in which the lymphoid tissue throughout the body was so greatly increased that he concluded that the condition consisted not of tumor formation in the true sense so much as an hypertrophy or hyperplasia of the preëxisting lymphoid structures. He inoculated other mice with the lymphatic material without results. On the other hand, through having kept mice in a cage with an animal affected in this way, he observed five similar formations in the mice introduced into the cage within a period of two years. Tyzzer[57] reports two instances of a condi-

[56] Haaland, *Ann. de l'Inst. Pasteur*, 1905, xix, 197.

[57] Tyzzer, *Fourth Report of the Caroline Brewer Croft Fund Cancer Commission*, 1907, 27.

tion somewhat resembling the condition described by Haaland, but he states that they presented the appearance of a "well-defined primary growth without appreciable hyperplasia of the lymphoid tissues elsewhere in the body."[58] Murray[59] reports four similar instances. In two, a general hyperplasia of the lymphoid tissue existed, and in two, the tumors were more localized. Both Tyzzer and Murray failed to transplant successfully the tumors to other mice.

1. The first example which I observed occurred in a white, female mouse (Original xvii). On each side of the neck were masses measuring 1.2 centimeters in diameter which were freely movable and not attached either to the skin or to the deeper tissues. The axillæ contained masses measuring from five to seven millimeters and the right inguinal region, a mass measuring five millimeters. All of these presented the same naked eye appearances. They were grayish-white in color, quite uniform and not lobulated. The liver was mottled, owing to the presence on the usual dark ground of many minute grayish points. The upper pole of the right kidney contained a grayish nodule measuring one and one-half millimeters and the left kidney showed two smaller nodules. Two small masses occurred in the retro-peritoneal tissues just behind the left kidney. Two masses measuring seven millimeters in diameter were attached to the mesentery. The lungs also contained several small foci, gray in color and sharply outlined. The spleen was considerably enlarged and mottled.

Under the microscope the structure of all of the different masses described proved to be the same. The masses were made up almost exclusively of small round lymphoid cells possessing a comparatively large pale vesicular nucleus and a narrow rim of cytoplasm. The cells were of the type found in the germinal centres of the lymph follicles. The stroma was delicate except where the larger blood vessels coursed. Mitotic figures were not numerous. In addition to the larger cells described, others of the usual lympho-

[58] Tyzzer states in his later paper (*op. cit.*) that he has met with 10 mice showing lympho-sarcoma; and Bashford and Murray in their last report (*op. cit.*) record another instance of generalized lympho-sarcoma.

[59] Murray, *Scientific Report of the Imperial Cancer Research Fund*, 1908, No. 3, 74.

cytic type were present. Small necrotic areas occurred here and there in some of the nodules.

The lungs showed much more replacement of the pulmonary tissue by the lymphoid growth than was noticeable to the naked eye. When the lymphatic nodules were small they were confined to the perivascular and peribronchial tissues, but the larger masses also invaded the pulmonary tissue. In addition, small accumulations of cells of the same general type occurred beneath the visceral pleura. Mitotic figures were fairly common. In other respects the lungs were congested and edematous, and contained numerous phagocytic cells containing pigment. It may be remarked that the pulmonary condition was similar to that described by Haaland.

The spleen was greatly modified, since both the pulp and the follicles were diffusely infiltrated with the lymphoid cells in such a way as to make the normal structure of the organ indistinct. Many myeloid giant cells were present, as is not uncommon in the spleen of the normal mouse. The sinusoids of the spleen were greatly distended with the cells, which, on the whole, appeared larger than the average. Mitotic figures were numerous.

The liver also exhibited an extensive invasion with the new cells. In some places the growth of these cells had replaced the normal tissue to such an extent that nothing of the liver structure remained visible. In other places the cells had grown into and distended the sinusoids, producing a compression of the rows of liver cells. Sometimes the new cells had spread out diffusely and at other times they had been confined to definite loculi. The interlobular tissue was more affected than other parts.

The new cells in the kidney were confined almost entirely to the cortex of the organ. The growth in the cortex was intertubular and periglomerular; in the medulla it surrounded the vessels only.

100 mice were inoculated with the material from the superficial masses, 70 were inoculated from the masses in the neck, 10 from the mass in the left axilla, 10 from the mass beneath the right pectoral muscles, and 10 from the mass in the right inguinal region. At the end of two weeks 60 of these mice remained alive, of which one developed a tumor. This growth was first noticed 120 days after the inoculation. When the growth had attained the size of

four by seven millimeters, a portion was removed for further inoculation and for histological study. The fragment which was left behind underwent absorption. 20 mice were inoculated with the excised fragment, but none developed tumors. Under the microscope the structure of this implantation nodule was in all respects similar to the original growths, except that the mitotic figures were more numerous, as were the capillaries. The stroma presented the character of the stroma of the original nodules.

2. The second example appeared in a white, female mouse (Original xxi) which showed a superficial tumor in the right inguinal region. The mass measured one centimeter and was freely movable. A portion of the tumor was removed by operation. It was grayish-white in color and somewhat lobulated. The fragment of tumor left behind did not increase appreciably in the next 14 days, the period during which the mouse survived. The autopsy showed a small mass one millimeter in diameter in the upper margin of the lower lobe of the right lung. It appeared to be a metastasis. Unfortunately the tissues removed at the autopsy were lost, and only the portions removed by operation remained for further study.

This tumor was composed for the most part of cells of the lymphoid type which were somewhat smaller in size than in the preceding case. While many of the cells resembled the lymphocyte, others were somewhat larger and possessed pale vesicular nuclei. The stroma consisted of a delicate reticulum enclosing small masses of cells. In the periphery there were many capillaries. Mitotic figures were not very numerous. Two appearances are highly important: in the first place there were many eosinophilic cells scattered through the tissue, and next there also occurred here and there, and particularly in the more central parts of the growth, large areas composed of a reticulum coarser than the usual reticulum and containing spaces enclosing large endothelioid cells, of which some were multinuclear. In addition these areas contained large and small lymphoid cells, a smaller number of eosinophilic and a large number of plasma cells. The endothelioid cells presented irregular margins and contained a large amount of cytoplasm that was strongly eosinophilic. The nuclei of these cells were either vesicular or solid and dense. A certain number of mitotic figures were present

among these cells. The large multinucleated or giant cells showed nuclei which were usually vesicular and contained a coarse chromatin network which was disposed in an irregular fashion in the cytoplasm. In these areas the lymphocytes were few in number, and the other cells relatively numerous. A small number of cells containing basophilic granules were also present.

The description just given indicates that the tumor in this animal possessed the histological characters of Hodgkin's disease, as has been described by Reed, Longcope and others, in human beings. It is true that the eosinophilic cells were perhaps less numerous in the mouse nodule than is the case usually in the human disease, but Longcope has pointed out that in the later stages of the disease in human beings the eosinophilic cells also diminish. Whether, therefore, we are to regard the nodule in the mouse as representing a late stage of the disease, or whether in this animal the eosinophilic cells tend to be less numerous, must be left undecided.

OVARIAN TUMOR.

I have observed two examples of tumors affecting the ovaries, and it is interesting to note that in both cases the large superficial tumors also present consisted of spindle-cell sarcomata.

1. The first example occurred in Original xviii. In this white mouse both ovaries were enlarged, firm in consistence, oblong in shape, and about eight times the normal size. Under the microscope the two organs presented the same appearances which were interpreted as representing papillary cyst-adenomata. There had taken place a great increase of the epithelial cells, which formed solid masses somewhat compressed and elongated into spindle cells and cysts of different sizes, usually small and more or less occupied by the papillary outgrowths from their walls. These outgrowths developed from narrow or somewhat thicker pedicles and spread out into a fan-like structure. The epithelial cells which covered the papillæ and the walls of the cysts were rich in cytoplasm and the nuclei of the cells were usually vesicular but sometimes dense. The more solid portions of the organs were at one time cystic but the cysts became occluded by the ingrowth of the papillæ. Acini

possessing a distinctly granular form and arrangement also occurred. Mitotic figures were rarely seen, but appearances suggestive of direct cell division were more frequent. Hemorrhage had taken place into some of the cysts. Sections stained by Mallory's connective tissue method revealed a delicate basement membrane of connective tissue surrounding the small cysts and the more solid portions as subdivided into small areas of quite definite form. These latter areas were filled with epithelial cells of a granular quality resembling somewhat the lutein cells of the ovary.

2. The second example of ovarian tumor occurred in Original Mouse xxvi. The left ovary was about five times the normal size and showed under the microscope numerous large cysts separated by smaller ones and the tubules of the ovary. The cysts and tubules were lined by high columnar epithelium, but cilia were not demonstrated. In some of the larger cysts the lining epithelium had become flattened. The tissue separating the cysts was composed to a large extent of smooth muscle cells arranged in strands or in bundles. Sections stained by Mallory's phosphotungstic acid and hematoxylin brought out the myoglia fibrils. The nuclei of these muscle cells were large and vesicular and showed a few mitotic figures. In certain portions the granular structures were few and the muscular fibres many, so that the appearance presented was that of a leiomyoma.

CONCLUSIONS.

There have come to our hands within a period of about two years and as a result of relatively non-strenuous efforts 26 mice which had developed spontaneous tumors, and according to our interpretations these 26 mice were the subjects of 41 primary tumors.

Our experience agrees with the observations of others that it is especially, and perhaps almost exclusively, the female mice which develop spontaneous tumors. In no instance has a male mouse possessing a spontaneous tumor come into our hands.

Our experience is further in agreement with previous observations which teach that by far the greater number of the spontaneous tumors of the mouse develop from the mammary glands. Of the 41 tumors which we have described, 29 arose in portions of the body corresponding with the distribution of the mammary glands.

Choosing the classification adopted by Apolant, we have found among the true tumors of the mammary glands representatives of each of his classes.

We also observed an example of an adenomatous tumor arising from a portion of the body corresponding with the distribution of the mammary gland which exhibited the structure of the typical sebaceous glands rather than the mammary gland, and which we have classified under the molluscoidal tumors of Haaland.

Metastases occurred in a certain number of the mice showing spontaneous tumors. When present they were exclusively in the lungs. We did not observe a single instance of metastasis to other organs, or to the lymphatic glands.

Next to the mammary glands the lungs showed in our series the most numerous primary tumors. According to our interpretations we encountered nine examples of primary pulmonary tumors, which were always associated with superficial tumors of the body. It is noteworthy that we have not come across a single instance of a primary pulmonary tumor among several thousand mice not showing superficial spontaneous tumors, which have been subjected to careful post-mortem examination. It would be easy to conclude, therefore, that the occurrence of spontaneous tumors in one part of the body in mice predisposes them to the development of spontaneous tumors of the lungs, but obviously such a deduction is not warranted at present. The first example of this type of tumor was described by Livingood.

Our observations of two primary sarcomata of the superficial tissues of the mouse is apparently unique. We believe that we excluded, probably, the possibility that the tumors were mixed sarcoma and carcinoma. Moreover, they exhibited properties unlike the superficial carcinoma of the mouse, since they were far more invasive locally. Our success in transplanting both of these sarcomata is also unique. In the first case a single successful graft which was not further transplanted was obtained; in the second case the sarcoma is being successfully transplanted at the present time.

We observed two examples of tumors developing from the lymphatic organs. One of these belonged obviously to the so-called lympho-sarcoma of mice. The other, we believe, is unique in so far

as its identification with the pathological condition described in human beings under the name of Hodgkin's disease is concerned. Furthermore, we succeeded for the first time, apparently, so far as the records in the literature go, in transplanting successfully a lympho-sarcoma. In one mouse an undoubted development of the transplanted graft occurred.

We also observed two examples of ovarian tumors, both of which were of the nature of papillary cyst-adenoma. These tumors arose in mice which were the subjects of spindle-cell sarcoma of the superficial tissues.

Our experience in the transplantation of the spontaneous mouse tumors agrees generally with that of other investigators. It would, however, appear that we have been on the whole more successful than others in the transplantation of the hemorrhagic class of mammary gland tumors, the so-called hemorrhagic cyst-adenomata. An explanation for this discrepancy cannot be readily given. It is, of course, possible, since such great variations are known to exist among mice as regards their susceptibility to tumor implantation, that the American mice are more subject to implantation of the hemorrhagic tumors than the European mice, but it is also possible that we have interpreted somewhat more broadly than others the class of hemorrhagic tumors. If the latter statement is true, the tendency was an unconscious one, and suggests that different workers will probably interpret these tumors quite differently.

Finally, this study indicates that mice are subject to a wide variety of spontaneous tumors, and suggests that the more widely the study is carried, the greater will be found the number and variety of tumors to which they are susceptible.

For the present we prefer to believe that spontaneous tumors are more frequent in female than in male mice, but that they also will be found to occur perhaps not very uncommonly among the latter. The point should be borne in mind that by far the greater number of spontaneous tumors of mice which have thus far come under observation have been present in old female mice. The business of breeding mice for sale leads to the retention of the female breeders until they become exhausted in this function by age, and the changing and weeding out of the males while they are still much younger.

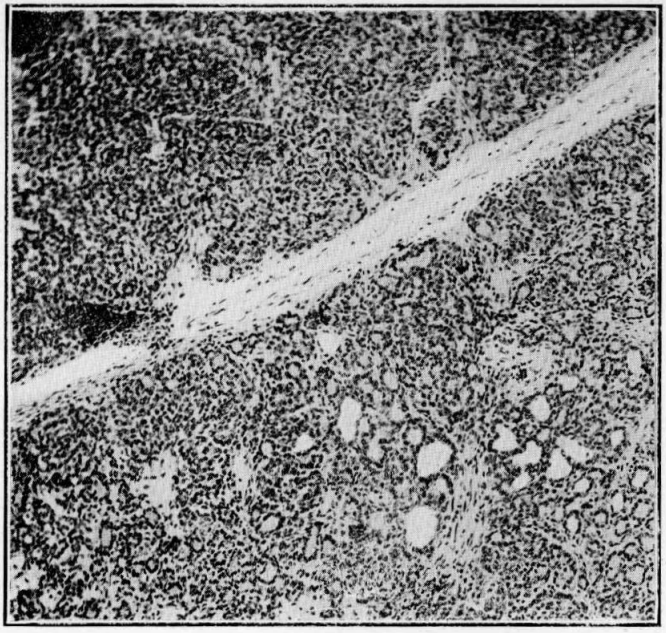


FIG. 1.

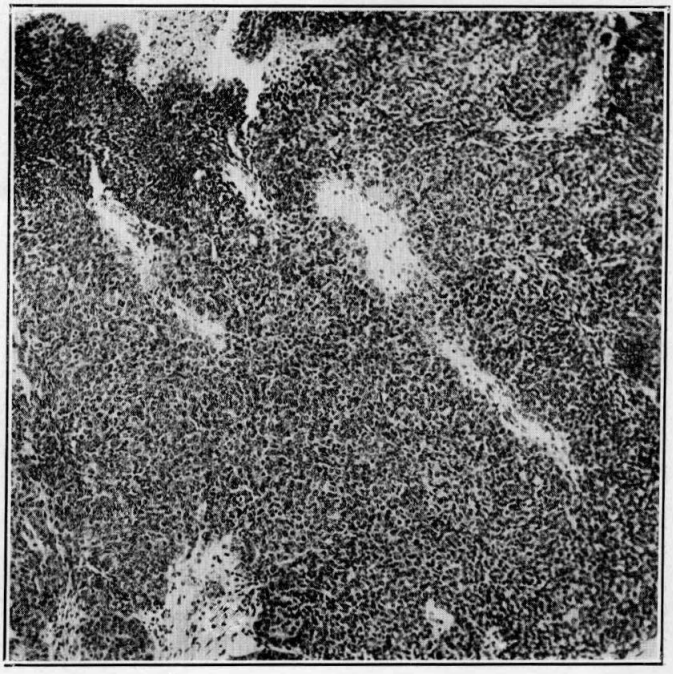


FIG. 2.

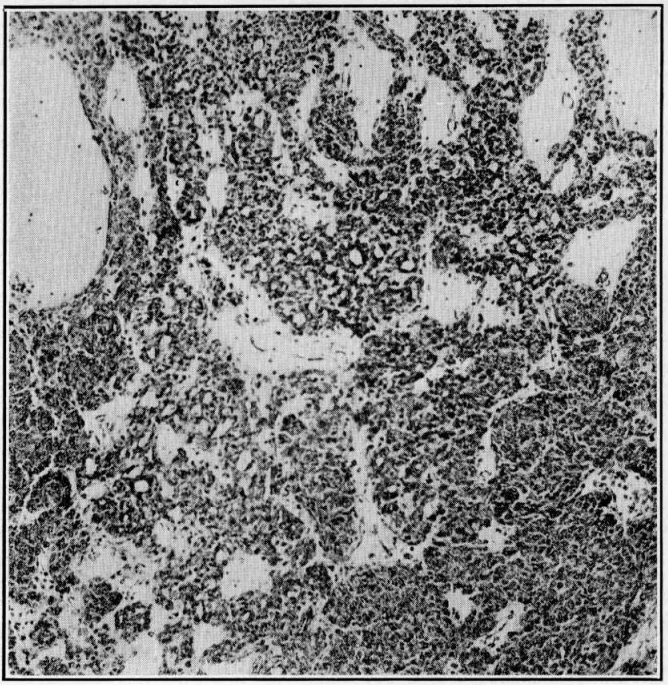


FIG. 3.

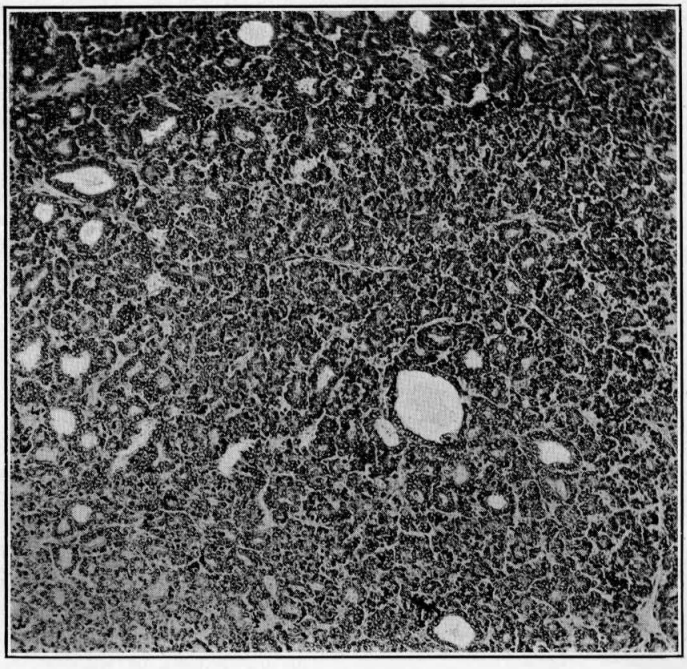


FIG. 4.

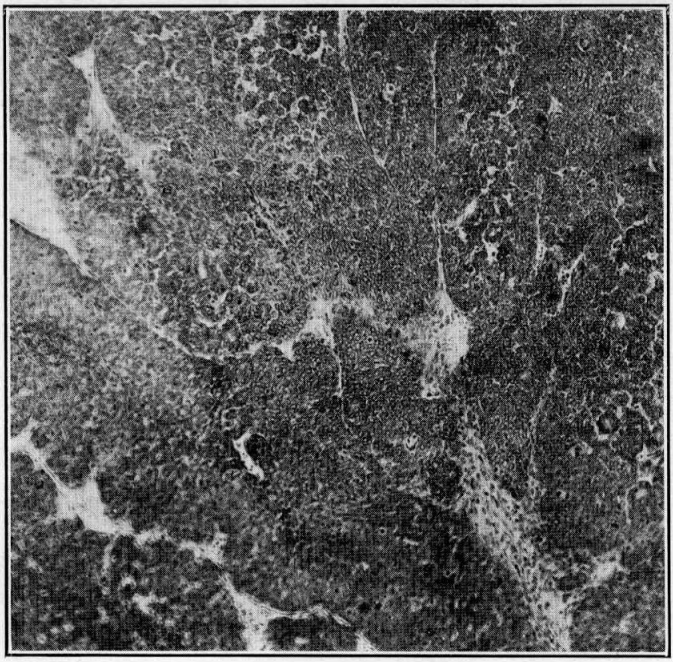


FIG. 5.

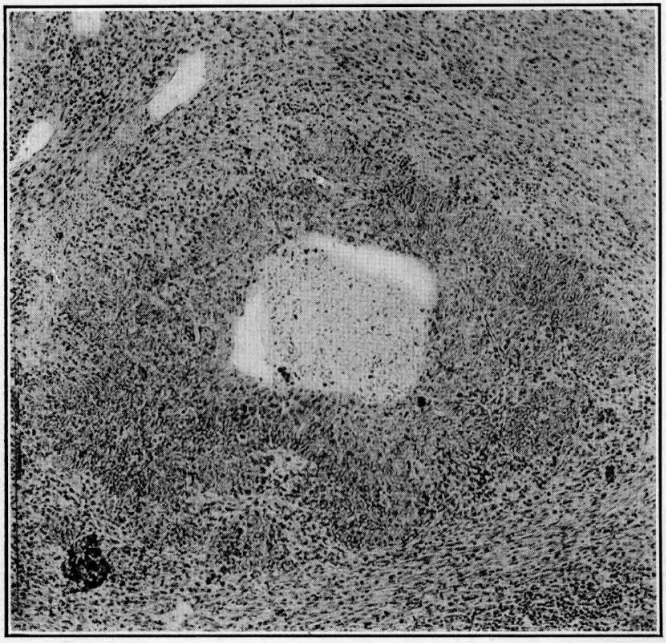


FIG. 6.

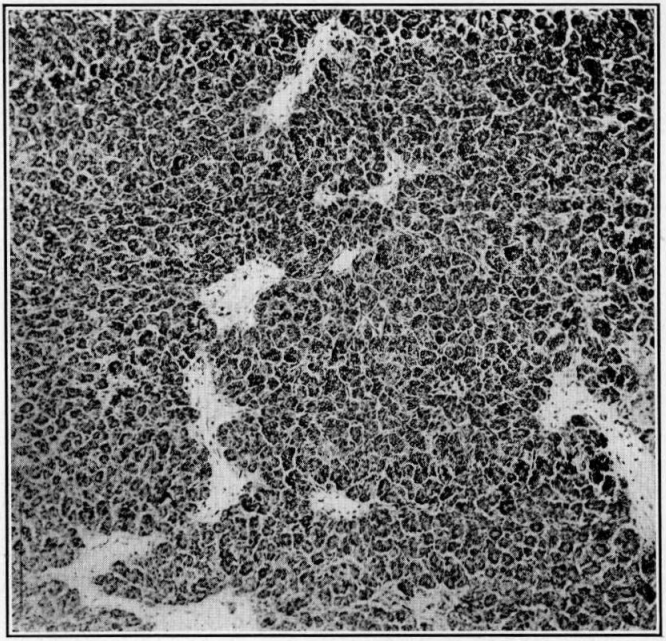


FIG. 7.

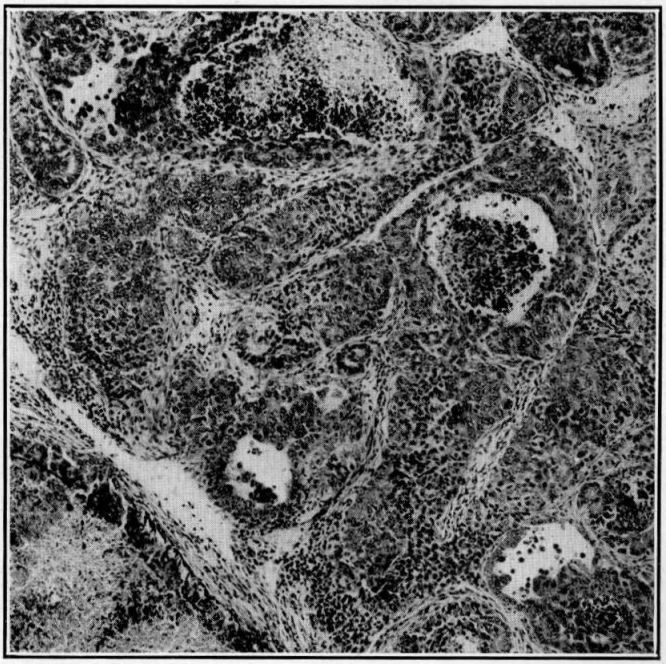


FIG. 8.

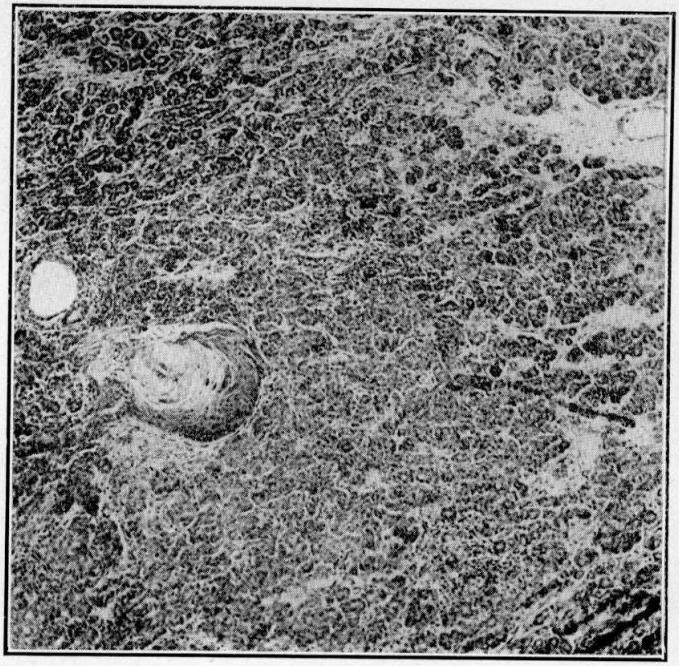


FIG. 9.

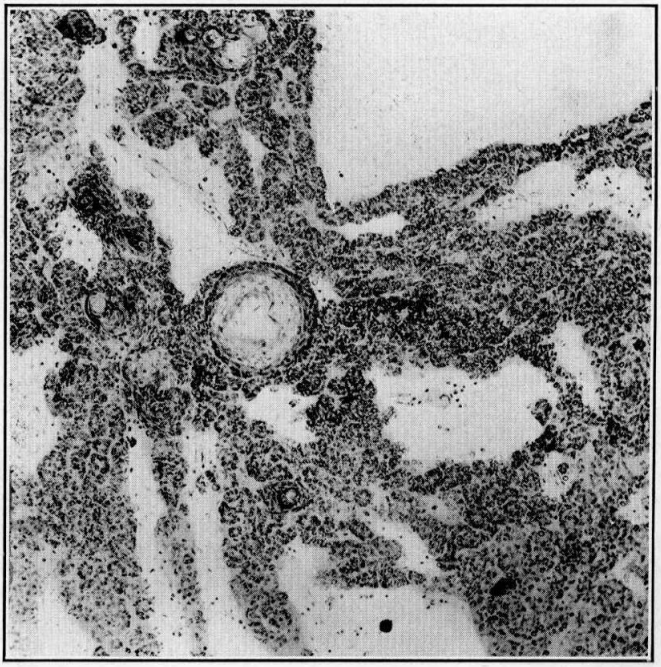


FIG. 10.

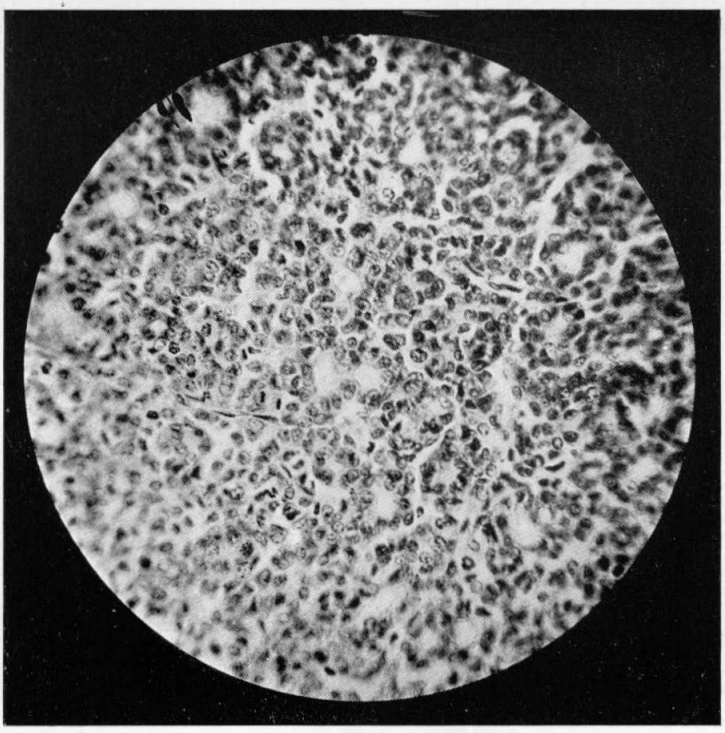


FIG. 11.

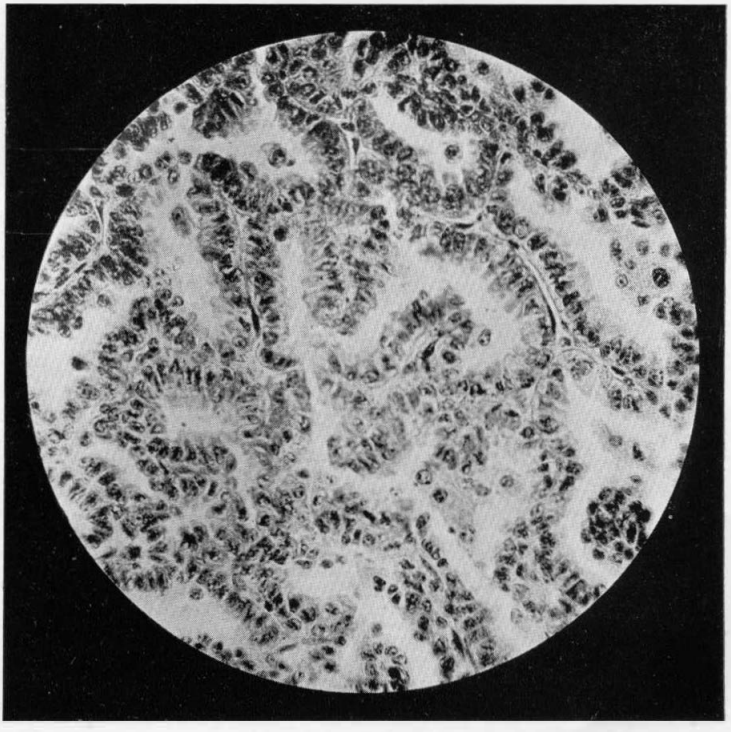


FIG. 12.

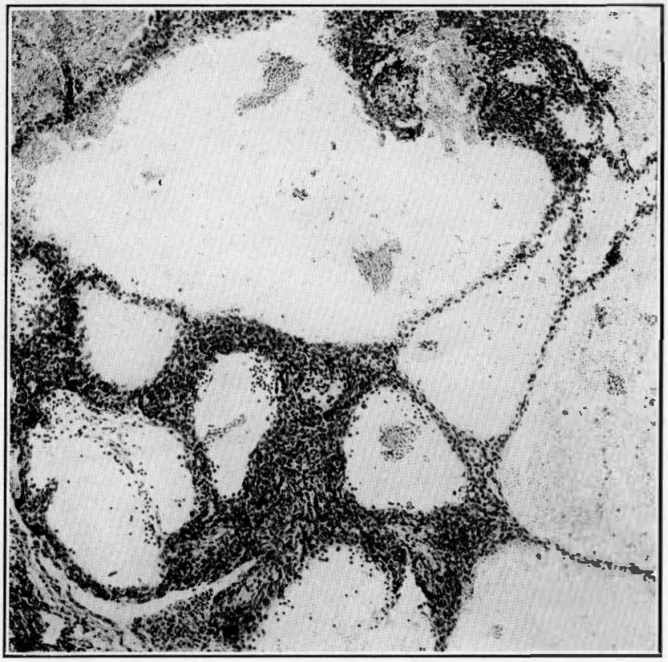


FIG. 13.

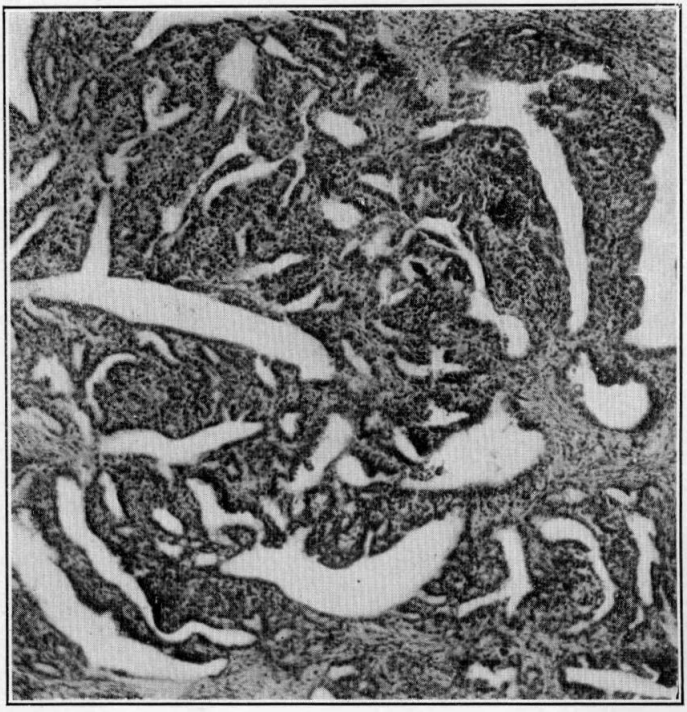


FIG. 14.

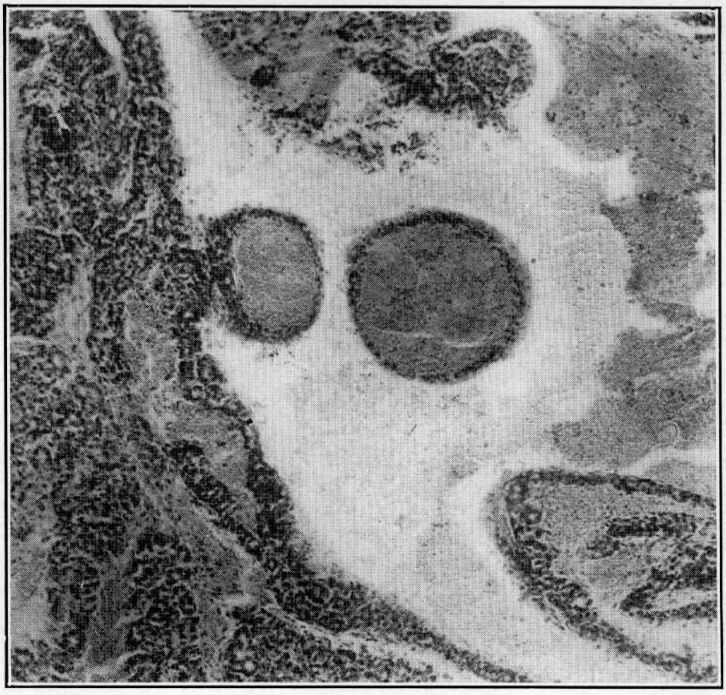


FIG. 15.

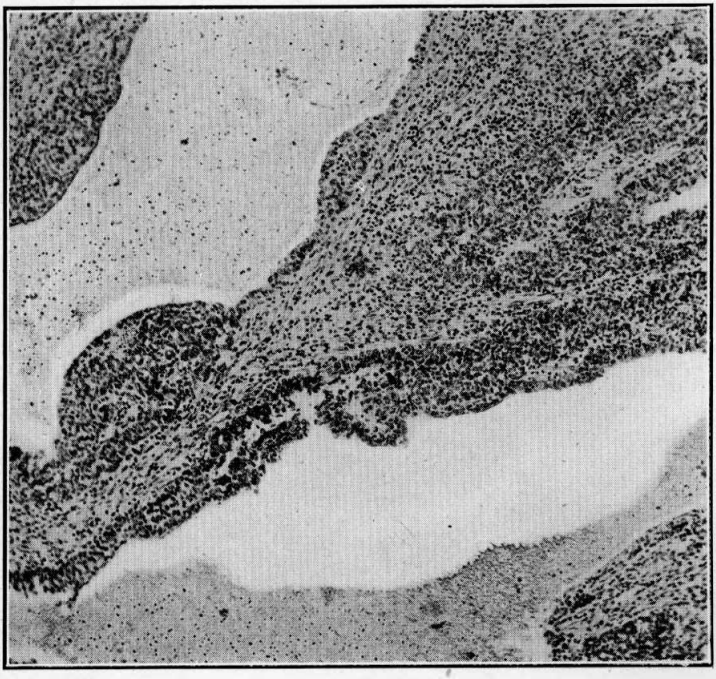


FIG. 16.

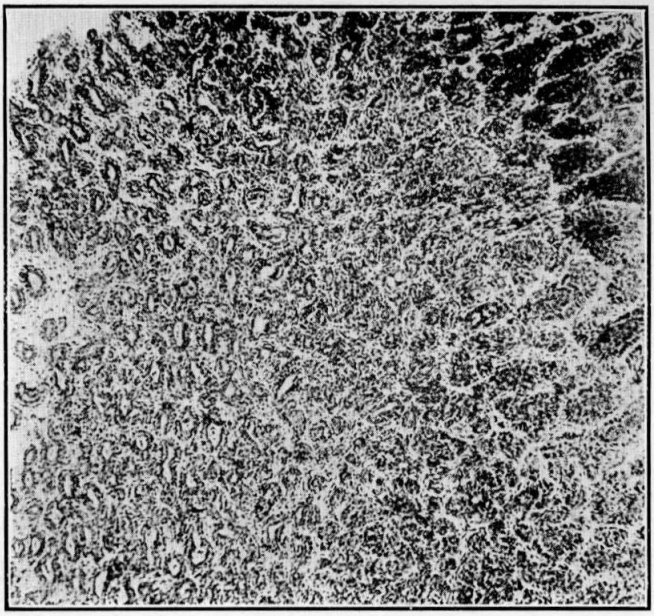


FIG. 17.

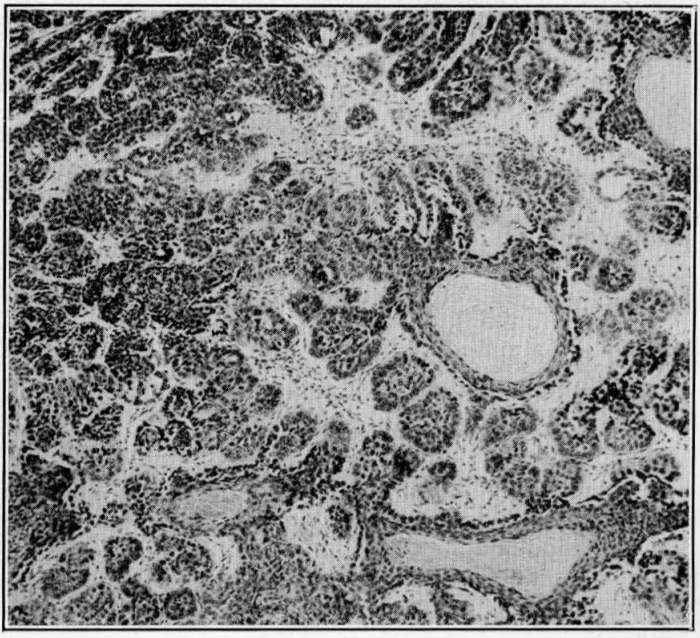


FIG. 18.

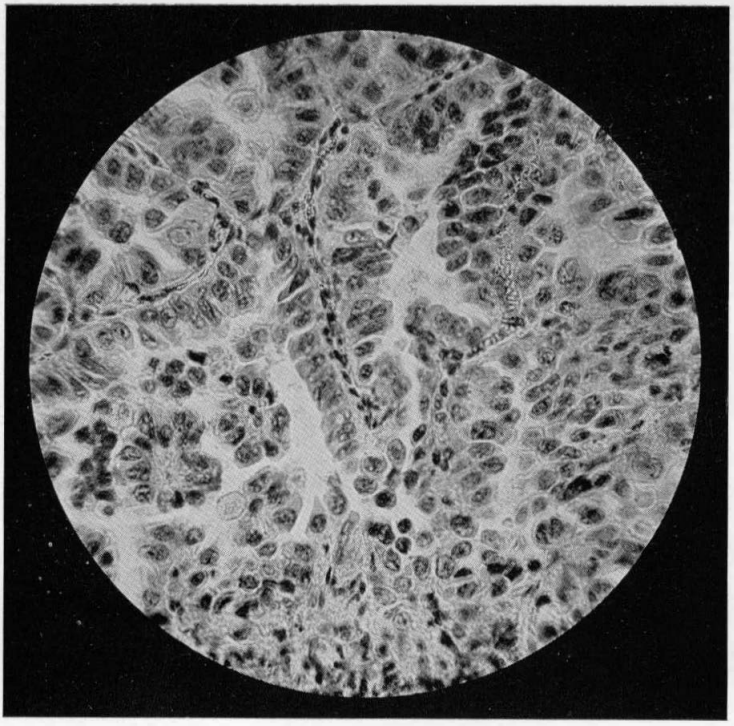
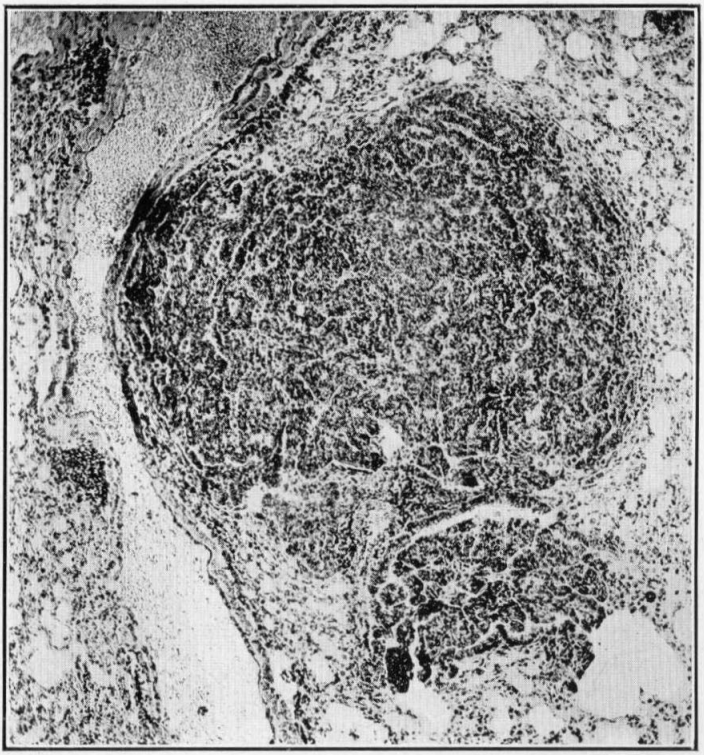


FIG. 19.



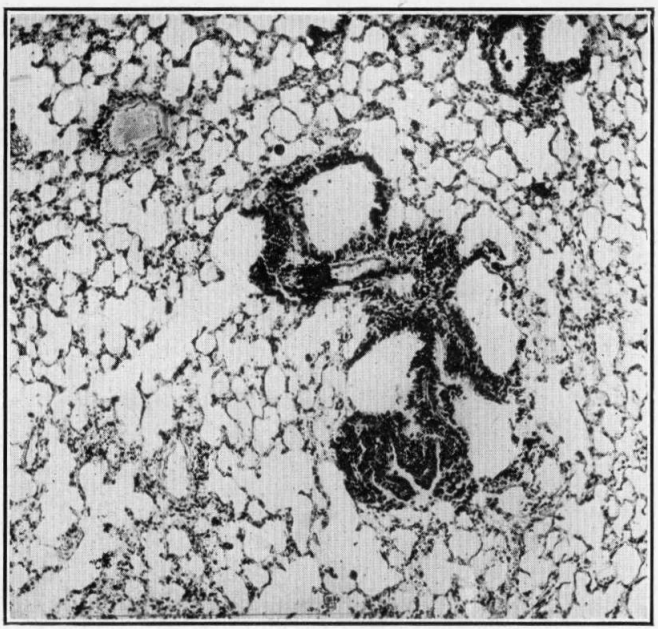


FIG. 21.

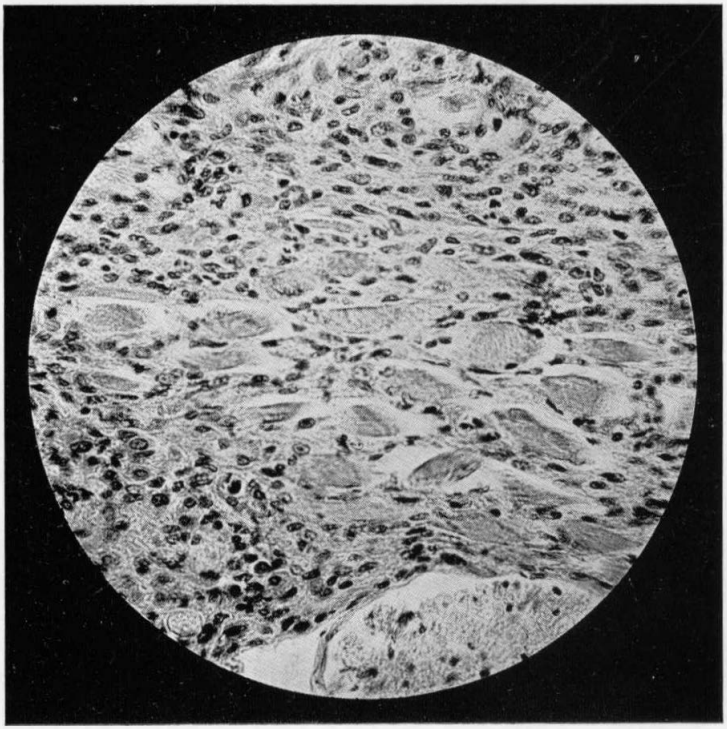


FIG. 22.

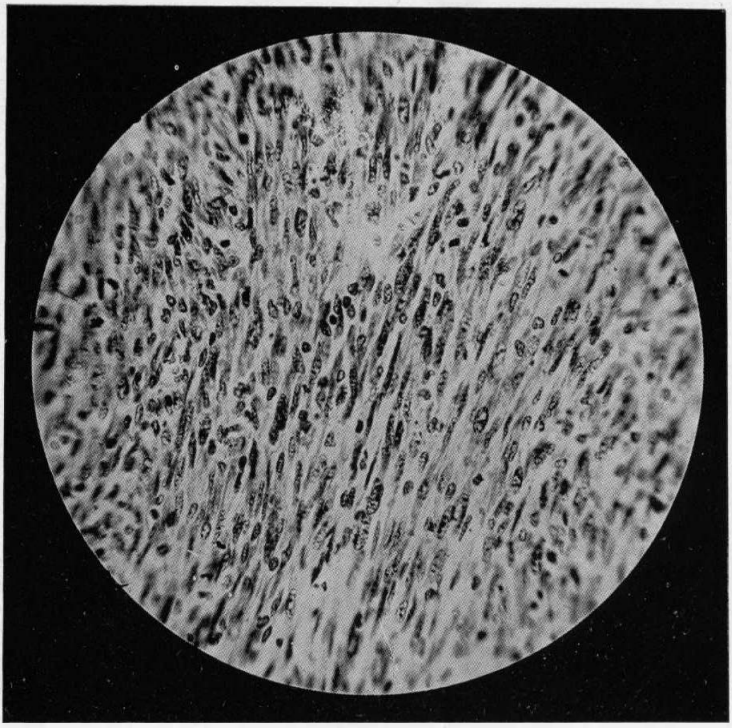


FIG. 23.

Consequently, far fewer males reach comparatively old age in the sense that the female breeders do, and this may be an important factor in accounting for the difference in susceptibility to spontaneous tumors hitherto observed in the two sexes.

EXPLANATION OF PLATES.

PLATE XVII.

FIG. 1. Original Mouse Tumor iv. Adeno-carcinoma. In the central and upper portions the growth is almost purely adenomatous, although there are also several nests of cells in which the glandular arrangement is not evident. The lower right hand portion shows the solid areas more clearly.

FIG. 2. Daughter tumor of fifth generation. The growth here is solid and there are no evidences of acini. The stroma is edematous. The presence of fat cells just below the center and to the left of it denotes the invasive character of the tumor.

PLATE XVIII.

FIG. 3. Daughter tumor of sixth generation. Shows apparent reversion to original type. It is composed almost wholly of acini and shows marked edema throughout. It is very similar to the primary tumor, though the stroma is not so well developed.

FIG. 4. Primary Tumor vi. The tumor is composed mainly of acini, some of which have become dilated and form small cysts. The stroma is not well developed. Solid nests of cells are not infrequent.

PLATE XIX.

FIG. 5. Daughter tumor of second generation. The growth is solid and there are no evidences of acini. The stroma is very delicate in character. A few blood-vessels with very thin walls are to be seen.

FIG. 6. Daughter tumor of the second generation. Lobules show necrotic centers. The stroma separating the lobules is well developed and very cellular. Numerous large blood-vessels with very thin walls are to be seen. The appearance here is very suggestive of a beginning sarcoma.

PLATE XX.

FIG. 7. Primary Tumor xiii. Adeno-carcinoma. The growth is composed of acini which are separated from one another by a very delicate stroma. Several blood-vessels are to be seen.

FIG. 8. Daughter tumor of third generation. The growth is composed of lobules, the central portions of which are necrotic. A few acini are to be seen between the lobules.

PLATE XXI.

FIG. 9. Primary Tumor xxiv. Adeno-carcinoma. This shows a small area of keratinization in the more solid portion of the tumor. Numerous acini are present.

FIG. 10. Primary Tumor xxv. The mass of keratin in the center is surrounded by numerous large cysts and a number of normal appearing acini. The cysts are filled with a finely granular and faintly staining material. In places the acini are widely separated by the serous exudate.

PLATE XXII.

FIG. 11. Primary Tumor xxv. Extra vascular lung metastasis. There are numerous acini, although the growth is composed mainly of nests of cells. Several mitotic figures are present. The stroma is very delicate.

FIG. 12. Primary papillary cyst-adenoma from the lung of the same mouse. This figure is to be compared with Fig. 11. The former is a metastasis from the large subcutaneous tumor shown in Fig. 10. The irregular papillary ingrowths are covered by a single layer of columnar epithelium. A few necrotic cells are to be seen in the cavities. The connective tissue framework is not well developed. The different characters of the growths shown in Figs. 11 and 12 justify us, we think, in concluding that the tumors are entirely independent of one another in their origin.

PLATE XXIII.

FIG. 13. Mouse Tumor iii. Daughter tumor of first generation. This shows very well the hemorrhagic type of adeno-carcinoma. There are numerous large cysts into which hemorrhages have occurred. Those cysts not showing hemorrhagic contents are usually filled with a serous exudate. The growth between the cysts is usually solid in character, though a few acini are to be seen.

FIG. 14. Mouse Tumor iii. Tumor of second generation. The growth shown here is to be compared with that of the first generation as shown in Fig. 13. The tendency here is towards the formation of irregular clefts and papillary ingrowths. This is the "spalten bildendes" type of Apolant. Hemorrhages are much less frequent in this tumor. The stroma is rather cellular and well developed in places.

PLATE XXIV.

FIG. 15. Primary Tumor x. This is also typical of the hemorrhagic type of adeno-carcinoma. In the center are two large blood-vessels in the midst of necrotic tissue. The walls of the vessels are very thin and are surrounded with a thin mantle of tumor cells. Hemorrhages are to be seen everywhere throughout the section. Numerous acini are present.

FIG. 16. Mouse Tumor xx. Daughter tumor of first generation. This tumor is composed of lobules, the central portions of which are necrotic, but the remarkable feature of the growth is the cellular stroma separating the lobules. In places the appearance of this tumor is very suggestive of a mixture of sarcoma and carcinoma.

PLATE XXV.

FIG. 17. Primary Tumor xxii. Molluscoidal type. This shows the radiating columns of cells at the periphery. Some of the columns show a lumen on cross section. The next figure shows that a number also contain masses of keratin. The appearance presented in the deeper portions is that of an adenoma.

FIG. 18. Primary Tumor xxii. Portion of same tumor which shows masses of keratin. The keratin occurs not only in large masses but is also seen in longitudinal sections of the smaller tubules. On the right the branching of the tubules is very evident.

PLATE XXVI.

FIG. 19. Primary lung tumor in Tumor Mouse xix. The structure here is typical of most of the primary lung tumors of our series. The papillary ingrowths are covered with a single layer of rather high columnar epithelium. The stroma is delicate in character. The cell outlines are distinct and they can easily be differentiated from one another.

FIG. 20. Original Mouse Tumor xiii. Primary lung tumor. This growth is more compact than any of the others of this series. To the left of the center there is a small bronchus, the lumen of which has been entirely occluded by the tumor growth.

PLATE XXVII.

FIG. 21. Original Mouse Tumor xiii. This shows what apparently is the point of origin of the primary lung tumor shown in Fig. 20. The growth was wedge-shaped and this corresponds with the apex which apparently was located at the termination of one of the bronchioles.

FIG. 22. Primary Tumor xviii. Sarcoma. The growth is composed of spindle-shaped cells which can be seen invading the surrounding muscle tissue. Some of the muscle cells are undergoing pressure atrophy, but the majority are well preserved.

PLATE XXVIII.

FIG. 23. Primary Tumor xxvi. Sarcoma. The cells are irregular in size and shape, but the dominant type is that of a spindle-celled sarcoma.

TRANSPLANTATION EXPERIMENTS IN MACACUS RHESUS WITH A CARCINOMATOUS TERATOMA FROM MAN.

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Although many attempts have been made to graft human tumors upon the lower animals none have been really successful. The experiment of Roux and Metchnikoff[1] with anthropoids was a failure. The use of the dog, rat, etc., has not given promising results. The remarkable experience Lewin[2] relates of the development of a granulation tissue tumor in a dog inoculated from a malignant adeno-cystoma of the ovary has not yet been satisfactorily accounted for.

With our present knowledge gained chiefly from studies of transplantable tumors of mice, rats and dogs, we can predicate that even when transplantation is easily accomplished within species it fails when attempted between nearly related species. Hence we should not expect that fragments of tumors removed from human beings should develop to any extent in other species of animals. However, a final decision on this point may well be deferred until a number of essays with a wide variety of tumors and several species of animals have been made. At present we can view the problem of engrafting tumors upon heterologous species from two points: first, in relation to true grafting in which the tumor fragment actually takes hold and grows; and second, in relation to mere ability to increase somewhat in size for a short time, owing to temporarily favorable conditions of nutrition and restraint of autolytic disintegration. This indirect form of growth, which Ehrlich[3] first described under the

[1] Roux and Metchnikoff, *Bull. de L'Acad. de méd.*, 1903, l, 101.

[2] Lewin, *Zeit f. Krebsforsch.*, 1906, iv, 55.

[3] Ehrlich, P., *Arb. a. d. k. Inst. f. exper. Therapie*, 1906, No. 1, 2.

name of zig-zag transplantation, does not always occur, and probably is dependent somewhat upon nearness of relationship between the species—one that supplies and one that receives the grafts. That some such factor operates at times is rendered probable by the far greater reactions of inflammation which occur when the species are widely separated than when nearly related. Dr. Flexner and I have made observations of this kind with a transplantable rat tumor ingrafted in mice, rabbits, and guinea-pigs. A similar interpretation can be put upon some of Loeb's[4] experiments.

It is now generally recognized that in order to secure satisfactory results the transplantations must be carried out with great care so as to avoid bacterial infection and consequent inflammatory reaction, and no foreign fluids should be allowed to come into contact with the graft. Unless these precautions are observed failure may result even with readily transplantable tumors, and when the fate of the grafts is to be followed day by day by accurate measurements, the inflammatory reactions about the fragments must be controlled. From this source alone actual increase in size may occur and it can be excluded as a factor only by excising some of the enlarged grafts and subjecting them to microscopical examination.

The history of the tumor, which follows in brief form, is convincing as to its malignancy.

S. L., female, white, age 14 years. Patient was first admitted to the Presbyterian Hospital in the service of Dr. A. J. McCosh on January 18, 1908. One sister when sixteen years of age had a dermoid cyst removed. Patient had an enlargement of the abdomen which had been present for two months. A diagnosis of a dermoid cyst of the ovary complicated by intestinal obstruction was made. The patient was operated upon the day of admission. There was found a smooth walled cyst the size of a foot-ball, which was friable and contained a mass of heterogeneous tissue. The cyst was first drained and then drawn into the incision, when a large amount of grumous gelatinous material escaped.

Pathological Report.—(Kindly supplied by Dr. Opie.) The tumor is made up of fibrous tissue, some striated muscle, and a number of hair follicles. There are also some papillomatous structures and epithelial whorls.

The patient appeared relieved until twelve days after the operation, when pain, vomiting and distention of the abdomen appeared, and with these symptoms a perceptible mass in the original locality. The mass continued to develop until February 9, when it was again operated upon. At the operation there

[4] Loeb, L., *Jour. of Med. Research,* 1902, viii, 1.

was found a mass of old organized and inorganized blood in the region of the hepatic flexure of the colon and considerable fibrin and masses of tissue looking like cyst elements (bone, cartilage, etc.) in the lower portion of the abdomen. The report from the pathological laboratory on the material obtained at this operation was "that it consists of masses of hyaline cartilage which are infiltrated with a few polymorphonuclear cells. The stroma is composed of fibrous tissue, fat and extravasated blood, and contains numerous cells of the polymorphonuclear and lymphoid type."

The patient was discharged three weeks later apparently well, but after another three weeks returned on account of a growth about the size of a cocoanut in the original locality. She was operated upon the following day, when there was found a mass filling the right lower quadrant of the pelvis, which was composed of a tenacious, hemorrhagic material involving all the structures in this locality and having no apparent point of origin. The serosa of the intestine contained a number of miliary nodules. It was with a portion of the material removed at this operation that the first transplantation experiments were conducted.

The material which was used for inoculation consisted of a soft, friable mass of the consistency of brain tissue showing hemorrhagic spots.

Microscopically the tissue consisted of hyaline cartilage, glandular columnar epithelium and a cellular stroma. The stroma is rich in cells and in places edematous. The cells are spindle-shaped or polygonal with rather large, pale vesicular nuclei. In places the stroma is finely granular and contains numerous fine vacuoles, this appearance probably being due to edema. It is exceptional to find a definitely fibrillated area, the tissue probably being too young for this. Not infrequently one finds a small clump of embryonic striated muscle cells in the stroma. Small hemorrhages are present. Mitotic figures are fairly numerous. Imbedded in this stroma are masses of hyaline cartilage. These masses are usually small and almost round in shape, though in some instances they are larger and more irregular in form. They are scattered throughout the sections and do not appear to bear any definite relation to either stroma or clumps of epithelial cells. They are very cellular and many of the cells are undergoing mitosis. Some of the masses are sharply differentiated from the surrounding stroma by a layer of closely packed cells, the nuclei of which are oval or elongated, with the long axis pointing either toward, or parallel with the periphery. Mitotic figures are present in the central as well as in the peripheral portions.

The epithelial type of cell is present everywhere, either in the form of irregular clumps without any definite arrangement, or in the form of an acinar or gland-like arrangement lining irregular clefts and tubules. In most instances the cells lining the tubules or clefts are arranged in several layers, though, exceptionally, places are seen in which the tubules are lined by a single layer. Goblet cells are not infrequent among those lining the tubules. The cells are usually large, with vesicular nuclei, though nuclei rich in chromatin are not infrequently seen. Without having regard to the arrangement of the cells, mitotic figures are very numerous.

Owing to the rapidity of the growth after recurrence of the tumor, Dr. McCosh offered it for inoculation experiments. We obtained the tumor immediately after its excision, wrapped in sterile dressings to prevent infection and evaporation, and made the implantations into macacus monkeys, rats and mice. The technic used was that followed in the transplantation of rat and mouse tumors. The tumor was cut into small fragments, no fluid of any kind being used for the subcutaneous inoculations, and one of the fragments placed in the distal end of a hollow needle. After cutting away the hair over the site of inoculation, the surface was cleansed with alcohol and the alcohol removed with sterile gauze. The needle was then introduced into the subcutaneous tissues and by means of a sterile platinum wire, just fitting the lumen, the fragment was pushed out of the needle into the tissues beyond. The inoculations were always made beneath the skin of the abdomen. Every precaution was taken to avoid infection. The time elapsing between the removal of the tumor from the patient and its inoculation into the animals was about forty-five minutes, the chief loss of time arising from the distance between the Presbyterian Hospital and the Rockefeller Institute.

Monkey 1.—This animal was inoculated in eight places with the tumor fragments. In four places it received tumor fragments alone; in two places, tumor fragments plus a drop of a saturated solution of scharlach R. in olive oil; in two places, tumor fragments plus a drop of a saturated solution of sudan III in olive oil; in one place, scharlach R. solution alone; and in one place sudan III solution alone.

7th day. There are distinct masses to be felt at the points where the fragments were lodged. These measured from 2 to 3 mm. in diameter.

9th day. Fragments about the same size. At this time the larger portion of two of the fragments were excised. The fragments to which the scharlach R. and sudan III solutions had been added at time of inoculation do not appear as large as those inserted without any chemicals. The material excised was used to inoculate two other monkeys.

11th day. The nodules not operated upon remain about the same size. Considerable swelling at sites of operation.

13th day. The nodules are somewhat smaller than at previous examinations.

16th day. Excised one of the nodules for histological study. Fragment removed measured about 2 mm. in diameter.

18th day. Fragments smaller, but still perceptible.

20th day. Fragments just perceptible.

22d day. Fragments disappeared.

The material excised from Monkey 1 was used to inoculate Monkeys 2 and 3.

Monkey 2. This monkey was inoculated in five places with material excised from Monkey 1.

4th day. All the fragments are easily felt and measure 2 mm. in diameter.

5th day. Excised fragment for histological study. The fragments measure 3 mm.

7th day. No change in size.

11th day. Fragments somewhat larger, measure 4 mm.

15th day. Fragments measure about 3 mm.

19th day. Fragments smaller.

21st day. Fragments disappeared.

Monkey 3.—The animal was inoculated in five places with material excised from Monkey 1.

4th day. Fragments measure 2 mm. in diameter.

7th day. One of the fragments remains about the same size, the others somewhat smaller.

12th day. Fragments somewhat larger and measure 4 mm.

15th day. Fragments disappeared.

As stated in the protocols, several of the fragments were removed for histological examination.

The first fragment examined was excised from Monkey 1 on the eleventh day after inoculation. The sections show a moderate amount of connective tissue separating the islands of cartilage which are several times the size of the islands in the transplantable tissue. The epithelial cells have all disappeared and the stroma of the transplanted fragment is much less cellular than in the original tumor, there are more connective tissue fibrils and the cells of the stroma are more elongated than in the original tumor. In other words, the tissue which surrounds the islands of cartilage is a reaction product chiefly, although part of it is possibly derived from the old

stroma. The cartilage appears normal, has not been invaded with connective tissue, and a small number of the cells are in active mitosis.

The second fragment examined was also obtained from Monkey 1, but was excised on the sixteenth day. Sections from this fragment show that there has been no marked change in the masses of cartilage, but the intervening connective tissue between the small masses is much more cellular. Many of the cells in the connective tissue are oval and resemble the stroma cells of the original tumor, and the amount of fibrillated tissue is less than in the fragment removed on the eleventh day. A few mitotic figures are still present.

The next fragment examined was the one removed from Monkey 2. This fragment had been nine days in Monkey 1 and five days in Monkey 2, or in the two monkeys for fourteen days. Microscopically the fragment is seen to be encapsulated with fibrous tissue, which in the center is quite hyaline. The cartilage masses are degenerating, (1) through loss of nuclei chiefly in the center and by degeneration of nuclei at the periphery, and (2) by a diffuse blue (hematoxylin) staining in the center of some of the masses, probably due to a calcareous deposit. In this situation the cartilage cells are shrunken, clear spaces exist in the matrix about them, and the nuclei are deformed and pyknotic. In the periphery of some of the cartilage masses mitosis is still to be seen.

The next fragment examined was also from Monkey 2. This fragment had been for nine days in Monkey 1, and for twenty days in Monkey 2, or twenty-nine days in both animals.

Microscopically the sections are composed almost entirely of fibrous and adipose tissue, and accumulations of small (lymphoid) cells and numerous eosinophiles. Nothing remains of the tissue transplanted.

Following the operation of March 23 the patient became progressively worse, the growth of the tumor being even more rapid than before, and she died on May 4, 1908. At each dressing of the operation wound a portion of the mass would adhere to the gauze or could be easily detached with forceps, and with one such piece another transplantation experiment was made.

Histologically the main difference between the material received at this time and that at the preceding operation consists in this: the growth now contains no cartilage and is composed of the cellular stroma and masses of epithelial cells often appearing as acini. Mitoses were numerous in both stroma and epithelium.

Monkey 4. This animal had not previously been inoculated. It received six fragments subcutaneously.

4th day. Fragments cannot be felt.

8th day. Distinctly visible. Measure 2 to 3 mm. in diameter.

12th day. Fragments disappeared.

Monkey 5. This was also a new animal. It received four fragments subcutaneously, and 2 c.c. of an emulsion, made by teasing the tumor in sterile ascitic fluid, was injected into the peritoneal cavity.

4th day. All fragments show apparent growth and measure 2 to 3 mm.

5th day. Removed one fragment for histological study.

8th day. Fragments measure about 3 mm.

12th day. Barely perceptible.

14th day. Disappeared.

Monkey 2. This animal had previously been inoculated with fragments from Monkey 1. These fragments had shown a marked increase in size, but at the time of reinoculation, on the fifteenth day, had already begun to be absorbed and measured about 3 mm. The monkey was now reinoculated in four places with new tumor material.

4th day. The fragments from first inoculation measure 2 mm. in diameter, those from the recent inoculation measure 2 to 7 mm.

5th day. One of the new fragments measuring 7 mm. was excised. A portion was used to inoculate monkey 6 in six places, and the remainder was saved for study. The fragment excised was rather sharply circumscribed and firm in consistence. Complete disappearance of fragments.

8th day. New fragments measure 7 mm.

12th day. Fragments measure 3 mm.

14th day. Barely perceptible.

20th day. Disappeared.

Monkey 3.—This animal had also been previously inoculated with a fragment excised from Monkey 1. These fragments had shown a marked increase in size, but at the time of reinoculation on the fifteenth day had undergone complete absorption.

It was reinoculated in four places with the new material.

4th day. The fragments measure from 4 to 7 mm.

8th day. Measure 6 to 7 mm.

12th day. Measure 3 mm.

14th day. Measure 3 mm.

20th day. Disappeared.

Monkey 6.—This was a new animal and was inoculated in six places with the fragment excised on the fifth day from Monkey 2.

The animal died two days later from an intercurrent disease. The fragments, which appeared pale, were saved for sections.

Some of the tumor material used to inoculate the monkeys in this second experiment was also used to inoculate five rats and five mice. These animals were examined every second day for some time, but at no examination was there any evidence of the fragments having increased in size.

Microscopical examination of the fragments excised on the fifth day from Monkey 5 shows that it consists of fibrous tissue enclosing masses of small cells (lymphocytes) and some leucocytes. No epithelial cells or cartilage are present. The increase in size of the fragment was caused in this case by inflammatory reaction.

Examination of the fragment removed on the fifth day from Monkey 2, which was used to inoculate Monkey 6, showed practically the same conditions noted above. The eosinophilic cells were more numerous than in other instances. The fragments removed from Monkey 6, which died two days after inoculation, were all necrotic.

From the experiments recorded, it is obvious that for a time the grafts increase in size under the skin of the monkey and do not increase under the skin of mice or rats. The microscopical appearance of the excised fragments indicates clearly what the causes of the increase in size are. It was found, in the first series of experiments, that the inflammatory reaction about the graft had been comparatively slight, that the epithelial elements had not survived but had entirely disappeared, and that the cartilage had not only remained alive for sixteen days, but still showed mitosis at the end of that period and had increased several fold in size as compared with the largest islands contained in the original tissue. Examination of sections made from the tumor material used in the second series of experiments shows that no cartilage was present and in the masses removed from the site of inoculation the epithelial cells and stroma of the original graft have been replaced by inflammatory tissue. As stated above, two series of experiments were conducted. In the first series only one monkey was inoculated with the nodules developing in this one, and two others were inoculated in six places each.

In the second experiment, with fresh tumor material, two new

monkeys were inoculated, and at the same time two of the old monkeys were inoculated.

Reference to the detailed description given for each monkey shows that in the three monkeys of the first series of experiments the measurements of fragments gave almost exactly similar figures on the corresponding days, but the examination of the second series in which new tumor material was used shows that the same relations do not exist.

These points are very well shown in the accompanying table.

Day.	Approximate size of piece injected.	First series.			Second series.				
		Monkey 1.	Monkey 2.	Monkey 3.	Monkey 4.	Monkey 5.	Monkey 2.	Monkey 3.	Monkey 6.
4	0.5 mm.		2	2	0	2	4.5	5.5	0
5	"		3						
7	"	2	3	2.5					
8	"				3	3	7	7	
9	"	2.5							
11	"	3	4	4	0	1	3	3	
14	"	3	3	0	0	0	1	3	
16	"	2	2	0	0	0	0	0	
18	"	1	1	0	0	0	0	0	
20		?	0	0					

There were four monkeys in the second series; two had never been inoculated and two had been used in the first set of experiments. Reference to the measurements obtained with the new monkeys shows that there was progressive increase in size of the fragments up to the eighth day, but that following this there was a rapid retrogression leading to complete absorption. In the two monkeys which were reinoculated, the increase in size was about twice that noted for the fragments in the other two monkeys of this series. The maximum size was reached on the eighth day, following which there was a progressive diminution in size, though in one monkey on the fourteenth day the fragment measured three millimeters. Complete disappearance was first noted on the sixteenth day. The differences noted in this series between the monkeys which were inoculated for the first time and those which were reinoculated are probably due to the greater reaction in the subcutaneous tissues of the latter, corresponding perhaps to the reac-

tion first reported by Arthus[5] which occurs in the subcutaneous tissues of rabbits following a third or fourth injection of horse serum.

Although the attempts to implant the tumor fragments obtained from a human subject upon monkeys failed, yet it can be said that certain of the fragments increased considerably—three or four times the size of the original fragments—in the subcutaneous tissues of the monkey and the increase continued up to the sixteenth day after the transplantation. During that period organic connection of the transplanted tissue with the monkey had taken place by means of a granulation tissue which had surrounded and invaded the fragments. It was found that all the teratomatous elements of the tumor did not survive or grow, but that only one element, the cartilage, was able to survive and increase in size. At the end of eleven days the epithelial cells had entirely disappeared and no trace of them could be found. It is possible that some of the cellular stroma of the original tumor persisted and formed part of the enveloping stroma of the cartilaginous masses. The oval cells of the stroma of the original tumor are indistinguishable from the oval cells of the transplanted fragments. That the cartilage of the implanted fragments has remained alive is shown by the excellent condition of its cells and the absence of leucocytic invasion, and that it has actually grown is indicated by the mitoses which are almost as numerous in the specimen secured sixteen days after implantation as in the original tumor, and by the obvious increase in the size of the cartilaginous masses. Thus it has been found that cartilaginous tumor tissue from men can remain alive and proliferating for sixteen days at least when implanted beneath the skin of certain monkeys, while epithelial cells, undergoing active mitosis, derived from the same tumor, quickly die and disappear.

The fact is worth noting that the results of the implantation of teratoma of human origin have been wholly different and entirely negative in certain lower animals—rats and mice—which are further removed in respect to evolution from man than is the monkey. I would, therefore, point out that it is not permissible to conclude from one set of experiments on widely removed species

[5] Arthus, *Compt. rend. Soc. de biol.*, 1903, v, 817.

that tumor implantations between heterologous species is absolutely impossible.

Finally, I am of the opinion that the results which we have obtained in the experiments on monkeys are not to be interpreted as an actual partial success in transplanting tumors from man to the monkey, but rather as an illustration, among the highest vertebrates, of the existence of a certain kind of imperfect obstacle to tumor growth between species, which has been expressed by Ehrlich[6] in his conception of atreptic immunity. In other words, the tumor cells introduced into the body of the foreign species are not at once wholly destroyed by the fluids and cells of the host, but are gradually exhausted by the want of a special and peculiar nutriment which the foreign host cannot supply, although a sufficient quantity is carried along at the time of inoculation to enable the foreign tumor cells to survive for a period. On the exhaustion of this peculiar nutritious element, the transplanted cells undergo a form of slow starvation retrogression.

[6] Ehrlich, P., *Arb. a. d. k. Inst. f. exper. Therapie,* 1906, No. 1, 84.