RESISTANCE TO A TUMOR-PRODUCING AGENT AS DISTINCT FROM RESISTANCE TO THE IMPLANTED TUMOR CELLS.

Observations with a Sarcoma of the Fowl.*

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Plate 32.

No one of the problems opened to experiment by the transplantation of animal tumors has received more attention than that of the nature of resistance to these growths. The exact manner of the resistance, or resistances,—for there may well be several kinds,—is still undetermined; nevertheless one important fact has emerged, namely, that the fate of implanted tumor, as indicating this resistance, is influenced for the most part by the same conditions that influence the fate of implanted normal tissue. The conditions at the site of the graft's implantation, the age of the host, its health, species, even variety, and its relationship by blood to the previous host,—these and other factors influence in like manner engrafted tissue of both sorts.[1] Furthermore, resistance to transplanted tumor can be induced by a preceding injection of living tissue,[2] and whether this tissue be normal or neoplastic makes little difference.[3] By the same means too animals can be made resistant against implanted normal tissue which ordinarily would grow in them, for example embryonic tissue, so that it now fails to develop (Fichera, Rous). The numerous data thus far obtained go to show that the

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[1] Loeb, L., *Proc. Am. Phil. Soc.*, 1908, xlvii, 3. Fichera, G., *Arch. ed atti d. Soc. ital. di Chir.*, 1909, xxii, 1. Rous, P., *Jour. Exper. Med.*, 1910, xii, 344.

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

[3] Bashford, E. F., Cramer, W., and Murray, J. A., *Brit. Med. Jour.*, 1906, i, 207. Schöne, G., *München. med. Wchnschr.*, 1906, liii, 2517.

resistance which in some individuals prevents the growth of implanted tumor is a resistance directed against the graft as strange tissue, and is unconnected with the neoplastic qualities which this tissue happens to possess.

These are the findings with transplanted neoplasms. Of the specific factors concerned in resistance to the growth of spontaneous tumors, as distinct from non-specific factors, such as infection, mechanical disturbance, etc., practically nothing is known. Manifestly these growths cannot be looked upon as consisting of a tissue strange to the body in the sense that transplanted tumor tissue is strange. The not infrequent occurrence of spontaneous tumors in animals highly resistant to implanted growths (Bashford) proves this.

The present paper is a report of an attempt to learn, through the study of an avian tumor, something of the nature of resistance to spontaneous growths. The tumor employed is a spindle-celled sarcoma of the chicken, which has already been reported upon several times from this laboratory.[4] The growth, when transmitted by transplantation, behaves exactly like the transplantable tumors of mammals, being influenced by the factors which influence them in common with transplanted normal tissue. But from it there can be separated by drying, or filtration, or glycerinization, an agent, presumably living, which, under special conditions, will cause a sarcomatous change in the tissue of a previously normal fowl. The growth thus generated is derived from the host's own tissues and in this regard resembles the spontaneous tumors of mammals. A study of the forces influencing such a growth's development, and the circumstances which sometimes bring about its retrogression may well have a bearing on similar problems in mammalian oncology.

Natural retrogression of the spontaneous tumors of mammals is relatively rare; but retrogression can be induced, more or less successfully, by the Roentgen rays, radium, and ultraviolet light. The point of attack of these forms of radiant energy is still unknown. In the case of the chicken sarcoma when influenced by radiant

[4] Rous, P., *Jour. Exper. Med.*, 1910, xii, 696; for references to other articles on the growth, see Rous, P., and Murphy, J. B., *Berl. klin. Wchnschr.*, 1913, l, 637.

energy there arises at once the question whether the sarcomatous cells as such are primarily affected, or whether the agent responsible for the growth is injured, with a resultant lessened malignancy of the cells so that they can be destroyed by the body. My first experiments have been directed to this matter. Certain differences in the time of origin of growths caused by the dried tumor tissue, that is to say by an action of the tumor-producing agent, and of those resulting from the fresh tissue containing transplantable tumor cells have suggested the method of work.

METHOD.

With parallel safety-razor blades the fresh sarcomatous tissue is cut into a number of slices of equal thickness, ordinarily 0.08 to 0.1 cm. These are placed in Ringer's solution, carefully trimmed free of all except sound tumor, and are then separated into a number of batches. If the tissue is to be submitted to the Roentgen ray the slices of each batch, spread flat, are together sealed in a large hollow-ground slide under a large, thin cover-glass. If the ultraviolet ray is to be used the slices are repeatedly washed with Ringer's solution to rid them as far as possible of free albuminous matter, and, covered with a thin layer of the fluid, are exposed to the light in an open dish; or without the fluid they are sealed between two flat pieces of quartz glass previous to exposure. The control is treated in the same way except that it is not irradiated. Radium has not been employed thus far.

When the exposures have been completed the tissue is cut fine with sterile knives. In some of the experiments a little sterile infusorial earth was added to it. By means of trocars small portions (about 0.01 c.c. each) are now inoculated intradermally in the feather-free pectoral strip of a number of chickens. The remainder of the tissue is spread very thin in a dish, and placed *in vacuo* over sulphuric acid for twenty-four hours, which is sufficient time to render it completely dry. It is then restored to approximately its former bulk by the addition of an excess of Ringer's solution, or a very little distilled water, and inoculations are made with it as on the preceding day, using the same chickens and the feather-free strip of the other breast. Usually five batches of irradiated tissue and a control batch have been used, fresh and dry, making in all twelve inoculations to each fowl. The tissue bits are implanted several centimeters apart, in a line, and their order is varied from fowl to fowl. The tumors resulting are extremely discrete and are plainly visible at all stages of their development.

An Heraeus mercury-quartz lamp of 220 volts supplied the ultraviolet rays. The specimens were exposed at 25 cm. distance. The temperature of the preparations was at no time above 28° C.

For the Roentgen rays one or another of three soft tubes with a spark gap of 1.5 to 4.5 cm. has been employed. The specimens were placed within 5 to 10 cm. of the tube.

Five sets of observations with the ultraviolet rays and four with the Roentgen rays have been made by this method. For each experiment three to seven fowls were used, making in all forty-one inoculated, of which forty developed tumors. Retrogression soon occurred in some.

The growths from the inoculation of fresh material in intradermal sites arise in general almost immediately, resulting, as previous work has shown,[5] from an extremely rapid proliferation of the implanted cells. At the end of a week the little tumors may be one centimeter in diameter. They appear as raised, translucent bosses or buttons over which the epidermis is tense and smooth (figures 1 and 2). At the end of two or three weeks they very commonly ulcerate, or coalesce, so that observations beyond this period are not of great value. The growths from the dried material are exactly similar but appear much later, not until at least seven days have elapsed, and often twice or thrice this time. That desiccation completely kills the tumor cells seems certain from the findings of previous workers with the normal and neoplastic cells of the higher animals. Moreover, experiments by Dr. Murphy show that the embryonic tissue of the fowl does not survive drying; and drying renders completely innocuous another transplantable chicken tumor (Chicken Tumor XVIII), propagated in this laboratory.

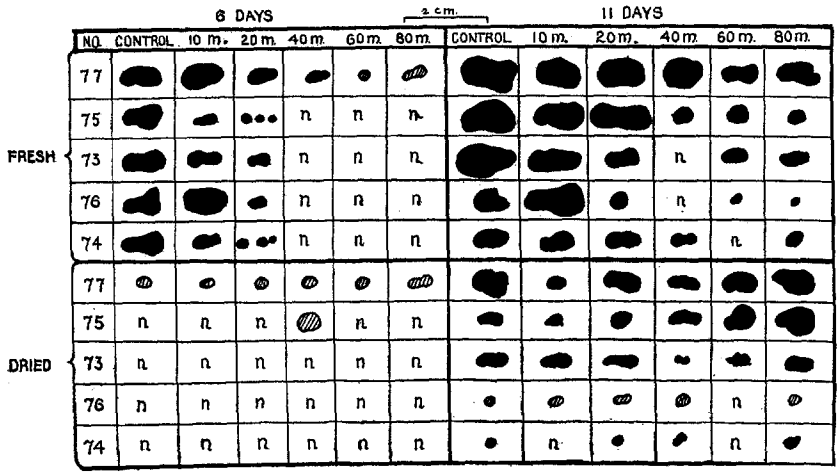
EFFECTS OF THE IRRADIATION.

The experiments have given almost diagrammatic results (text-figures 1 to 6). The Roentgen rays do not appreciably affect either the sarcoma cells or the growth's causative agent when the tissue has been exposed for eighty minutes, the longest time employed. This failure to be influenced is not surprising when it is considered how refractory many mammalian sarcomata are to the rays. The protocols of the individual experiments will not be given.

Ultraviolet light rapidly destroys the activity of the sarcoma cells and this without notably injuring the agent associated with them. In text-figure 1 its selective action is shown. At the end of six days good sized growths are found as the result of inoculation of the fresh control material, while the irradiated fresh tissue mani-

[5] Rous, P., and Murphy, J. B., *Jour. Exper. Med.*, 1912, xv, 270.

fests less tumor-producing activity, until with sixty and eighty minutes' exposure this is completely suppressed. The dried material has as yet given rise to no growths. At the end of eleven days the effects of irradiation and of drying are less marked. In four of the five fowls the dried material has given rise to growths; and in the size of these growths there is no evidence of influence of the irradiation. Differences among the tumors from the fresh material still

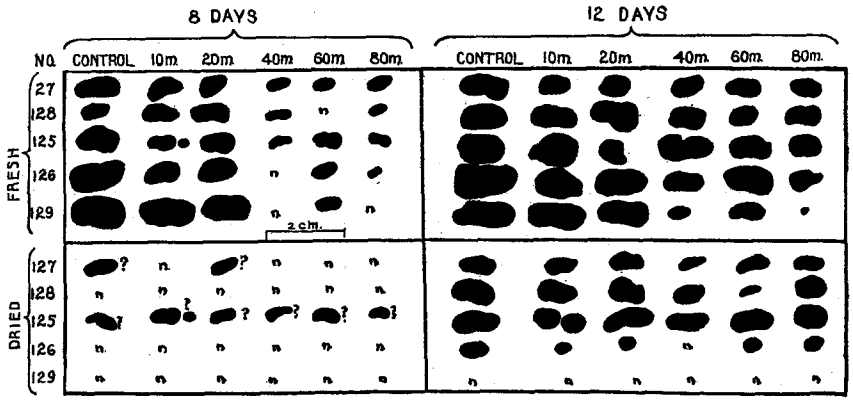


TEXT-FIG. 1. To show the effect of ultraviolet light on the activity of fresh and dried tumor tissue. In the first vertical column are the numbers designating the fowls. Then follow silhouettes to scale of the tumors. The measurements were taken six and eleven days after the inoculations. 10 m., 20 m., 40 m., etc. = 10, 20, and 40 minutes' exposure; n = no tumor. Cross-hatching = induration.

It will be seen that the irradiation has a marked injurious effect on the element in the fresh tissue (the living tumor cells) which gives rise to tumors immediately after implantation. The tumor-producing agent which resists drying is unaffected by the irradiation.

indicate this influence, but less strikingly than before, since now at length the material irradiated for sixty and eighty minutes has given rise to growths. These growths have arisen after about the same time and are of about the same size as those derived from the dried material. In text-figure 2 similar findings are presented but the differences are not so clear cut, since the longest period of irradiation has failed to rid the fresh tissue of the activity specifically associated with its fresh state.

The interpretation of these results is plain. The text-figures show, first, that in the sarcomatous tissue there are two elements capable of producing the growth, one of which will withstand drying while the other will not. The labile element, which we know to be the living and transplantable tumor cells, is so sensitive to the ultraviolet rays that sixty to eighty minutes' exposure will completely destroy its activity; whereas the stable element, the tumor-producing agent, is at most only slightly affected by this irradiation.



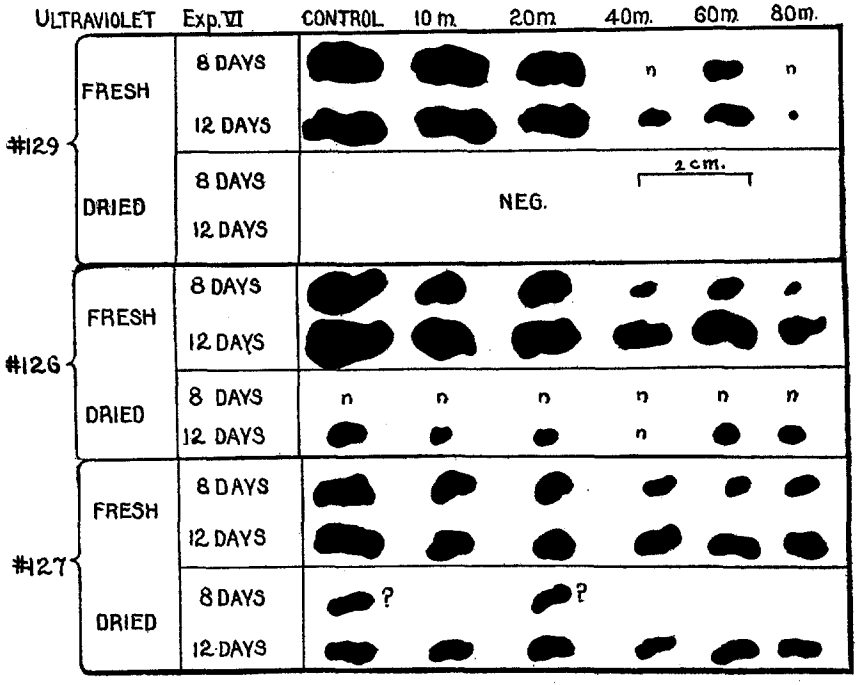
TEXT-FIG. 2. This chart is arranged in the same way and shows findings similar to those of text-figure 1.

TYPES OF RESISTANCE TO THE SARCOMA.

Taken from another point of view the irradiation experiments have demonstrated the presence in the fowl of two distinct resistances to the chicken sarcoma, directed, the one against the tumor cells, the other against the tumor-producing agent. These may exist separately or in one host.

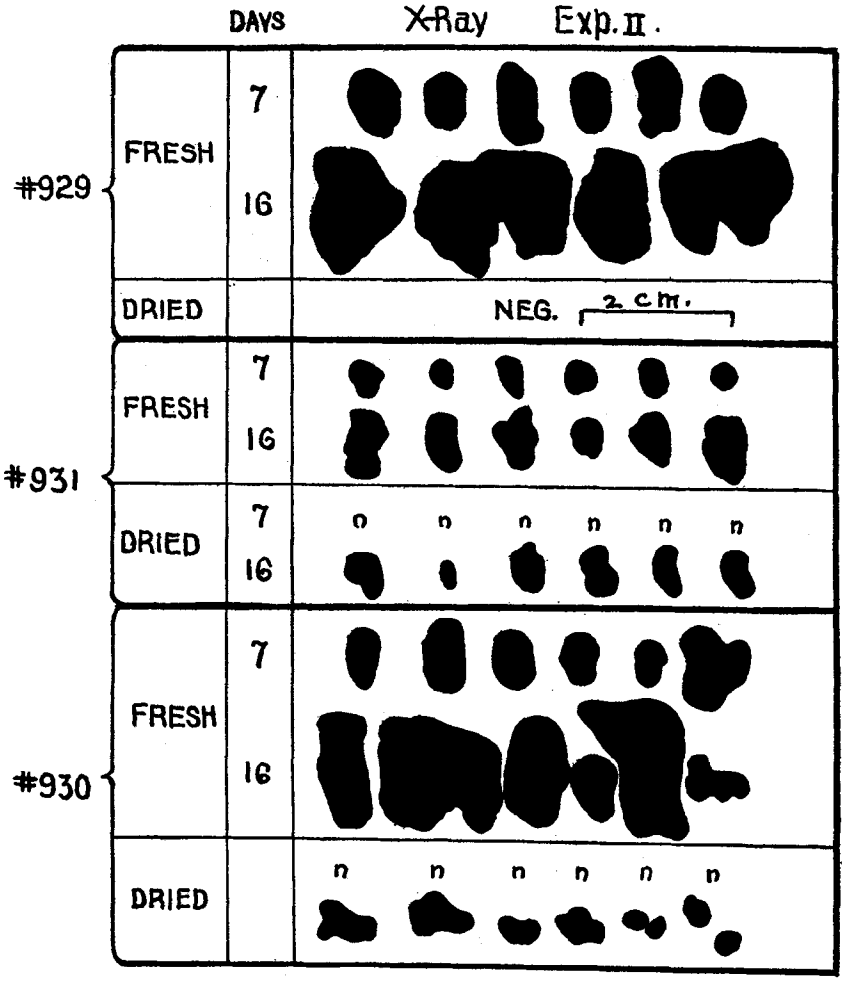
In text-figure 3 are given the findings in three of the fowls of text-figure 2 but now so arranged as to bring into contrast the fate of the fresh and dried material in the individual host. The fresh material produced tumors in all three fowls but with a very different degree of success. In one instance, No. 129, in which it gave rise to large tumors the dried material failed to engender growths, and in another, No. 126, this latter acted only slowly. In the remaining

instance growths developed rapidly from the dried material and the fresh material proved only slightly more active, the results with it being poor as compared with those in the first two hosts. In text-figure 4 precisely the same facts are shown of some fowls of X-ray experiment II, and here the complicating effect of a variation in tumor size due to injury from the irradiation is not



TEXT-FIG. 3. Some of the results shown in text-figure 2, but now so arranged as to demonstrate individual differences in the resistance of the fowls and the presence of two sorts of resistance. In No. 129 the fresh tissue rapidly gave rise to tumors, whereas the same material, when dried, failed to engender them. That this is not due to lack of activity on the part of the dried stuff is shown by the findings in the other two fowls. In fowl 126 the fresh tissue rapidly engendered tumors and the dried material in due time caused them. In No. 127 the dried material was active in the production of growths, whereas the fresh material was much less successful than in the fowls previously mentioned. It is evident that some hosts have a relative resistance against a tumor-producing element in the fresh tissue, while in some a resistance is directed against the tumor-producing element which survives drying. ? = possibly a tumor, possibly only induration.

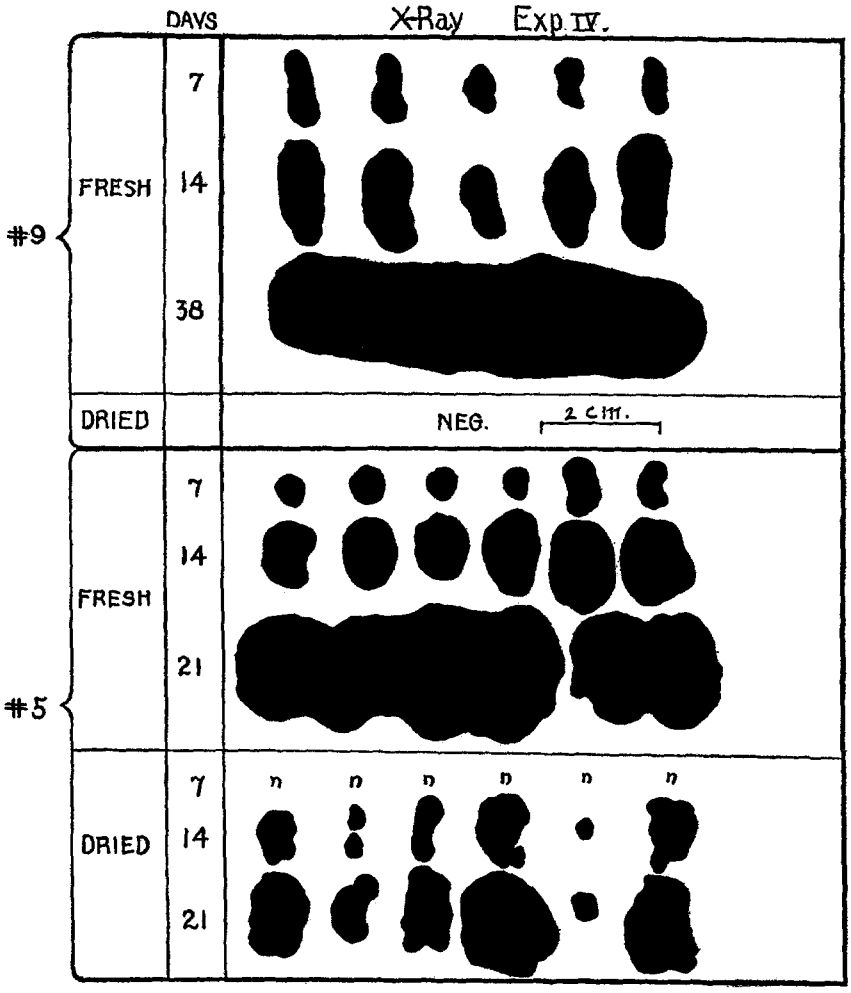
present. In text-figures 5 and 6 some phases of the matter are again illustrated. The fowls in which the dried material is very active



TEXT-FIG. 4. This illustrates the same facts as text-figure 3. The periods of exposure to the X-rays are not given since the material was practically unaffected by them.

are not necessarily those in which the fresh material does badly, but often ones in which it does very well. Other charts showing the same facts might be given. The chickens of all the experiments

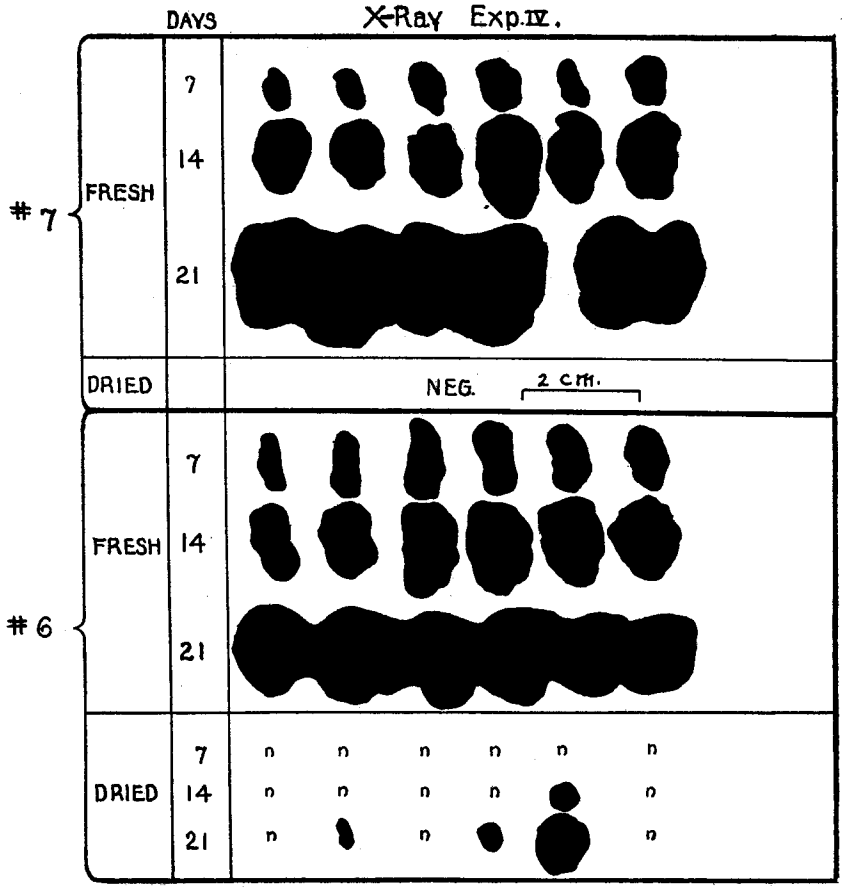
can be separated into four classes: those in which the fresh material gave rise to tumors and the dried gave none; those in which both



TEXT-FIG. 5. This illustrates further some of the facts of text-figures 3 and 4 and shows that good growth from dried material is not necessarily associated with poor results from the fresh tissue.

gave rise to tumors with nearly equal facility; others again in which neither gave rise to growths; and finally a majority in which both

inoculations resulted in growths, those from the fresh material appearing earlier and maintaining a marked general advantage of size. In other words the chickens showed themselves resistant or susceptible in widely various degrees to one, or another, or both the



TEXT-FIG. 6. This chart shows again the differences in individual fowls.

labile and stable elements going to produce the tumor. And we know that these labile and stable elements are, respectively, the tumor cell and the tumor-causing agent.

A number of questions at once present themselves in relation to this conclusion. Resistance to the transplanted tumor cells is never

so clear cut as that to the agent. It is at best only relative. If fowls are really resistant to the implanted cells how does it happen that even in hosts most resistant to them growths from the fresh material appear somewhat earlier and at first are larger than those from the dried material? One reason is because in resistant fowls a profuse, local, round-celled reaction occurs about the fresh tissue implanted in intradermal sites, forming transiently a considerable nodule.[6] Perhaps also the tumor-producing agent is somewhat attenuated by drying. Theoretically the findings, in fact all of the differences between fresh and dried material, might be explained on the assumption that the chicken sarcoma is never really transplanted but comes only from infection by means of an agent largely impaired by drying. This is contrary to the facts and entails awkward secondary assumptions, for example, that fowls react differently to the stable and labile portions of the agent, some being susceptible only to the one, some to the other. But we know that the differences between fresh and dried material are primarily those of the tumor cells involved. Furthermore, investigations have shown that some fowls are naturally resistant to the action of the tumor-producing agent[7] and some to growth of the implanted tumor cells.[8] The present work goes only a step further in demonstrating that the resistances thus manifested are independent of one another.

It is interesting to consider in the light of these results the rôle of the causative agent in the growth of the sarcoma in the individual fowl. Histologically there is no suggestion that the agent takes part in this process. And yet in one class of susceptible fowls, as the present results show, it doubtless aids in the growth's extension. In another class the tumor's development following the implantation of tumor cells is probably from the first solely the result of the proliferation of these cells.

In the present state of our knowledge it is impossible to say whether findings with chicken tumors have a direct application to

[6] Rous, P., and Murphy, J. B., *Jour. Exper. Med.*, 1912, xv, 270.

[7] Rous, P., Murphy, J. B., and Tytler, W. H., *Jour. Am. Med. Assn.*, 1912, lviii, 1751.

[8] Rous, P., *Jour. Exper. Med.*, 1910, xii, 696; Rous, P., and Murphy, J. B., *Berl. klin. Wchnschr., loc. cit.*

FIG. 1.

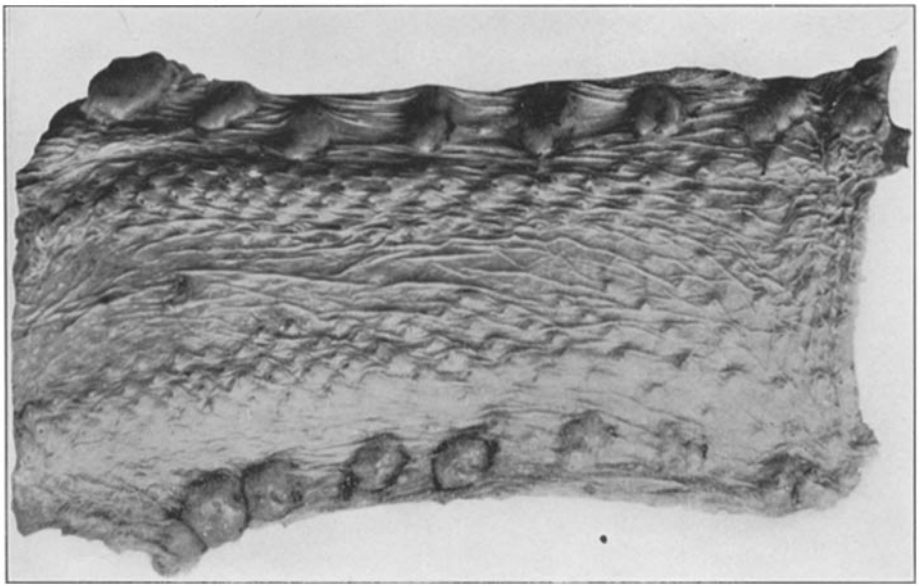


FIG. 2.

(Rous: Resistance to Tumor-Producing Agent.)

the problems of mammalian tumors. Were this so then the present observations would go far to explain how it happens that "spontaneous" growths can arise; as they sometimes do, in rats or mice highly resistant to transplanted neoplasms. For in these individuals resistance to implanted cells need by no means carry with it resistance to a tumor-producing agent.

SUMMARY.

Ultraviolet light rapidly kills the cells of a transplantable sarcoma of the fowl without notably injuring the etiological agent associated therewith. The Roentgen ray has little effect on either cells or agent.

Fowls manifest two sorts of resistance to the avian tumor, one directed against the implanted tumor cells as such, the other against the action of the etiological agent to cause a neoplastic change. In the individual fowl the two resistances appear to be independent of one another, though they may exist together or may both be absent. A recognition of them will perhaps explain some features in the biology of other tumors.

The work has been done with the assistance of Dr. Linda Lange.

EXPLANATION OF PLATE 32.

FIG. 1. A photograph taken eight days after the implantation of material exposed to the Roentgen rays, showing the discrete character of the intradermal tumors. Tumors have arisen, thus far, only from the fresh material. The dried tissue was put in the left pectoral strip. The large mass to the left of the upper end of the sternum is the crop.

FIG. 2. Intradermal tumors in the pectoral strips. The preparation has been turned so that the sternal keel, if present, would lie horizontally along its middle.

The upper line of growths (right pectoral strip) has resulted from the inoculation of fresh tumor tissue exposed in a glass tube to the ultraviolet light for various periods. In the lower line are growths from some of the same material exposed in the same way but in a quartz tube. At the extreme left of each line (left of the photograph) is a tumor from the control material. The periods of irradiation become longer as one proceeds to the right. The photograph was taken on the seventeenth day after the inoculations.

It will be seen that the ultraviolet rays have failed to act through glass, but through quartz their action has been such that at the end of seventeen days tumors are just beginning to appear from the material irradiated longest (fifteen and thirty minutes).