A TRANSPLANTABLE CARCINOMA OF THE GUINEA PIG.

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PLATES 29 TO 32.

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The successful transplantation of rat and mouse tumors has led to many attempts to transfer the tumors of other species. The venereal lymphosarcoma of dogs, transferred by coitus, has been studied by a number of investigators, especially Sticker.1 Von Dungern and Coca2 described, and succeeded in transferring an epidemic tumor of the hare. They3 were able to transfer the venereal dog tumor to foxes and the hare tumor to rabbits. The status of these growths must be considered doubtful since it has been well established that neoplasms in general are only transplantable within the species in which they occur spontaneously. Ribbert4 has described a fibroma of the dog which he inoculated successfully into two other series of dogs.

Rous5 succeeded in transferring a spindle-celled sarcoma of a fowl. Later he and his collaborators reported a number of other transmissible avian sarcomata. Fujinami and Inamato6 have transplanted a myxosarcoma of the fowl. More recently Schultze7 described a spindle-celled sarcoma of the rabbit which he had propagated through twelve tumor generations. In the laboratories of the Imperial Cancer Research Fund of London another rabbit sarcoma has been studied.

Up to the present a transplantable tumor of the guinea pig has not been reported. Few data are obtainable concerning the tumors of this species; apparently they are rare. Sternberg8 reported a metastasizing adenocarcinoma of the mammary gland of the guinea pig. He did not attempt transplantation.

1 Sticker, A., Ztschr. f. Krebsforsch., 1904, i, 413.
3 von Dungern, München. med. Wehnschr., 1912, lix, 238.
The tumor which I have transplanted occurred in the mammary gland of an old female guinea pig which, judging from the condition of the breast, had recently suckled offspring. This animal was removed from the Institute breeding pens, with a number of others suffering with abscesses. It had a large fluctuating abscess in the submental region and a large, rather firm, sharply circumscribed tumor in the left mammary gland. This tumor lay in the substance of the gland directly beneath the skin, but was not attached to the subcutaneous tissue or musculature. It was hemispherical in shape, of a grayish white color, and measured 4.5 by 4 by 3.6 cm. The mass was enveloped in a delicate, translucent capsule. It was removed and cut across. Just beneath the capsule was a zone of grayish white, translucent tissue 0.5 to 1 cm. wide. A milky fluid exuded from its cut surface. Toward the center of the tumor this zone gradually faded off into a rather granular necrotic material. The color of this portion of the growth varied from yellowish white to ocher. The diagnosis of cancer was made from frozen sections of the outer zone of grayish white tissue. Examinations of the regional lymph glands and other organs for metastases were negative.

Bits of the peripheral layer of the tumor were inoculated by means of a trocar into the subcutaneous tissue of the groin and into the muscles of the upper leg of sixteen young guinea pigs. Most of the animals were between two and three weeks old. Animals of this age were chosen because it has been frequently shown that young individuals are especially favorable hosts for tumor grafts.

First Tumor Generation.

Oct. 30, 1914. Sixteen young guinea pigs were inoculated with two small pieces of the tumor in the subcutaneous tissue of the left groin and the muscles of the left upper leg. The pieces varied from 0.15 to 0.2 cm. in diameter. On Jan. 15, 1915, one of the animals showed a swelling in the muscles of the leg at the site of inoculation (Fig. 1). The growth was irregularly rounded and protruded sharply from the surface of the leg. It measured 2.1 by 1.7 by 1.2 cm. The subcutaneous graft in the left groin could not be detected on palpation. On Jan. 20, 1915, the animal was anesthetized with ether and the major portion of the tumor removed. It lay within the muscles, protruding sharply from the general contour of the leg. Externally it was covered with a thin sheet of muscle. The mass was encapsulated on its external surface with a thin, trans-
lucent layer of connective tissue. The inner surfaces were firmly fixed to the muscles. Nearly all of the tumor was removed in small pieces. These consisted of pink, friable tissue, some portions of which were hemorrhagic. A few circumscribed areas of necrosis were noted. The incision in the skin was closed with silk. Most of the tissue was used to inoculate another series of animals. The remainder was fixed in Zenker's fluid for microscopic study.

The other animals of this series were under observation for over 6 months. None of them developed tumors.

Histology of the Primary Tumor.

Sections of the original tumor show it to be a carcinoma. The cells are arranged in acini, or more or less irregular clumps, supported by a delicate connective tissue stroma. The proliferating portion is confined to a narrow layer surrounded by a thin capsule. In one section the cancer cells have penetrated this capsule and invaded the mammary gland (Fig. 5). The individual cells in the growing portion are large, with a large vesicular nucleus. They stain well. A few mitotic figures are seen. Toward the center the cells appear shrunken and the nuclei stain intensely, while still further in all cell structure is lost. The tumor is well vascularized, but not hemorrhagic.

A few eosinophils are found along the borders of the tumor. In several places its cells have invaded the fatty tissue of the gland (Fig. 5). The interacinar connective tissue is increased throughout the section. Many of the neighboring acini and ducts show pressure changes. They are flattened and the epithelium is atrophied. The wall of one of the larger ducts adjacent to the tumor has become infiltrated with tumor cells and the growing tissue extends as a considerable mass into the duct lumen. There is a marked collateral hypertrophy of the lining epithelium and the interacinar mass is partly covered by a double layer of non-malignant columnar cells (Fig. 7).

Of great interest are certain changes in the breast that cannot be attributed directly to the tumor. One finds the atrophy, overgrowth of connective tissue, and chronic inflammatory changes seen in the breasts of old animals of other species. Some of the acini at considerable distance from the tumor show proliferative changes. Their epithelium has proliferated until they are nearly occluded with a densely packed mass of cells (Fig. 6). Apparently the cells in lesions
of this type show no tendency to break through the acinar walls. A few acini reveal still more advanced changes and have almost lost their character. The remains of such an acinus consist of an irregular clump of cells with an ill defined basement membrane. As a whole, the changes are similar to the so called precancerous lesions noted by Haaland\(^9\) in the breasts of old female cancerous and non-cancerous mice. McCarty\(^10\) has also described the same conditions of the mammary glands of women suffering from carcinoma of the breast.

**The Tumor of the First Transplantation.**

It has been stated in the protocol that a tumor developed slowly in one animal inoculated with small pieces of the spontaneous tumor (Fig. 1). The greater portion was removed, but it began to recur 3 weeks after operation.

Mar. 3, 1915. The operated tumor remained quiescent for about 3 weeks and then began to grow rapidly. On this date it measured 2.4 by 1.5 by 1.5 cm. The enlarged inguinal lymph gland on the same side was felt as an oval disc measuring 1.9 by 1.4 by 0.6 cm. (Fig. 2). Growth of the subcutaneous graft had not taken place.

Mar. 8, 1915. The animal was found dead. The leg tumor measured 2 by 1.6 by 1.2 cm. It was an irregular flattened sphere with a smooth projecting surface. The deeper portions had invaded the muscle. On section the growth was divided into numerous lobules by strands of connective tissue. Considerable brownish yellow, necrotic material was noted. The peripheral portions of the tumor were composed of friable, translucent, yellowish white tissue which exuded a milky fluid.

The disc-shaped mass in the inguinal region proved to be a metastasis in which the lymph gland had been replaced almost entirely with tumor tissue. It measured 1.2 by 1.1 by 0.6 cm. and it was made up of the same type of tissue as the leg tumor. About 1.5 cm. above the metastasis there were two tiny, discrete, hemispherical, grayish white nodules on the external surface of the abdominal muscle. These were doubtless a part of the tissue supposed to have been inoculated subcutaneously.

On opening the abdominal cavity a large, irregular, ovoid tumor was found. It measured 6.6 by 5.0 by 3.8 cm. and lay on the right side between the liver and

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cecum (Fig. 4). Broad bands of connective tissue connected it with the neighboring viscera and peritoneum. Its surface was smooth and glistening. The mass was divided by fissures into four principal lobules which in turn were separated into a large number of small lobulations. The color varied. The sounder portions were grayish white and translucent. Considerable hemorrhage had occurred beneath the capsule, which resulted in a gray and red mottling. The capsule was thin and well vascularized.

On section the surface lobulations were found to extend throughout. The four principal ones were separated by broad bands of connective tissue. At both ends and along the superior border was a layer of translucent, grayish white, nearly homogeneous tissue. The balance of the tumor was practically comprised of pinkish or reddish gray necrotic material.

Other tumors were not found in the abdominal cavity. Gross or macroscopic metastases were not observed in the other organs or glands.

Doubtless the large abdominal tumor was responsible for the death of this animal. From the location and general character of the tumor it seemed evident that it resulted from direct transplantation. Probably the cannula, with which the subcutaneous inoculation had been made, pierced the abdominal wall, and a small portion of the tumor lodged in the mesentery. The inguinal tumor was unquestionably a metastasis from the leg mass to the inguinal lymph gland.

Second Tumor Generation.

Series A.—Jan. 20, 1915. Thirty young guinea pigs were inoculated in the leg muscles and in the subcutaneous tissue of the groin with small pieces of tumor from No. 31.

Feb. 26, 1915. One animal, No. 26, had developed a tiny shot-like nodule in the leg and groin.

Mar. 8, 1915. Animal 26 was found dead. In the muscles of the leg at the site of inoculation were three round, raised, grayish red nodules varying in size from 0.2 to 0.4 cm. In the subcutaneous tissue of the groin 6 small glistening gray nodules were found embedded in the fascia. Microscopically these consisted of living tumor tissue, doubtless that which had been implanted. Metastasis to the other organs had not taken place. Bronchopneumonia caused the animal's death.

Mar. 8, 1915. Two other guinea pigs, Nos. 9 and 38, had developed small tumors in the leg and groin. The greater portion of the leg graft of No. 9 was removed and inoculated into the leg muscles of twenty young animals (3rd Tumor Generation, Series A).

May 1, 1915. The operated tumor recurred very slowly at first, but later grew rapidly. On this date it measured 2 cm. The animal was again anesthetized and a considerable portion of the leg tumor removed; bits of it were injected into twenty-five others (3rd Tumor Generation, Series B).
May 11, 1915. The leg tumor of No. 38 had grown slowly. At this time it measured 1.8 cm. in diameter. The major portion of it was removed, chopped fine, and drawn into a syringe. Five young guinea pigs were inoculated in the leg muscles with small amounts of the hash.

May 15, 1915. The tumor of No. 9 enlarged rapidly after the last operation, but soon began to soften and become a huge, almost spherical swelling. It involved the whole upper leg and hip and extended to the mammary region (Fig. 3). It measured 4.7 by 4.2 by 4.2 cm. In the leg of Guinea Pig 38 a large diffuse wedge-shaped nodule had appeared.

June 18, 1915. Animal 9 was found dead. The leg tumor had begun to ulcerate several days before. At autopsy it was found to be largely necrotic. The inguinal lymph glands draining the tumor were enlarged. Gross tumors were not found in other organs. Microscopic examination of the enlarged inguinal lymph glands revealed a metastatic tumor in one of them. Metastatic tumor cells were observed in sections of the kidney. They were scattered in irregular aggregates in the external portion of the medulla. The process seemed to have begun in the interlobular capillaries with consequent invasion and obliteration of the tubules. Metastases were not found in the lungs or other organs.

Series B.—Mar. 8, 1915. Twenty young guinea pigs were inoculated by means of a cannula in the leg muscles and in the peritoneal cavity with bits of the leg and abdominal tumors of No. 31, obtained some hours after death. The animals were under observation over 4 months, but none of them developed a tumor.

Third Tumor Generation.

Series A.—Mar. 30, 1915. Twenty young guinea pigs were inoculated with cannulas in the left leg with bits of tumor from No. 9.

Apr. 16, 1915. No. 103 had a small tumor at the point of inoculation.

May 11, 1915. Three animals, Nos. 103, 77, and 67, had developed tumors of a rather uniform size in the inoculated leg muscles. The largest measured 2.3 cm. and the smallest 1.8 cm. On operation most of the tumor of No. 103 was necrotic. Five young guinea pigs were inoculated in the leg with the better portions of the growth (4th Tumor Generation, Series A). Twenty others were injected in the leg with small pieces of tumor incised from the leg of No. 77 (4th Tumor Generation, Series B).

June 16, 1915. The tumor of No. 67 was incised and several small portions of it were inoculated into the upper leg of 15 young guinea pigs (4th Tumor Generation, Series C).

The Transplanted Tumor.

There has been a gradual increase in the number of takes. In the later generations the tumors have appeared earlier and have grown more rapidly and reached a larger size.
Microscopically the transplanted tumor consists of a zone of variable width of rapidly proliferating epithelial cells surrounded by a thin capsule. The cells may be grouped in acini, but they usually lie in small irregular clumps. Large numbers of them may be undergoing mitotic division (Fig. 9). Degenerative changes similar to those observed in the spontaneous tumor are frequent. At the growing edge small blood vessels are numerous. Occasionally they have the form of thin-walled sinuses (Fig. 8). One is struck by the slight reaction about the tumors; practically no round celled infiltration is observed about their borders. The connective tissue capsule is well defined but thin.

SUMMARY.

An adenocarcinoma of the mammary gland of an old guinea pig has been successfully transplanted through eight successive series of animals. It now appears much earlier and grows more rapidly. The number of takes also has increased. In two instances metastasis to the regional lymph glands (inguinal) has been observed. Once microscopic metastases were found in the kidney. The so called precancerous changes observed in the breasts of women and mice suffering from mammary carcinoma were found in the mammary gland of the spontaneous tumor animal. It is hoped that the tumor may soon be utilized for experimental purposes.

EXPLANATION OF PLATES.

PLATE 29.

FIG. 1. The tumor in the leg of Guinea Pig 31 of the 1st Tumor Generation, 81 days after inoculation.
FIG. 2. Recurrence in the leg of the same animal, at autopsy 47 days after partial removal. Note the metastasis to the inguinal lymph gland.
FIG. 3. Large cystic tumor of the leg of Animal 9 of the 2nd Tumor Generation, 4 months after inoculation. On two different occasions large portions of the growth had been removed.

PLATE 30.

FIG. 4. The abdominal tumor found at autopsy of No. 31 of the 1st Tumor Generation. It has been reflected to show the bands of attachment.
PLATE 31.

Fig. 5. Cells of the spontaneous mammary tumor invading the fatty tissue of the gland.

Fig. 6. Epithelial inclusions in the lumen of the mammary acini of the original animal.

Fig. 7. Growth of the cells of the spontaneous tumor through the wall and into the lumen of one of the larger milk ducts. The growth of the cancerous epithelium has been accompanied by a collateral growth of the lining columnar cells. Note the pressure changes of the smaller ducts.

PLATE 32.

Fig. 8. The characteristic border of growing neoplastic cells with many blood sinuses in a section of transplanted tumor.

Fig. 9. The transplanted tumor in a later generation showing the usual arrangement of cells. Many of them are undergoing mitosis.
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