VARIATIONS IN A CHICKEN SARCOMA CAUSED BY A FILTERABLE AGENT.*

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PLATES 41 TO 49.

Marked alterations in structure and behavior are not uncommon in tumors which run their course in a single individual; but, as might be expected, they are more frequent and better marked in growths of which the existence is prolonged by transplantation. The present paper has to do with the changes that have taken place in a spindle-celled sarcoma of the fowl¹ during its propagation for a period of about three years, and in numerous individuals. The findings have especial claim to attention because the sarcoma, otherwise a typical tumor, can be transferred to new hosts, not only by transplantation, but by means of an agent which is separable from the neoplastic cells, and is probably a living organism. Possibilities of variation are thus introduced which do not appear in the case of tumors that depend for their transmission solely upon the survival of a parent strain of cells.

In several previous communications, the characters of the chicken sarcoma have been taken up in detail.² Under ordinary circumstances its growth takes place by a proliferation of the cells already neoplastic, and apparently in that way only; while the metastases are referable entirely to autotransplantation. The transmission of the disease to other susceptible hosts is most readily accomplished by implanting in them a bit of the sarcomatous tissue, which, as experiment has shown, will survive in the new site, and by its proliferation give rise to the new tumor. The growth is at present developing in

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¹ Rous, P., *Jour. Exper. Med.*, 1910, xii, 696; 1911, xiii, 397.

² Rous, P., *loc. cit.*; Rous, P., and Murphy, J. B., *Jour. Am. Med. Assn.*, 1912, lviii, 1938; *Jour. Exper. Med.*, 1912, xv, 270; Rous, P., Murphy, J. B., and Tytler, W. H., *Jour. Am. Med. Assn.*, 1912, lviii, 1751, 1840.

the thirty-second successive series of fowls to which it has been thus transferred. Despite our knowledge that the individual neoplasms arise practically *in toto* through a proliferation of the cells introduced from the last host, it is uncertain whether the present tumors are strictly the offspring by cell division of the original growth. For a transforming action of the causative agent upon normal tissue which would be negligible in the individual case might well bring about in the course of time and many transplantations a total change in the growth's make-up. At all events the tumors of the thirty-two series of transplantations represent the most direct possible continuance of the original sarcoma strain. In addition, many growths have been studied which were produced by the causative agent acting alone, either as a filtrate or as dried or glycerinated tumor tissue, and many more which may have resulted either from an action of the agent or the survival of tumor cells, since they developed after the implantation of sarcomatous tissue damaged *in vitro* but not killed. In all, the primary and secondary growths of 217 fowls have been carefully gone over.

LOCAL VARIATIONS.

The structural variations occurring under ordinary conditions in different parts of the same sarcoma will first be taken up. Certain of these, due to degeneration or to attempts at differentiation, are found in nearly every specimen.

A typical growth consists of spindle cells, more or less attenuated, coursing this way and that in irregular strands. When rapid proliferation is taking place the cells near the border of the growth may be short and blunt, or even irregularly rounded (figure 1). As the mass increases in size and the cells once at its edge come to lie deep within it, they cease to divide so actively, gradually assume an attenuated spindle form, and produce intercellular fibrils. A similar progressive differentiation is often noted in the tumors of human beings.

For the most part the active cells in the best nourished portions of the sarcoma are the ones which enter the blood vessels and are distributed to distant parts (figure 2). The secondary foci partake of the character of the emboli from which they arise, and they may

consist, when small, entirely of active cells of blunt or rounded form (figure 3), appearing for this reason markedly different from the older tissue of the primary mass. But as time passes and the metastases enlarge, their cells gradually become spindle-shaped like those in the parent growth. In many instances the metastases consist from the beginning of spindle cells.

Degenerative changes are of several sorts. In old tumors the spindle cells may become extremely attenuated (figure 4), if necrosis does not overtake them. More frequently cell death occurs early, often preceded by a hydropic change. In the latter case the cells may become greatly swollen and multinucleated. A myxomatous change is not infrequent, and is seen especially at the center of fairly nourished, slowly growing tumors in resistant hosts. It cannot be looked upon as intermediate to the ordinary necrosis.

Very striking accommodative changes are sometimes seen. Their dependence on special conditions of pressure and nutrition in the organ containing the tumor is usually very evident. In cross section growths in voluntary muscle have often an alveolar structure,³ the result of replacement of the muscle fibers with retention of their outline. Growths in the lungs may present a very complex picture. The pulmonary air spaces may contain masses of actively dividing spheroidal tumor cells, easily distinguishable from desquamated epithelium; whereas nearby in the interstitial tissue the neoplastic cells are of spindle form in strands (figure 5). The structure of the ovary leads to a somewhat similar diversity there. In the liver, kidney, spleen, and heart the sarcoma is evenly constituted of spindle cells. In the gizzard, an organ of very close texture, the growth is often non-invasive, and its spindle cells are compactly arranged and often are attenuated. All these various pictures may be seen in the metastases of a single tumor. It is not improbable that pressure changes have something to do with the gradual development of the attenuated spindle form in the older portions of primary growths.

Certain striking histological differences are sometimes observed in secondary tumors seated close to one another in the spleen or liver. Of two neighboring foci in the liver one may consist of sharply defined, rounded, or even cuboidal cells, arranged in columns within

³Rous, P., *Jour. Exper. Med.*, 1911, xiii, 397.

the portal spaces, and superficially very suggestive of newly formed bile ducts; while the other has the usual spindle-celled form. In the spleen there are sometimes found, in addition to nodules of the ordinary sarcoma, sharply circumscribed growths consisting entirely of short, closely crowded, fusiform cells (figure 6) in which mitoses are unusually numerous.

At first sight it would seem unlikely that these diverse secondary nodules are true metastases of a single tumor. Instead it might be assumed that they have arisen in part by localizations of the sarcoma-producing agent in connective tissue cells of unusual potentiality, lying within the affected viscus. But against this assumption are our experiments which show that the agent rarely if ever acts to cause secondary nodules,⁴ and the direct proof that these latter are in general the result of a dissemination of cells from the primary growth. Furthermore all morphological gradations can be traced between the ordinary sarcoma and the peculiar tumors, and as the latter enlarge, their cells take the spindle form. When one considers how markedly local differences in nutrition and pressure influence the appearance of the sarcoma, and the fact that the histology of each beginning metastasis depends to some extent on the character of the embolus from which it has arisen (embolus of rounded cells, embolus of spindle cells), the assumption of a secondary localization of the agent seems unnecessary. On the other hand, the occasional occurrence of such a localization can not be absolutely ruled out.

CHANGES WITH INCREASED MALIGNANCY.

Some of the most marked changes in the sarcoma have accompanied its increase in malignancy. The original tumor was a discrete, fairly encapsulated mass, firm and gristly, with a striated cut surface. At its center was an irregular core of coagulation necrosis. The tumors of the first few generations were quite similar, but often contained one or more cysts full of pale yellow, mucinous fluid. With the great increase in transplantability and rapidity of growth which took place between the second and sixth tumor gener-

⁴Rous, P., and Murphy, J. B., *Jour. Exper. Med.*, *loc. cit.*; Rous, P., Murphy, J. B., and Tytler, W. H., *loc. cit.*

ations, the character of the neoplastic tissue altered markedly. It became soft, friable, and translucent, with little striation and no encapsulation. Rapid breaking down *en masse* occurred often, and in these cases amyloid was sometimes found in the spleen and liver.⁵ Hemorrhage into the tumor substance with the formation of large, ragged cavities full of brown or green or bloody fluid was very frequent. The cells had often a very irregular spindle form, or were short and blunt or even rounded. Their increased activity is perhaps best illustrated by the change in their behavior toward striped muscle. In the earlier generations they had supplanted this by penetrating in strands between its fibers, bringing about their gradual atrophy and disappearance; but now they often apposed themselves directly to the fiber substance, which disappeared before them as though eroded; or else they penetrated the sarcolemma at one or more points and proliferated and extended rapidly within it⁶ in a manner met with in only the most malignant of mammalian growths.

The gross form of the neoplasm was much altered with the increase in malignancy, as would naturally follow from its greater invasive power. The earlier series of growths resulting from implantation in the pectoral muscles were discrete, and frequently projected sharply from the body contour. But the later ones at this site have been much less definite in form, though still considerably firmer than the normal tissue and easily distinguished on palpation. They may first become evident to the naked eye as diffuse swellings which little by little gain in prominence through expansive growth and the emaciation of the host. Only in somewhat resistant fowls is the very discrete character of the original attained. In these the sarcoma may be encapsulated and very firm, owing to the large amount of non-neoplastic, fibrous tissue about and within it. No better example of tumor malignancy as affecting tumor form could be desired. The course of a growth in the individual fowl may be predicted with considerable accuracy from the findings on a single palpation.

⁵For amyloid changes in the organs of mice with tumors, see Lubarsch, O., *Centralbl. f. allg. Path. u. path. Anat.*, 1910, xxi, 97.

⁶Rous, P., *Jour. Exper. Med.*, 1911, xiii, 397.

INTERCURRENT VARIATIONS.

By selective transplantation the sarcoma has been kept for many months in a highly malignant state. From time to time without evident cause changes in its morphology have manifested themselves. In some hosts the tumor consists of spindle cells much smaller than in other fowls of the same age and variety; and in some the growth closely approaches in appearance a round-celled sarcoma (figure 7) though many of its cells have protoplasmic tails or queues, and there is always some differentiation to the spindle form. Most striking are the giant-celled growths.

Giant cells were numerous in some portions of the original tumor, as appears from sections of it recently made (figure 8). Nevertheless, in the nodules resulting from transplantations into the original host they were so few that the impression given was that of a practically pure spindle-celled sarcoma. In the subsequent tumors in other hosts, they were for a long time met with only infrequently and these later growths may well be called pure, spindle-celled sarcomata. But in the nineteenth transplantation generation, giant cells again appeared as an important constituent of the tumor and growths characterized by large numbers of them have since been frequent.

In appearance the giant cells (figure 9) are entirely different from those occurring about foreign bodies in the fowl or associated with avian tuberculosis. They are not arranged in foci but lie scattered among the neoplastic spindle cells, from which their development by a process of enlargement, accompanied sooner or later by degeneration, can be directly traced. They may reach a diameter of 100 microns or more, and are usually oval, with one or two blunt processes, or forks, and a single large nucleus, features which give them a superficial resemblance to ganglion cells. At first the cytoplasm is finely granular and faintly basic, as is that of the spindle cells round about; but later, as the cell enlarges, the cytoplasm stains with eosin and may become vacuolated. The single nucleus, eccentrically placed, is sometimes pyknotic, but more often vesicular and swollen, with its chromatin in a central mass (fish eye nucleus). In the smaller cells mitosis may take place, and in the larger ones

nuclear budding is frequent. Specimens are not rare with from two to forty scattered nuclei of remarkably various size and appearance. Phagocytic activity of the giant cells is frequent. Erythrocytes, leucocytes, or even small tumor cells may be ingested by them. They may be abundant in the best nourished portions of the sarcoma, indeed at its advancing edge (figure 10). They are present, as a rule, in the metastases from primary growths containing them.

The significance of the giant elements is no clearer than in the case of the sarcomatous giant cells of mammals and the conditions which lead to their appearance have not yet been recognized. In stained preparations or under the dark-field microscope they show no distinctive inclusions. During a long period they were observed only in the sarcomata which resulted from transplantation, as distinguished from those engendered by the filterable agent; but of late some change must have taken place in the agent, since now growths caused by it not infrequently contain giant cells. That peculiarities of the host have a considerable determining influence is shown by the fact that a single lot of causative virus, in the form of a filtrate or of dried or glycerinated tumor tissue, will give rise in some fowls to a pure spindle-celled growth, in others to the giant-celled form. In general the giant-celled form is relatively slowly growing, yet in hosts with an evident, though partial, resistance, tumors of either sort may be found. All things considered it would seem probable that the giant cells represent a perversion which may take place in any of the neoplastic spindle cells when under the influence of special conditions. Some of these conditions are supplied by the host, some by the growth's causative element, and others, doubtless, by local circumstances. The cells belong to the second group of giant cells in sarcomata distinguished by Mallory;⁷ namely, to the group of giant cells arising out of tumor cells, in contradistinction to those developing in the non-neoplastic stroma.

HEMORRHAGIC FORM OF THE SARCOMA.

Hemorrhage in association with the sarcoma first occurred in the eighth tumor generation, some fifteen months from the time of the original transplantations. The growth had attained its max-

⁷ Mallory, F. B., *Jour. Am. Med. Assn.*, 1910, 1v, 1621.

imum malignancy in the sixth generation. The first bleedings that were noted took place into areas of necrosis at the center of tumor nodules, and were of slight extent and sharply localized. In the ninth and tenth generations several fowls died suddenly of hemorrhage from visceral nodules and this cause of death has since been frequent. The findings at autopsy are remarkable.

The host is nearly always one in which the tumor has grown rapidly and disseminated widely, though, because of the premature termination of the disease, the secondary growths may have had little chance to develop, and emaciation may be but slight. The lung and heart tumors are seldom hemorrhagic. Bleeding in the occasional metastases in the spleen is kept within bounds by the stout capsule of that organ. Extensive hemorrhage in the primary tumor may result in much breaking down there; but the fatal bleeding usually takes place from a liver metastasis, rarely from a focus in ovary or kidney. At autopsy the peritoneal cavity is found to be more or less distended with blood clot, arranged often in layers of different ages and degrees of firmness; and when this clot is lifted it is discovered to have a direct connection with one or several ruptured blebs or hemorrhagic tumor masses on the surface of one of the organs mentioned (figure 10).

The liver substance may be riddled with discrete, spherical cavities up to one centimeter in diameter, filled with fresh or old blood clots, which, reaching the surface, take the form of blebs. That the tumor cells have in some way caused the hemorrhage is usually evident. In one liver all gradations may be found from microscopic foci of the sarcoma in which hemorrhage has just begun, to large blood cysts which show tumor cells in only a relatively small area of their wall, or to large tumor masses which show relatively small hemorrhages in their interior. Not a few of the blood cysts have a zone of pressure necrosis about them without demonstrable tumor; but the absence of neoplastic tissue under these circumstances is scarcely surprising.

When hemorrhage takes place into the sarcomatous ovary it often converts a number of the follicles into large blood cysts, through the rupture of which death may come about. The hemorrhages in the kidney are usually small and well circumscribed, but they may

dissect into the surrounding tissue and be fatal. The intravenous injection into a normal fowl of the tumor-producing agent in the form of a Berkefeld filtrate has led in one instance to the development of a small sarcoma in the kidney, with a dissecting hemorrhage from which the fowl died.

Although the relationship between tumor and hemorrhage can scarcely be questioned in these cases, there are instances in which it is not so evident. Multiple hemorrhages in the liver of the chicken may undoubtedly be due to other causes than the sarcoma. Occasionally we have found them in the fatty liver of fowls which had not been inoculated with the sarcoma but had died of some other disease. In inoculated hosts dying of a large pectoral sarcoma but having visible metastases only in the lungs they are fairly frequent. In these instances, microscopic search usually brings out the presence of minute patches of tumor in association with some at least of the hepatic hemorrhages (figure 11), and there seems little doubt but that all should be attributed to sarcomatous foci, destroyed for the most part by the bleeding they have induced.

The structure of the growths provoking hemorrhage is often somewhat different from that of the ordinary sarcoma. The cells are short and blunt or nearly spherical, and lie loosely grouped; intercellular fibrils are few, and there is little supporting stroma. These circumstances appear to favor a wide extravasation once the rupture of a vessel has occurred. Vessels are not especially abundant, as they are in the hemorrhagic adenocarcinomata of mice, but the tumor cells are evidently invasive and make their way into many of the blood channels.

DISTRIBUTION OF THE METASTASES.

One other alteration in behavior observed during the routine propagation of the growth deserves brief mention, namely, the change in the distribution of the metastases. Elsewhere this subject has already been dealt with to some extent.⁸ In the earlier generations of the tumor, the heart was most frequently affected after the lungs. In the later ones the heart has been relatively free from secondary growths and the liver has very often shown them. During the fall

⁸Rous, P., *Jour. Am. Med. Assn.*, 1910, lv, 1805.

and winter months metastases in the ovary have been rare, but now for two successive years they have been a feature of autopsies performed during the spring and summer (figure 12), or, in other words, during that period when the ovary is functioning actively, when trauma in the organ is of daily occurrence, and when its blood supply is greatly increased. The same factors have not infrequently been observed to determine secondary localizations of tumors of the mouse or man, and they may well be effective in the present case. There is some evidence to show a seasonal localization of the chicken sarcoma in the testicle as well as in the ovary, but our observations on this point have been few, since female birds have in general been used as hosts.

SARCOMATA CAUSED BY THE FILTERABLE AGENT.

Taken together, the sarcomata produced by direct injection of the filterable agent do not differ from those resulting from the growth of a bit of the transplanted sarcomatous tissue; and they manifest the same variations. Peculiarities of the individual host are important in determining these. A single lot of agent, in the form of dried, or glycerinated, tissue or a Berkefeld filtrate, will produce in some hosts a pure spindle-celled sarcoma, in others a giant-celled growth, and in yet others a growth composed of fusiform or rounded cells, and perhaps hemorrhagic. All are malignant tumors of the connective tissue, grading into one another histologically.

Attempts to obtain growths of unusual morphology by bringing the agent into contact with special types of cells have as yet been unsuccessful, and so too with attempts to obtain modifications in tumor form by attenuation of the agent *in vitro*. The growths caused by the treated agent take a long time to appear, and frequently retrogress, but otherwise they are not peculiar. The same is true of the sarcomata arising after the implantation of sarcomatous tissue which has been damaged by heat or by saponin. That the tumor cells fail to undergo a structural modification under these influences is scarcely surprising for heating does not alter the structure of transplantable mouse tumors.⁹

⁹ Loeb, L., and White, E. P. C., *Centralbl. f. Bakt., 1te Abt., Orig.*, 1910. lvi, 325.

DISCUSSION.

The variations manifested by the chicken sarcoma are not more striking than certain ones which have been noted in rat and mouse tumors (Bashford, Lewin) and which are due without doubt to intercurrent changes in a single strain of tumor cells. Lewin, for example, describes an adenocarcinoma which within twelve generations assumed the form of a carcinoma solidum, a carcinoma alveolare, and a cancrioid. The various forms of the chicken tumor are all sarcomata which grade into one another histologically. It would be unnecessary to suppose for them any other cause than intercurrent changes in a single strain of tumor cells were it not that an etiological agent for the tumor has been found,—an agent which accompanies the growth at all its stages, and is capable of causing, under special conditions, a neoplastic change in tissue hitherto normal. The presence of this agent leads one to ask whether the variations in the chicken sarcoma are not due in part at least to changes in it, or perhaps to its action on cells of unusual type or potentiality.

The latter possibility can be almost ruled out. Our many experiments to bring about an action of the agent on a tissue other than those it usually affects have uniformly failed. For example, when the agent, as a Berkefeld filtrate, is injected intravenously into fowls with induced lesions of various tissues the resulting tumor always arises from the spindle cells of connective tissue. On the other hand, there is no doubt but that changes in the causative agent lead to some modifications in the sarcomata of which it is the cause. When the agent is attenuated by heat, it gives rise to tumors which grow slowly and retrogress frequently. And whereas formerly the tumors caused by the agent were all spindle-celled sarcomata, now it not infrequently engenders giant-celled growths. Many of the variations in the transplantation sarcomata, expressing themselves as morphological changes or otherwise, may well be due to modifications in the agent under the play of conditions in successive hosts. The question as to whether the transplantation sarcomata observed during the last three years have consisted entirely of descendants of the original strain of tumor cells or are in part the result of a

neoplastic transformation in successive hosts does not affect this matter.

SUMMARY.

Variations are described which have from time to time occurred in the structure and behavior of a transplantable, spindle-celled sarcoma of the fowl, a growth caused, as elsewhere shown, by a filterable agent. Of late the growth has frequently given rise to fatal hemorrhages from its substance. In some of the recent, rapidly growing tumors the cells have tended to be spherical, showing only a very tardy and imperfect differentiation to the spindle form. A giant-celled form of the growth is sometimes met with. Despite their diversity the tumors grade into one another and in the final analysis are all to be considered as spindle-celled sarcomata. Attempts to obtain an action of the etiological agent upon cells other than those it usually affects have failed, as have attempts to bring about changes in the histology of the sarcomata by attenuating the agent.

Some of the lesser morphological variations in the sarcoma are undoubtedly due to local conditions in the host, and of the more important changes some have been associated with an increase in the growth's malignancy. For others the determining conditions have yet to be discovered. On the whole the variations described are not more marked than those occasionally manifested by the transplantable mammalian tumors, and traceable to the changes in a single strain of tumor cells during their propagation in successive hosts. In mammals the ultimate reason for these changes is not known. In the case of the chicken tumor some of them are undoubtedly the expression of changes in the growth's causative agent.

EXPLANATION OF PLATES.¹⁰

PLATE 41.

FIG. 1. Marginal portion of a sarcoma which is replacing voluntary muscle. The cells at the edge of the growth (to the left of the picture) are for the most part oval or rounded; those further in are definitely spindle-shaped. The replacement of the muscle fibres has resulted temporarily in an alveolar structure. *m* = muscle fibres in process of disappearance; *r* = rounded tumor cells.

¹⁰ All the microscopic specimens were stained with methylene-blue and eosin

FIG. 1.

FIG. 2.

(Rous and Murphy: Variations in Chicken Sarcoma.)

FIG. 3.

(Rous and Murphy: Variations in Chicken Sarcoma.)

FIG. 4.

(Rous and Murphy: Variations in Chicken Sarcoma.)

FIG. 5.

(Rous and Murphy: Variations in Chicken Sarcoma.)

FIG. 6.

FIG. 7.

(Rous and Murphy: Variations in Chicken Sarcoma.

FIG. 8.

FIG. 9.

(Rous and Murphy: Variations in Chicken Sarcoma.)

FIG. 10.

(Rous and Murphy: Variations in Chicken Sarcoma.)

FIG. 11.

(Rous and Murphy: Variations in Chicken Sarcoma.)

FIG. 12.

(Rous and Murphy: Variations in Chicken Sarcoma.)

FIG. 2. Growth of the sarcoma into the lumen of a blood vessel, showing the rounded type of tumor cell frequently cast off into the blood stream. The vessel is full of nucleated erythrocytes. *e* = endothelial lining of the vessel; *t* = tumor cells loosened from the main mass; *n* = nucleated red cell lying between the tumor cells.

PLATE 42.

FIG. 3. Colored photograph of a small metastasis in the lung. It consists of rounded cells similar to those in figure 2. Their staining reaction sharply distinguishes them from the pulmonary tissue.

PLATE 43.

FIG. 4. Attenuated spindle cells of the older portions of the chicken sarcoma.

PLATE 44.

FIG. 5. Portion of a diffuse metastasis in the lung. *b.v.* = vessels filled with nucleated erythrocytes; *a.s.* = rounded cells in the air spaces; *s.p.* = spindle-shaped tumor cells in the pulmonary tissue.

PLATE 45.

FIG. 6. Peculiar type of tumor tissue occurring in some metastases of the spleen.

FIG. 7. Portion of a sarcoma the cells of which are nearly spherical. In deeper portions of the same tumor the cells had well marked queues or tails, or were of blunt spindle shape.

PLATE 46.

FIG. 8. A section of the original sarcoma, showing tumor giant cells and infiltration of the skin.

FIG. 9. Invasion and replacement of liver tissue by sarcoma of the giant-celled type. *l* = columns of liver cells.

PLATE 47.

FIG. 10. Hemorrhage from secondary nodules in the liver. The clot partially overlies the nodule from which the bleeding occurred. Some of the others are hemorrhagic.

PLATE 48.

FIG. 11. Drawing of a minute metastasis in the liver, with a hemorrhage in its midst. *t* = tumor tissue; *h* = hemorrhage (nucleated erythrocytes).

PLATE 49.

FIG. 12. Metastases in the ovary. The sarcomatous masses hang by pedicles like true ova.