

EXPERIMENTAL PRODUCTION AND TRANSMISSION OF TAR SARCOMAS IN CHICKENS.

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PLATES 30 TO 32.

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The experiments described in the following pages were undertaken with the view of gaining information as to the filterable causative agents of transplantable chicken tumors. This group of neoplasms,¹ representing a variety of typical sarcomas, resembles in all particulars, histologically and biologically, the transplantable tumors of other animals, yet many, if not all, of those closely studied have the unique quality of being transmissible through the agency of a cell-free filtrate^{2,3} or a desiccate of the tumor. Furthermore, the distinctive histological and biological characters of the individual neoplasms are transmitted equally as well by the filtrate or desiccate as by the living tumor cells. The nature of these filterable agents has been discussed repeatedly because of their fundamental significance in the general question of tumor etiology. Four main conceptions have been put forward to explain the facts but two of them, namely that the chicken sarcomas are inflammatory conditions resulting from infection, not true tumors, and the other that the viable neoplastic cells pass through the filters or resist drying, are so unlikely that they need not be discussed. Rous has been inclined to interpret the evidence as indicating that the causative agents are in the nature of filter-passing organisms. Another interpretation hinted at by some authors

¹ Fujinami and Inamoto, *Verhandl. jap. path. Ges.*, 1911, 1, 114. Rous, P., *J. Exp. Med.*, 1910, xii, 696. Tytler, W. H., *J. Exp. Med.*, 1913, xvii, 466. Rous, P., and Lange, L. B., *J. Exp. Med.*, 1913, xviii, 651.

² Rous, P., *J. Exp. Med.*, 1911, xiii, 397. Rous, P., Murphy, Jas. B., and Tytler, W. H., *J. Am. Med. Assn.*, 1912, lix, 1793.

³ Rous, P., and Murphy, Jas. B., *J. Exp. Med.*, 1914, xix, 52.

(*i.e.*, Doerr⁴) presumes the existence in these tumors of a substance produced by the tumor cells and causing the malignant transformation of other cells in a manner analogous to the action of the d'Hérelle substance which produces changes in the bacteria with which it is associated, appears to multiply in such association, and is transmitted to subsequent generations.

Our purpose was to engender in chickens a malignant tumor, preferably a sarcoma, by artificial means and to investigate whether or not such a tumor would contain a filterable agent capable of transmitting the tumor. A positive finding might be taken to favor the conception that an inanimate principle is responsible for tumors. Whatever the outcome, however, it would have an interesting bearing on the subject.

The method selected was the injection into fowls of an extract from the residue of distilled coal tar. It is well known that such a substance applied repeatedly to the skin will induce carcinoma,⁵ but recently Russell has shown that the subcutaneous injection of it in rats is quite as regularly followed by the development of sarcoma.⁶ There seems to be no doubt that the experimentally induced tumors are true neoplasms. Histologically they have the same characteristics as the spontaneously developed growths. They metastasize more or less freely, and some of them are transplantable though as a group they are subject to the same limitations in this respect as spontaneous tumors.

Production of Tar Tumors in Chickens.

As it was desirable for our purposes to secure a sarcoma rather than a carcinoma, the tar preparation was injected instead of painted on the surface. With the hope of hastening the process hashed up tissues of chicken embryos were added to the first tar injected and the later injections were made into the embryomas that developed. The tar product used had been previously found to be extremely active in the production of cancer in mice.

⁴ Doerr, R., *Klin. Woch.*, 1922, i, 1489, 1537.

⁵ Yamagiwa and Itchikawa, K., *Gann. Japan Z. Krebsforsch.*, 1916, x, 1. Tsutsui, H., *Gann. Japan Z. Krebsforsch.*, 1918-19, xii, 17. Fibiger, J., and Bang, F., *Compt. rend. Soc. biol.*, 1920, lxxxiii, 1157.

⁶ Russell, B. R. G., *J. Path. and Bact.*, 1922, xxv, 409.

Preparation of Tar.—The tar product used throughout these experiments was prepared from a residue obtained from a coke oven in which the tar had been distilled at a temperature of approximately 377°C.⁷ About 250 gm. of the hard, black, pitch-like residue was pulverized to a fine powder and extracted with 2½ liters of benzene for 24 hours. It was then filtered to remove the undissolved particles and the filtrate distilled until the volume was reduced to approximately 500 cc. The tarry residue was then washed with 1 liter of a 12 per cent NaOH solution in water and the same procedure was repeated with 1 liter of 30 per cent H₂SO₄. The heavy precipitate thrown down by these treatments was removed by gently warming the mixture on a steam bath until the precipitate coalesced, when the liquid fraction was decanted. The latter portion was washed four times with water. The tar fraction which remained in the benzene solution was evaporated to drive off the excess benzene, leaving a residue of about 300 cc. of the product.

Experiment 1.—Ten full grown Rhode Island Red hens were injected in the right breast with 0.3 cc. of hashed 7 day old chick embryos and in the left breast with 0.5 cc. of a mixture of equal parts of tar residue and hashed embryo. At 2 week intervals thereafter, throughout the duration of the experiment, 0.2 cc. of the tar was injected into the resulting mass in the left breast. The rate of growth of the embryonic tissue in the two sides showed considerable variation. In four birds the larger nodule developed in the tar-injected side, while in four the embryonic tissue alone grew more rapidly, and in two the growth was about equal. With one exception the normal embryomas reached their maximum size in from 1 to 2 months and then slowly retrogressed to complete absorption. One of these, however, continued to grow for 5 months, by which time it had attained a size of 5.6 by 4.3 cm., when it became stationary and has since shown no growth. Pieces removed at operation have shown it to be made up of bone and cartilage.

By the end of 5 months only three fowls remained alive and two of these had progressively enlarging tumors in the tar-injected side.

Chicken Tumor A.—In *Chicken 1* the growth started after a period of retrogression of the embryoma. During the 5th month the nodular mass characteristic of the embryoma was replaced by a smooth well rounded tumor which continued to grow progressively till it caused the death of the animal. Small pieces were removed from time to time for histological examination. Hereafter we will refer to this neoplasm as *Chicken Tumor A*.

At autopsy the left breast muscle was almost completely replaced by a large tumor composed of two rounded masses connected by a wide base. The larger measured 5 to 6 cm. in diameter and the other about 4 cm. In places the tumor was bordered by reactive tissue for the most part associated with the remains of the tar. The tumor

⁷ We are indebted to the Barrett Company for the preparation of this product.

was firm, almost gristly, sharply differentiated from the muscle, homogeneous, semitranslucent, with a slightly yellow tinge. There was no necrosis or hemorrhage. Tar droplets were found scattered through it. The remains of the old embryoma persisted in the right breast as a thin yellowish plaque about 5 by $3\frac{1}{2}$ cm. and less than $\frac{1}{2}$ cm. thick. There were deposits of fat scattered through this firm mass which on section appeared to be made up of bone and cartilage. No gross metastases were found in any of the organs. Some congestion of the lungs was present and the liver was mottled with whitish areas from pin-point to pin-head size. The egg duct contained a normal sized egg with the shell crushed but the membrane intact. The other organs appeared normal.

Histologically the tumor was made up of medium size spindle cells, closely compacted and coursing in irregular bundles. The remains of the embryoma, composed mostly of bone and cartilage, were found at the edge of the tumor. No microscopic metastases were noted.

Chicken. Tumor B.—Chicken 9 showed the beginning of an active tumor growth in the tar-injected side by the end of the 3rd month and this progressed till it caused the death of the fowl by the 7th month of the experiment. This neoplasm will be designated Chicken Tumor B.

At autopsy a large tumor was found in the left breast, measuring 8.1 by 6.5 cm., almost replacing the pectoral muscles (Fig. 1). In the skin of the right breast there was a nodule about the size of a pea, the result of an autotransplant introduced by us several weeks previously. A crater-like cavity in the middle of the primary tumor was filled with blood and necrotic debris. On cutting through the tumor it was found to be uniformly grayish white in color, firm to the touch but easily crushed. There was no necrosis except a small area associated with the cavity resulting from the removal of a piece of the tumor. The borders were sharply defined from the muscle but the striæ occurring in the tumor followed the same general line as the fibers of the muscle. The liver contained a rounded mass approximately 2 cm. in diameter and two smaller ones (Fig. 2). On section the larger showed a beginning necrosis in the center with radiating lines extending to the periphery. The lungs were almost replaced by firm whitish nodules varying from the size of a pin-head to that of a large pea (Fig. 3). In general consistency the hepatic nodules were similar to the nodules in the lungs. The other organs appeared normal.

Histologically the tumor tissue was of rather uniform appearance. It consisted of irregular, interlacing bundles of slender spindle cells, and a small amount of intracellular slightly undulated fibrils. The tumor cells did not vary much in shape or size but a small number of rounded forms were noted, sometimes containing more than one nucleus. The typical nuclei were long and thin, some rod-like. Mitosis was rare. There were a few foci of mononuclear round cells and some lacunæ, from which doubtless tar remains had dissolved out, surrounded by foreign body giant cells. In places on the surface the connective tissue was honeycombed with such lacunæ, enclosed by the same type of giant cells. The cells and the general structure of the metastases resembled the original tumor but more rounded elements were present in the lung metastases, and the spindle cells were in general shorter and thicker.

Transplantation Experiments. Chicken Tumor A.

At intervals during the development of Chicken Tumor A pieces were removed and transplanted into other Rhode Island Red fowls from the same stock. In all, 56 chickens were inoculated, 26 of these being young fowls from 5 weeks to 3 months old. Not a single progressively growing tumor resulted, although temporary growth with later retrogressions took place in a few cases.

Transplantation of Chicken Tumor B.

The same general procedure was carried out with this tumor.

First Generation.—In all, 50 adult Rhode Island Red chickens received grafts from Chicken Tumor B and in several of these a temporary nodule developed but with one exception they retrogressed. In this one chicken, however, the tumor grew continuously but with great slowness so that at the end of 3 months it was only about 1.5 cm. in diameter, but during the next month active growth began and proceeded with great rapidity till the whole breast was replaced. Autopsy showed a tumor similar in general character to the original one, both in the gross and microscopically, but no metastasis had taken place.

Second Generation.—Grafts from the single tumor arising in the

first generation were grafted into both breasts of 15 young and 19 adult Rhode Island Red chickens. Of these 17 developed tumors which grew progressively in 14 instances while in 3 they retrogressed after attaining considerable size. Several of the tumor-bearing chickens were killed early but of those allowed to survive 7 showed metastases. The lung was involved in 6 cases, the heart in 3, the ovary in 3, the peritoneal cavity in 2, and the liver and stomach in 1.

Third Generation.—Transplants from the preceding generation yielded tumors in 8 out of 13 chickens inoculated and in all except 1 of these they grew progressively. The lungs were involved in 4 instances and the heart, liver, stomach, skin, muscle, and peritoneal cavity in 1 instance each.

The tumor has been carried in this manner through eleven generations, during which passages there has been some increase both in the growth rate and in the number of takes. The general character of the growth has not changed except that in the more rapidly growing tumors there is a greater amount of necrosis, of a dry yellowish character. We have encountered no cystic degenerations; and hemorrhages into the tumor are rare and never extensive. It is not uncommon to find that tumors growing in the breast muscle tend to follow the large vessels up towards the axilla and in several instances the tumor has extended through the axilla to the pericardium (Fig. 4). Occasionally we have observed scattered nodules on the outer surface of the esophagus extending down to and involving the pouch about the stomach. The distribution suggests that the tumor had been disseminated through the lymphatics.

Of the 77 fowls having progressive tumor something over 50 per cent had metastases. The lungs were involved in 31, the ovaries in 11, the heart in 7, the stomach in 7, the intestines in 6, the pericardium in 4, and the liver in 6. In 1 instance each, the skin, trachea, striated muscle, crop, and pancreas, was the site of metastasis. With the exception of the original chicken no discrete metastases have been found in the liver, the involvement in all of the 6 instances being due to direct extension of the tumor through the chest wall or the diaphragm.

Histologically the structure of the tumor in the later generations is similar to that of the original growth except for a greater irregularity

in the form and size of the cells (Fig. 5). Many of these are plump and irregular in outline, the nucleus large and intensely stained, and some are pycnotic. By incomplete division, giant cells with several or many nuclei are formed. Variability in size and shape of the cells is more evident in the metastases than in the primary tumor. Mitosis is frequent, in some tumors remarkably so (Fig. 6). Necrotic areas occur frequently and in some instances only a thin layer of tumor cells is found on the surface of the tumor mass.

The invasive character of the growth is evidenced by the infiltration and destruction of various tissues as muscle, fat, liver, constituents of the normal structure being surrounded and widely separated by the malignant growth. This is most strikingly seen where striated muscle is being replaced by the sarcomatous tissue, for here the tumor cells not only grow in the spaces between the fibers but invade the fibers themselves (Fig. 7). Frequently only remnants of the muscle fibers are to be seen either in the center or on the circumference of the sarcomatous cast. In such places, the structure simulates in cross-section a sarcoma with polygonal cells and alveolar structure, but on longitudinal section the spindle shape of the plump cells is still recognizable.

Filtration and Desiccation Experiments.

The tumor described here is not so active as one of the three types of sarcomas,—spindle-celled sarcoma, osteochondrosarcoma, and intracanalicular sarcoma,—studied at The Rockefeller Institute and perhaps metastasizes with less regularity; yet it is far more malignant than two other types of tumor described. In general behavior and histological appearance the tar tumor is most like the simple spindle-celled sarcoma of the group just mentioned, but a notable difference is to be observed in the fresh specimen as compared with this transplantable tumor of spontaneous origin. The latter tumor is usually soft and contains great quantities of mucoïd material, while the tar tumor is much firmer and is free from any mucilaginous material. The general character, however, of the tar tumor unquestionably justifies grouping it with the other transplantable chicken sarcomas and all of these closely studied have proved to be transmissible through the agency of a cell-free filtrate or desiccate of the tumor material.

A priori there would seem to be every reason to expect that the tar tumor would be found to possess the same quality.

Filtration Experiments.—About 20 gm. of the tumor material relatively free from necrosis was chopped into fine bits and thoroughly ground with sterile sand. About 400 cc. of Ringer's solution was added and the suspension shaken for 20 minutes. It was then centrifuged to remove the sand and solid particles, and the supernatant fluid decanted. A small amount of a suspension of *Bacillus prodigiosus* was added immediately before filtration in order to test the filters. The extract was then passed, part through a Berkefeld N and part through a Berkefeld V filter. Sterile infusorial earth (Kieselguhr) was added to each of the filtrates and from 5 to 10 cc. injected into the breast muscles of normal Rhode Island Red chickens. Cultures of the filtrate on agar remained sterile, showing that the filters were impervious to *Bacillus prodigiosus*.

This filtration test was carried out seven times with material from seven different tumors selected from the first to the eleventh generations and, in all, 50 chickens have been inoculated with filtrate. The fowls were followed for from 3 to 5 months, and in a few instances still longer. Not one tumor developed.

Desiccation Experiments.—A quantity of fresh tumor material was chopped fine, spread in a thin layer on a glass plate, and placed in a desiccation jar over sulfuric acid. The air was evacuated and the jar placed in a freezing room till the tumor was completely dry (24 to 48 hours). The material was then ground to a fine powder, taken up in Ringer's solution, and injected into the breast muscles of normal chickens.

This test has been carried out with five different tumors and 42 chickens have been injected with the material. None of these have developed tumors during the period of observation (3 to 6 months).

DISCUSSION.

The immediate object of the experiments has been accomplished in that a sarcoma of the chicken has been engendered by experimental processes which in its histology and its general behavior on transplantation resembles closely some of the spontaneous chicken tumors. The tar tumor fulfills all the criteria necessary to identify it as a neo-

plasm. It grows with considerable rapidity, metastasizes freely, and is highly invasive in character. It is as typical a neoplasm as the transplanted chicken tumors of "spontaneous" origin. Nevertheless we have been unable to demonstrate in association with the growth a tumor-producing substance separable from the tumor cells. It can be transplanted at the present only by the employment of living cells. However, the data thus far obtained have the limited value of negative evidence, for the possibility cannot be excluded that another tar tumor or a different technical procedure might yield a positive result.

A point of perhaps fundamental importance should be mentioned; namely, the suitability of the soil necessary for the effective action of tumor filtrates. The causative agent of the chicken tumor which this growth so much resembles (Chicken Tumor I) is capable of producing malignant transformation in the mesodermal tissue of the embryo,⁸ having no effect on the ectodermal or endodermal tissues, and in the adult^{3,9} it gives rise to tumors only when brought in contact with young or reactive connective tissue. The tar tumor considered in the present paper had its origin in embryonic tissue and quite possibly filtrates from it may be capable of affecting only a specific type of embryonic cell. This problem is under investigation and attempts are also being made to obtain other strains of tar tumors.

SUMMARY.

Two spindle cell sarcomas have developed as the result of the injection of coal tar into induced embryomas in chickens. One of these, transplanted to other chickens for eleven generations, is highly invasive in character, and metastasizes freely. Attempts to transmit this neoplasm by a filtrate or desiccate of the tumor have failed of success.

EXPLANATION OF PLATES.

PLATE 30.

FIG. 1. Lateral view of the chest wall of Chicken 9 (Chicken Tumor B) showing the original tumor induced by the injection of tar. The tumor has been sliced

⁸ Murphy, Jas. B., and Rous, P., *J. Exp. Med.*, 1912, xv, 119.

⁹ Rous, P., Murphy, Jas. B., and Tytler, W. H., *J. Am. Med. Assn.*, 1912, lviii, 1751.

through showing (*A*) healthy tumor, (*B*) necrotic area, (*C*) cavity containing the blood clot resulting from an earlier operation.

FIG. 2. Liver from the same animal showing two small and one large metastases indicated by arrows.

PLATE 31.

FIG. 3. Lung from the same animal. The white areas are metastases which almost replace the entire lung.

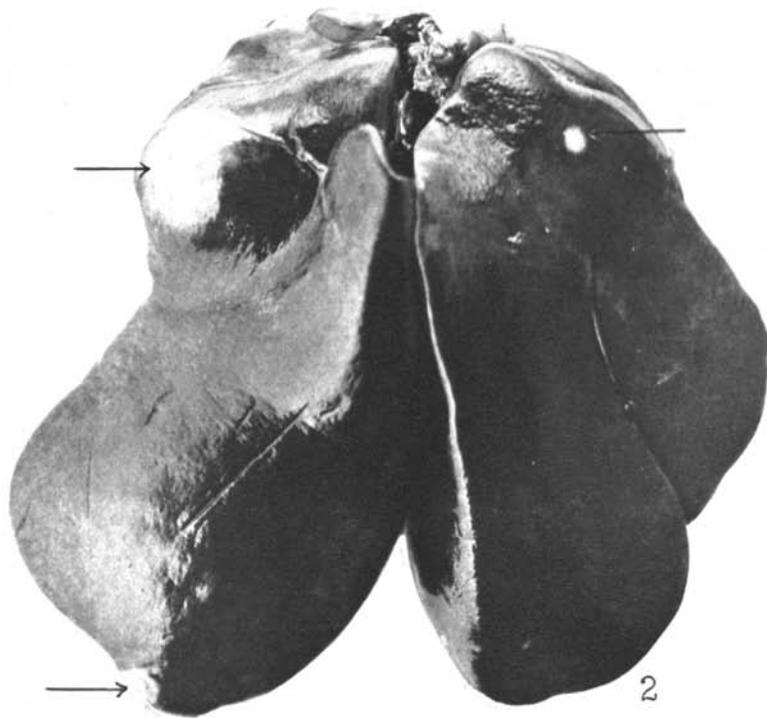
FIG. 4. Heart from a chicken inoculated in the breast muscle with a transplant from the tar-induced tumor (third generation). The tumor in this case followed the large vessels through the axilla and down to the heart. *A*, mass of tumor surrounding the large vessels; *B*, metastasis in the heart muscle.

FIG. 5. A photomicrograph of a section taken from a later generation of the tar tumor.

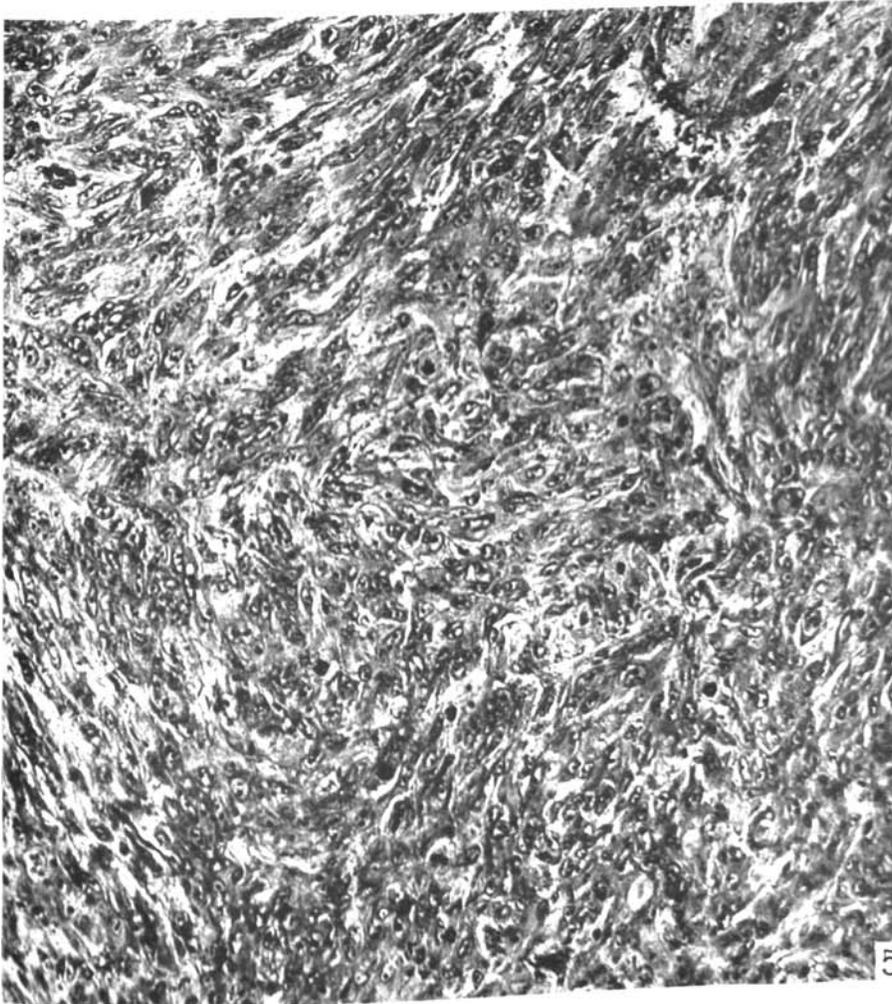
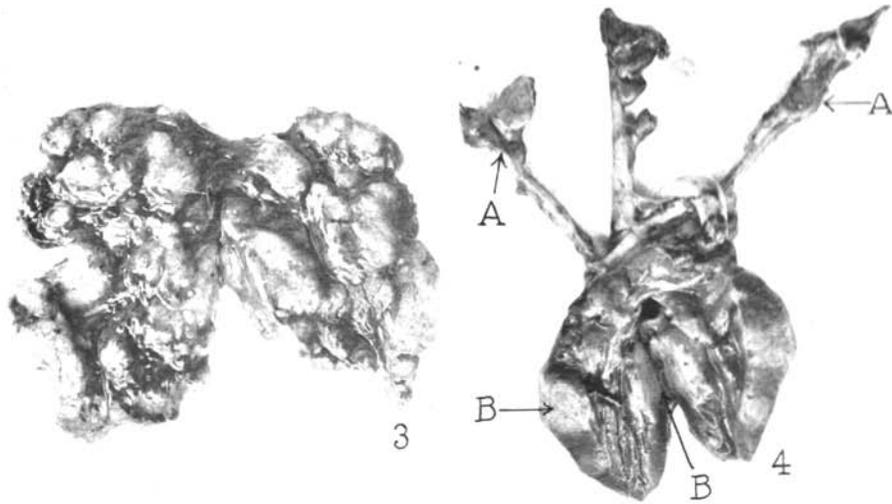
PLATE 32.

FIG. 6. A section of a transplant of the tar tumor showing a remarkable number of mitotic figures.

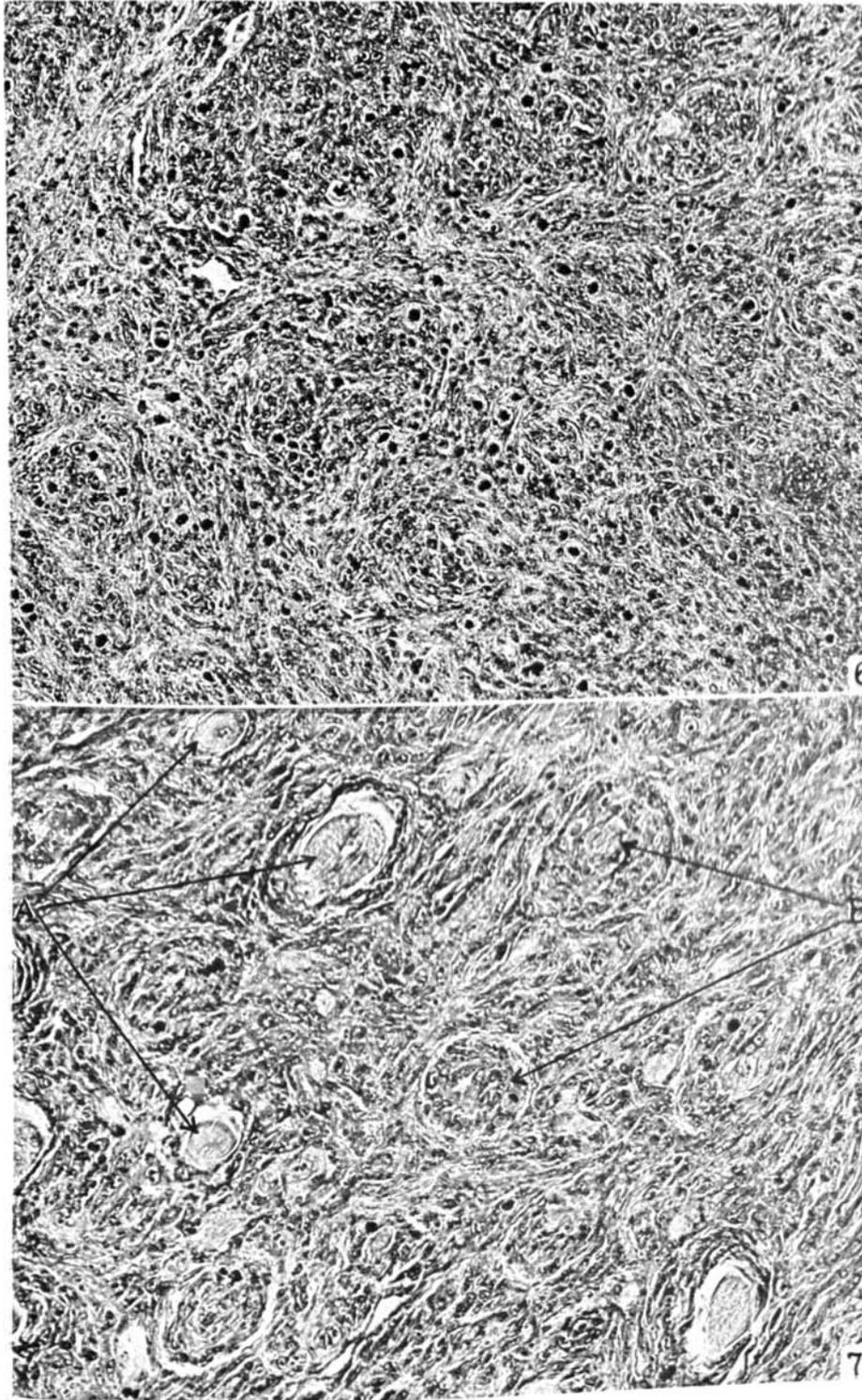
FIG. 7. Section showing the tar sarcoma invading striated muscle. *A*, remains of muscle fibers partly invaded by tumor cells; *B*, muscle fibers completely replaced by tumor cells.



(Murphy and Landsteiner: Tar sarcomas.)



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