A TRANSPLANTABLE NEW GROWTH OF THE FOWL, PRODUCING CARTILAGE AND BONE.*

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PLATES 79 TO 83.

Neoplasms of the common fowl have been described by numerous authors, and include tumors of various classes. Up to the present time four transplantable tumors of the fowl have been reported, one of which is the growth here dealt with. Fujinimi and Inamoto in 1910 reported at a meeting in Japan a myxosarcoma which they had succeeded in transferring to other fowls.¹ In the same year Rous² described the transplantation of a spindle-celled sarcoma which at times showed myxomatous tendencies. This tumor was highly malignant, usually leading to the death of the animal, and often producing widespread metastases. The tumor reported by Fujinimi and Inamoto appears to have been not so malignant, though sometimes metastasizing and causing death. The tumor which forms the subject of the present paper is an osteochondrosarcoma and has been mentioned in an earlier note³ from this laboratory, in which was also reported the fourth transplantable chicken tumor, a spindle-celled sarcoma. For purposes of reference the osteochondrosarcoma is known in this laboratory as Chicken Tumor VII. It has great interest from at least two points of view. Its cells regularly undergo a process of metaplasia and differentiation to form cartilage and bone; and the growth is transmissible, like the spindle-celled sarcoma of Rous, by an agent separable from the tumor tissue and filterable through Berkefeld filters.⁴ The filtrate,

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¹ Fujinimi, A., and Inamoto, K., *Verhandl. d. Jap. path. Gesellsch.*, 1911, 114.

² Rous, P., *Jour. Exper. Med.*, 1910, xii, 696; 1911, xiii, 397.

³ Rous, P., Murphy, J. B., and Tytler, W. H., *Jour. Am. Med. Assn.*, 1912, lviii, 1682.

⁴ Rous, P., Murphy, J. B., and Tytler, W. H., *Jour. Am. Med. Assn.*, 1912, lix, 1793.

when brought into contact with connective tissue under suitable circumstances, gives rise to the characteristic growth in which is laid down cartilage, followed often by bone.

DESCRIPTION OF THE ORIGINAL GROWTH.

The fowl bearing the original growth was obtained while yet alive from a dealer in poultry. It was a Plymouth Rock hen, in good condition, of approximately pure breed, and apparently about a year old. On the lower portion of the keel of the sternum was an irregularly spherical mass, so symmetrically disposed that the keel passed almost through its center. It measured 6.6 by 5.7 by 4 centimeters. The growth was smooth, nearly as hard as bone, and the skin over it was slightly stretched but not firmly attached (figure 1). A wedge-shaped piece of it was taken out under ether anesthesia. The tissue thus obtained was found to be firm, white, and fibrous, with some translucent areas suggesting cartilage. Small bits were at once implanted in the original fowl, by means of a trocar (Bashford needle). Two such grafts were placed in the pectoral muscles, and four subcutaneously, on each side.

An examination of the fowl thirty-six days later showed that the original mass had not grown, except perhaps at the edges of the recent incision. The gap left by the removal of tissue had persisted almost unchanged, and was filled with purulent material. At six of the ten inoculation sites small nodules were felt. The fowl was in poor condition, so it was killed, and bits of the tumor tissue were inoculated into twenty young, pure-bred Plymouth Rock hens, the trocar again being used. Two of these died of intercurrent disease, but in twelve of the eighteen that survived growths developed, appearing in general within thirty days. The propagation of the tumor has since been easy, and it is now in its seventh transplantation generation.

GENERAL MORPHOLOGY OF THE GROWTH.

The general morphology of the transplantation tumors does not differ from that of the original or spontaneous growth. The latter was removed at autopsy together with the lower half of the

sternum, and was cut transversely. It was well encapsulated. In the gross specimen the sternal keel could be traced to the growth's center, but here it was lost in a mass of red, spongy, bony tissue which radiated from it a distance of one to one and one half centimeters (figure 2). Peripheral to this the tumor was hard and white, with fine strands of opaque, fibrous tissue separating more translucent, homogeneous areas. The secondary nodules, globular and well defined, were composed of the opaque, fibrous tissue. The fowl was emaciated. No metastases were found.

Microscopically the capsule of the original growth was found to consist of fibrous connective tissue containing isolated muscle bundles. The growth itself was made up of a zone of what may be termed prechondral tissue, enclosing and grading into a mass of hyaline cartilage through which ran the sternal keel. From this latter numerous irregular, bony trabeculae radiated into the cartilage.

HISTOLOGICAL FINDINGS.

The outer, or prechondral, tumor tissue is an irregular connective tissue composed of sparsely scattered cells in a relatively large amount of fibrillar intercellular substance. The cells closely resemble normal connective tissue cells of the young fibroblast type, having large vesicular nuclei, some with two nucleolar masses and some showing amitotic division or nuclear budding. The cells lie, singly or in groups, in slit-like spaces, and in the groups are larger and more globular. Blood vessels are numerous, and their walls consist usually of endothelium only. The intercellular substance gives the staining reaction for collagen.

Deeper in are occasional islands in which the cells are polyhedral or globular, and lie in irregular spaces suggesting capsules, while the intercellular substance is here relatively homogeneous, though non-staining. These islands strongly suggest cartilage, and in fact all transitions are found between them and actual cartilaginous areas lying further toward the center of the tumor. The cartilage cells are globular, in well formed capsules, and the ground substance stains deep blue with hematoxylin, taking an intense blue at the capsule rims. With aniline blue the ground substance takes a light, diffuse stain and also shows numerous fine, deeply staining, collagen fibrils.

Near the center of the growth the cartilage undergoes a transition to a non-calcified, osteoid tissue, which itself undergoes calcification and partial resorption. It is in this way that the radiating bony trabeculae already mentioned appear to have been formed. The deeply staining cartilage masses, at their inner border, shade into a zone with a non-staining, homogeneous ground substance, the cartilage cells at the same time showing various stages of transition to cells with the general morphology of bone corpuscles (figure 7). Still further

in, calcification is taking place with the formation of trellis-like figures, staining deep blue with hematoxylin, between the cells of the osteoid tissue. Into this calcified zone, which in most places is quite narrow, marrow spaces, with their blood vessels and osteoclasts, are advancing, and resorption is taking place. Thus are formed irregular bony trabeculae. The intertrabecular marrow spaces contain a loose connective tissue, for the most part only sparsely cellular, but in places packed with cells that resemble fibroblasts. Occasionally small collections of granular leucocytes, mostly of the myelocyte type, are seen. Blood vessels are small and not numerous. Deep in the mass the keel of the sternum is recognizable as two roughly parallel bony plates (figure 4). The bony trabeculae which radiate from the outer surface of these into the tumor tissue closely resemble the trabeculae of the medullary cavity. The periosteum can be traced from the base of the sternum up to the lowest of these bony processes on each side, but here it is lost in tumor tissue. Toward its free edge the sternal keel is broken through in many places and here the newly formed bone is continuous with the medullary trabeculae. The edges of the gaps show active erosion by osteoclasts.

The secondary subcutaneous and intramuscular nodules consist practically entirely of prechondral tissue of the sort described. This tissue is much more cellular than in the original tumor, the cells lying close together with but a small amount of intercellular substance. Many of them have two nucleoli, and some show amitotic division in progress, or, more rarely, a mitotic figure may be seen. At its margin the prechondral tissue extends out between the muscle bundles, while external to this there is, amid the muscle, a diffuse increase of connective tissue.

To recapitulate briefly: The outermost, youngest zone of the tumor is made up of cells of fibroblastic type scattered sparsely in a collagenous intercellular substance. In its deeper portions this tissue is undergoing a transformation to cartilage, its cells taking the character of cartilage cells, while the ground substance becomes homogeneous and basic staining. The original growth is well encapsulated, and seems nearly, if not quite, stationary. The secondary tumors have grown rapidly, are more cellular than the primary growth, and are invasive in tendency.

GENERAL RESULTS OF TRANSPLANTATION.

The growth has been propagated in series of chickens by transplanting small bits of the fresh, peripheral, neoplastic cartilage. In general, Plymouth Rock chickens have been used, and by means of a trocar the grafts have been placed deep in the pectoral muscles. In one series of four fowls prechondral tumor tissue was placed in one breast, in the other cartilage from the same tumor. Growths

TEXT-FIG. 1. Chart showing the results of implantations in the right and left pectoral regions of the twelve susceptible fowls of the first transplantation generation. The tumor growth is represented as a black band, the thickness of the band being that of the average diameter of the growth at any one time, and its length that of the number of days that the growth endured. The angular outlines of the bands are referable to the fact that they were plotted from measurements taken at considerable intervals. Rapid growth followed by slowing or retrogression is characteristic of Chicken Tumor VII.

From the figure it would appear that the tumors began to grow immediately on the implantation of the grafts. This was probably not the case, but a more correct chart cannot be made since the first examination did not take place until thirty days after the implantation. 809, 810, etc. = index number of the individual fowl. R and L = right and left pectoral regions.

TEXT-FIG. 2. The fate of the osteochondrosarcoma in the first five tumor generations. One negative series of the sixth generation is also figured.

developed in all four hosts, but only from the cartilage. The results of the transplantation from the original fowl are shown in text-figure 1, which also illustrates well the general behavior of all the propagated growths. The varying success of the tumor in its first five transplantation generations is shown in text-figure 2.

The percentage of successful inoculations from the primary growth was very high,—60 per cent. of the fowls yielding tumors and growths developing at 40 per cent. of all the inoculation sites. The first transplantation of spontaneous mouse tumors does not ordinarily yield nearly so high a proportion of growths. It is interesting to note that in Haaland's⁵ series of seventy-eight spontaneous mouse tumors that were transplanted, the greatest average success was obtained with growths that on transplantation showed a general tendency to retrogress. In the case of Chicken Tumor VII this tendency is very marked.

Passage from host to host has scarcely increased the transplantability of the chicken tumor (text-figure 2). Furthermore, at present (the seventh tumor generation) it grows little if any more rapidly than when first transferred. But the prechondral tissue frequently infiltrates the surrounding muscle; and in one recent case a metastasis was found. For these reasons the tumor is best considered as an osteochondrosarcoma.

The development of Chicken Tumor VII after the implantation of a graft follows certain regular lines. The immediate fate of the implanted tissue has not yet been traced, but where it is placed in susceptible fowls there appears after ten days or two weeks a discrete, smooth, firm nodule which usually enlarges rapidly. It is ellipsoidal with its long axis in the direction of the muscle fibers. In one instance a mass four centimeters in diameter developed within thirty days after the implantation of a tumor bit not larger than four by one millimeters. There is often some invasion of the tissue surrounding such a nodule, but its growth is predominantly expansive in character and often it can be shelled out of its capsule, which at this time is usually well developed. Microscopically the tumor is found to consist of the peculiar prechondral tissue that

⁵ Haaland, M., *Fourth Scientific Report of the Imperial Cancer Research Fund*, 1911, 1.

has been described in connection with the original growth. Cartilage is already forming at its center, as islands which gradually enlarge and merge into a mass that seems homogeneous to the naked eye, and as the mass grows larger degenerative changes occur at its center. With the gradual transformation to cartilage there takes place a slowing of the tumor's growth rate (text-figure 1), and after about fifty days nearly all the tumors become stationary, and many begin to retrogress. In some of the growing tumors, the proliferation of the prechondral rim and its transformation to cartilage slowly continue. If the host lives long enough ossification occurs within the cartilage and nearly the whole tumor may be converted into bone. The bony change takes place quite independently of whether the tumor is growing, stationary, or retrogressing, but in any event bone does not appear in the growth until the third or fourth month.

Instances in which this tumor has led to the death of the host are as yet few. Even when quite large it does not appear to cause ill health. At present there are in the laboratory numerous fowls which for many months have carried the growth in the breast as a stationary, retrogressing, or slowly growing lump of bony hardness. In the instances in which the tumor proved fatal the host has gradually emaciated and at length become comatose for a day or so before death. At autopsy no changes were found in the viscera other than those attendant upon emaciation and secondary anemia.

SPECIMEN PROTOCOLS.

A Progressively Growing Tumor.—Fowl 504, 7th generation A. In each breast of this fowl there was implanted a graft from No. 321, 6th generation A. In the right breast there soon developed a growth like a slightly flattened sphere, lying within the muscle, which increased rapidly in size up to the time of the fowl's death, sixty-seven days after the implantation. At autopsy the mass measured 6.4 by 5.6 by 4.5 cm. It projected sharply from the breast of the emaciated host. It was not attached to the sternum, but the pressure of its enlargement had arched inward the membrane stretching between two sternal ribs lying directly behind it. The growth was very firm and on section discrete, grayish white, of smooth, close texture, and translucent. Its center was honey-combed with blood channels, and showed at one point the old clot of a hemorrhage, at another a few bony spicules. Microscopically the growth consisted of hyaline cartilage with a thin rim of prechondral tissue. The central blood channels were walled only by endothelium. No metastases were found anywhere. It is doubtful if death was due to the tumor.

A Metastasizing Tumor.—Fowl 46, 2d generation C. A bit of tumor from No. 824, 1st generation A, was implanted in the muscle of each pectoral region of this fowl. Three weeks later a nodule about 1 cm. in diameter had developed at one site and after two months a similar growth appeared at the other. The tumors grew slowly and at length became stationary, remaining so for many weeks before the fowl's death which occurred from intercurrent causes seven months after the inoculation. At autopsy the tumors were found to be shaped like slightly flattened spheres. They measured 5 and 1.7 cm. respectively, in their greatest diameter, were discrete and stony hard, and consisted of a narrow, gristly rim of cartilage about a mass of spongy bone and red bone marrow.

On cutting away the sternum a characteristic tumor mass, 0.9 cm. in diameter, was disclosed, projecting sharply into the thoracic cavity and attached by a narrow base to the inner surface of the lowest right rib at the junction of its sternal and vertebral portions. Its situation was such that it could not be attributed to the primary implantation. It contained cartilage.

A Stationary, Bony Tumor.—Fowl 823, 1st generation A, and 6th generation A. This fowl was inoculated with two grafts of the original osteochondrosarcoma, and developed as a result one small tumor which was largely removed at operation, the remainder of it retrogressing. One hundred and five days after its disappearance a reinoculation was done, this time with material from No. 353, 5th generation B. A tumor developed which within a month reached a diameter of 2.5 cm., but then ceased to grow. Four months later, when the fowl was killed, it measured only 2 cm. across. At autopsy it proved to be a roughly spherical, bony nodule, with a smooth surface and a thin connective tissue capsule. It was made up of an outer shell or lamella of bone enclosing spongy bone and red marrow and a few islands of cartilage. The general health of the host had been good throughout.

HISTOLOGY OF THE METAPLASIA.

The formation of cartilage which takes place regularly in the transplanted growths (figures 5, 9, and 10) comes about in the same manner as in the original tumor. The islands of cartilage, at first isolated in prechondral tissue, gradually enlarge and become more numerous until to the naked eye their fusion appears complete. As a matter of fact, the microscope shows that they are still separated by thin strands of the prechondral tissue. In its interior the cartilage sooner or later undergoes degeneration, with loss of staining power and gradual death of the cells.

The change to bone also occurs in the same way as in the primary tumor. The cartilage undergoes a transformation to osteoid tissue, becomes calcified, and is actively eroded by osteoclasts to form trabeculæ between which red bone marrow appears (figure 6). There is thus formed an irregular mass of spongy bone. The

process, as in the primary tumor, seems to be one of progressive resorption, with no production of new bone except as is obtained by the continuous conversion of cartilage into a calcified, osteoid tissue. Osteoblasts appear to play no part in the bone formation.

The bone cells are somewhat irregular in morphology, but the majority closely resemble normal bone corpuscles, being small, flattened, sharp angled, dark staining cells, lying in clear cut spaces. The ground substance is dense and homogeneous. The marrow spaces show large blood spaces, walled with endothelium and distended with red blood cells. Most of the red cells are not peculiar, but many are more globular than the normal and have a bluish grey cytoplasm (after eosin and methylene-blue) and pale, rounded, vesicular nuclei. Occasionally a mitotic figure is seen in one of these cells.

In the intervascular tissue are seen numerous spindle cells of the type seen at the growing border of the tumor. These cells occur also in more globular form, many with few or no cytoplasmic processes, but with the same large vesicular nuclei and pale staining cytoplasm as the prechondral cells. Small numbers of cells occur, however, of the same size and shape as these globular cells, and with the same type of nucleus, but with a strongly basophilic cytoplasm which may contain occasional eosinophilic granules. The nuclei occasionally show mitosis. These cells closely resemble the strongly basophilic cells seen in the development of human blood, and designated as *Wanderzellen*, myeloblasts, etc., by various authors (Maximoff, Askanazy, Schridde, Naegeli, and others). With these cells occur mononuclear cells filled with eosinophile granules, and the ordinary polymorphonuclear eosinophile leucocytes. These four types of cells, the globular, pale staining connective tissue cell, the basophilic cell with few or no granules, the myelocyte, and the polymorphonuclear leucocyte, are often seen lying together in nest-like collections, the last two types always being in excess. The suggestion is very strong that the myelocyte type takes its origin from the basophilic cell, which in turn seems to be related to the globular connective tissue cell. Whether these latter take their origin from tumor cells, representing thus another metaplastic change, has yet to be determined.

The striking characteristic of the retrogressing neoplasms is the replacement of tumor by fibrous connective tissue which from its maturity and uniformity would appear to be derived from the host tissues. The tumor is separated by this into small, sharply defined nodules, which consist of cartilage, or, in the case of the more out-lying ones, of dense collagenous tissue that is still living. At the margin of the mass are many good sized blood vessels, surrounded usually by numerous lymphocytes. In old, stationary growths cartilage and prechondral tissue may practically disappear leaving a rounded, bony nodule consisting of a smooth outer lamella, or bony shell, and a spongy interior of trabeculæ and red marrow.

INTRAVENOUS INOCULATION.

Three fowls of the 5th generation A received at the time of their original intramuscular inoculation an intravenous injection of a suspension of the finely ground tumor in Ringer solution. All fowls developed tumors in the breast muscle as a result of the inoculation at that site. Two of them showed at autopsy no visceral lesions. In the third fowl, No. 352, dying 136 days after the injection, there were found, in addition to the large, bony, pectoral growths, two tumor masses attached to the ovary, and each the size of a hen's egg, in which coils of small bowel were involved. There were also irregular disseminations of tumor tissue, up to 0.5 cm. in thickness, on the parietal and visceral surfaces of the peritoneum. Microscopically both the ovarian and the peritoneal growths showed characteristic prechondral tissue and cartilage. The ovarian tumors also showed extensive hemorrhage and necrosis. Whether these visceral growths resulted from the material injected intravenously, or were secondary to the pectoral tumors, cannot be definitely said. The relatively slight metaplastic changes observed in them favors the latter conjecture since it points to a recent origin for them.

BACTERIOLOGICAL EXAMINATION.

Smears taken from the tumor tissue and stained by various methods have failed to reveal any structures suggesting living organisms, as has also examination with the dark-field microscope.

Cultures were made on all the usual laboratory media, both aerobically and anaerobically. No growth resulted, save on two tubes which showed evident contamination.

IMMUNITY.

Several experiments have been made in the attempt to demonstrate the occurrence of an immunity, natural or acquired, against the tumor. The results obtained up to the present hardly seem to justify definite conclusions, owing partly to the comparatively small number of animals tested, and partly to a lack of uniformity in the material used for reinoculation, as shown by the results in control fowls. The following is a summary of the findings.

In the 3d generation B there were inoculated three Plymouth Rock hens, three black Minorca hens, three brown Leghorn hens, and six mongrel pigeons. No tumor growth took place in any of the pigeons, suggesting, for that species at least, the occurrence of a natural specific immunity, as is the case with animal tumors in general. Tumors developed, however, in both of the alien breeds of the same species as the original host. A difference of breed does not, then, constitute a barrier to the transplantation of Chicken Tumor VII. In this respect the tumor contrasts sharply with Chicken Tumor I (Rous⁶), which in its earlier generations could be transplanted only to fowls of close blood relationship to the original host.

Reinoculations have been made in several series of animals, including fowls which remained negative from the first inoculation, fowls bearing growing or retrogressing tumors, and fowls in which the growth had retrogressed completely. Reinoculation of fowls negative from the original implantation gave tumors only in one series of three fowls. These had been inoculated originally with tumor emulsion, which gave completely negative results. The other fowls of this class, twenty-two in all, still showed no growth after the second inoculation. Although the value of the result was lessened, for a part of this series, by the unsuitable nature of the material used for reinoculation, the findings suggest the occurrence of a strong natural immunity in certain individuals of the species.

⁶ Rous, P., *loc cit.*

In the case of fowls bearing tumors, or in which tumors had retrogressed, reinoculation gave a very small percentage of tumors as compared with the general results in normal fowls. The interpretation of the findings is complicated by several factors, which are important, one being the general poor health of fowls which have been confined for some time in close quarters. The inference is apparently justified, however, that a certain degree of resistance develops in animals in which the tumor has grown for any considerable period. If this is so it offers one explanation for the general tendency to retrogression which Chicken Tumor VII exhibits.

DISCUSSION.

The osteochondrosarcoma described occurred as a unique instance among some thirty primary chicken tumors brought to this laboratory during the last twelve months. It appears to have arisen from the periosteum, all the layers participating in the growth. Injury must be thought of as a contributory cause, because of the tumor's position. Its symmetrical arrangement and histological structure at first suggested that it was congenital in origin, but no evidence confirmatory to this view has been obtained.

In its general characters Chicken Tumor VII is like certain mouse tumors, also osteochondrosarcomata, described by Haaland⁷ and Murray.⁸ Murray's tumor was successfully transplanted and retained its ability to produce cartilage and bone. From his description it appears that these tissues were laid down in a spindle-celled parenchyma, much as happens in the case of Chicken Tumor VII. It is interesting in this connection to remember that dogs are not infrequently the subject of mixed tumors of the mamma which contain an epithelial element, cartilage, and bone, and occasionally give metastases showing one or more of these tissues. The development and differentiation which takes place in Chicken Tumor VII is somewhat similar to that occurring in the normal growth of the chick embryo, but in other ways is notably different. The tumor cells in their early form are relatively simple looking con-

⁷ Haaland, M., *Ztschr. f. Krebsforsch.*, 1907, v, 125.

⁸ Murray, J. A., *Third Scientific Report of the Imperial Cancer Research Fund*, 1908, 69.

nective tissue cells, and they become changed into elements which, save for the fact that they are hypertrophic, are wanting in none of the morphological characters of normal cartilage cells. At the same time an intercellular substance is formed which shows the morphological features and the staining reactions of the ground substance of normal cartilage. But between the connective tissue stage and the cartilage stage is an intermediate one during which the cell produces a large amount of collagenous material. Such a stage is not prominent in the development of the normal cartilage of the bird.

In many instances of Chicken Tumor VII the cartilage at length undergoes degeneration and death, possibly from insufficient blood supply. In others, or in other parts of the mass the cartilage is changed to an osteoid tissue which later undergoes calcification and erosion by osteoclasts, with resulting formation of bony trabeculae. The bone formation takes place, as a rule, only in connection with the marginal healthy cartilage, so that a peripheral bony zone may be found enclosing a soft degenerated center (figure 3).

The process of bone formation differs in its order from that of normal endochondral bone in the chick, as described by Lillie,⁹ for in the production of normal bone the cartilage is first calcified and then eroded, while at the same time osteoid tissue is deposited on the trabeculae by osteoblasts. Whether in Chicken Tumor VII the bone marrow develops from neoplastic tissue or by an ingrowth of tissue elements from the host is unsettled, but the former process is suggested by the findings as an origin for many of the marrow elements, as is also the possibility of even certain of the blood cells having arisen from tumor tissue. Both hypotheses, of course, require more extensive confirmation.

SUMMARY.

An osteochondrosarcoma of the common fowl, designated in this laboratory as Chicken Tumor VII, has been successfully transplanted to seven successive series of hosts. The original growth contained bone and cartilage, was attached to the sternal keel of an otherwise healthy chicken, and appeared to have arisen from this

⁹ Lillie, F. R., *The Development of the Chick*, New York, 1908, 409.

structure. In the growths derived from its transplantation cartilage is regularly laid down, followed later by bone in case the host lives long enough. The prechondral tissue consists of spindle-shaped or multipolar cells of the fibroblast type. The histological character and the behavior of this prechondral tissue show that it is sarcomatous, a fact further proven by one recent case in which the tumor has metastasized. The secondary growth in this instance consisted of prechondral tissue in which a cartilaginous change was taking place.

The tumor could not be transferred to pigeons, the one foreign species tested, but grew readily in chickens of two alien breeds. Reinoculation experiments suggest the occurrence of a natural, individual immunity, and of a certain degree of acquired resistance. In one fowl visceral growths developed following an intravenous injection of tumor emulsion, although whether they were due to this cause or were secondary to the large implantation growths in the muscles is uncertain. Recently the tumor has been transmitted by means of the filtrate from a Berkefeld filter.

EXPLANATION OF PLATES.

PLATE 79.

FIG. 1. Original tumor with a part of the sternal keel, viewed from the right side. The skin has been stripped away except over the crest of the keel. About three fourths natural size.

FIG. 2. Section through the center of the tumor and across the keel of the sternum. The keel lies vertically in the midst of the growth but is lost in a mass of spongy bone. A wedge-shaped piece of tissue has been cut from one side of the tumor. The dark areas deep in the growth are areas of spongy bone. *n* = the notch left by excision of the tissue; *k-k* = the position of the keel; *st* = the sternum at the base of the keel. About natural size.

PLATE 80.

FIG. 3. Cross section of a bony tumor resulting from transplantation. The growth was eighty-five days old and had been stationary for some weeks before its removal. In this instance a broad zone of spongy bony tissue surrounds a degenerated center. The growth is well encapsulated. About natural size.

FIG. 4. Original tumor. Microscopic preparations showing the disappearance of the sternal keel amid the growth's bony trabeculæ. At the upper left hand corner of the photograph is a dark mass of cartilage, which is separated from the bone by a layer of osteoid tissue nearly free from stain. *k-k* = lamellæ of the keel. Stained with hematoxylin.

FIG. 1.

FIG. 2.

(Tytler : A Transplantable New Growth of the Fowl.)

FIG. 3.

FIG. 4.

(Tytler : A Transplantable New Growth of the Fowl.)

FIG. 5.

FIG. 6.

(Tytler : A Transplantable New Growth of the Fowl.

FIG. 7.

FIG. 8.

(Tytler : A Transplantable New Growth of the Fowl.)

FIG. 9.

FIG. 10.

Tytler : A Transplantable New Growth of the Fowl.

PLATE 81.

FIG. 5. Transplantation tumor (fowl 822, 1st generation). Duration of growth, thirty-five days. Microscopic preparation showing prechondral tissue and its transformation into cartilage. Stained with eosin and methylene-blue.

FIG. 6. Transplantation tumor (fowl 808, 1st generation), showing bony trabeculæ, osteoclasts, and marrow with myelocytes. Stained with hematoxylin.

PLATE 82.

FIG. 7. Original tumor. At the right of the picture there is cartilage, next to this osteoid tissue (nearly unstained), and to the left calcification of the osteoid tissue with marrow spaces advancing into the latter. Stained with hematoxylin.

FIG. 8. Hypertrophic cells of hyaline cartilage from a transplantation tumor (fowl 806, 1st generation). One cell has three nuclei. Stained with Mallory's chloride of iron hematoxylin.

PLATE 83.

FIG. 9. From a transplantation tumor (fowl 815, 1st generation), showing transformation of collagenous connective tissue to cartilage. Note the large blood sinuses. Stained with hematoxylin.

FIG. 10. Another portion of the same growth, more highly magnified, showing collagenous neoplastic tissue (prechondral tissue) with cells resembling fibroblasts. Part of a blood sinus walled by endothelial cells is also shown. Stained with Mallory's chloride of iron hematoxylin.