

SOME FACTORS DETERMINING THE LOCALIZATION OF
A CHICKEN TUMOR AGENT *

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PLATES 19 AND 20.

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In the production of many pathological processes, two main factors are concerned. The first, the general factor, which determines the nature of the condition, and the second, or local factor, determining the site of the inflammatory process.

In osteomyelitis for example, there is frequently the history of an antecedent injury to the affected part, and it may be taken for granted, that this injury in some way predisposes to a localization of bacteria, which may happen to be present in the circulating blood or elsewhere. In syphilis, tertiary lesions appear more frequently in superficial bones which are more exposed to injury than in the deeper ones which are well protected by overlying tissues. Kiedel and Zimmermann (1) have described an interesting case, showing that under certain conditions a syphilitic eruption may follow the lines of tattoo markings in the skin, and further, that certain of the pigments used in tattooing are more active than others in this relation, though whether because of a specific action of the dye substance, or of the degree of reaction that it induces is not known. That all inflammatory reactions are not equally effective in promoting the growth of organisms, is well shown by the work of Chesney and Kemp (2), who have demonstrated, that spirochetes will survive better and multiply more rapidly in a recent granulating wound, than in an older subacute lesion.

Various attempts have been made to study the question of localization experimentally.

Thus Gye and Kettle (3) have demonstrated the localizing effect of silica in tuberculosis. Levaditi and Nicolau (4) have shown that the trauma produced by the removal of hair is sufficient to determine a localization of the eruption,

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when vaccine virus is injected intravenously. Somewhat similar results may be obtained when the "virus" of epithelioma contagiosum is injected intravenously in birds, the lesions developing in this case around the roots of feathers which have recently been plucked (5).

Rous and Murphy and Tytler (6) while working with the filtrates of those chicken tumors, which can be transmitted by means of a cell-free filtrate, found that a larger number of tumors could be obtained, if a certain amount of tissue derangement was caused at the site of the injection. To produce this they added kieselguhr to the inoculum. This substance has been shown by Podwysoski (7) to produce a well marked tissue reaction when injected subcutaneously. Jones and Rous found that injury determined the localization of the growths developing after the inoculation of mouse tumor material into the peritoneal cavity (8).

Metastasis formation by "filterable chicken tumors" is common, probably because, as shown by Rous, Robertson and Oliver (9), the active agent is frequently present in the blood of chickens with developing tumors. The secondary growths may occur in the liver, lungs, etc., but during the spring season they are much more common in the ovary. It has been suggested, that this seasonal infection of the ovary is due to the frequent injuries received by that organ during the process of ovulation, and the resulting presence of much recent granulation tissue, which it seems is readily attacked by the tumor agent. Rous, Murphy and Tytler (6) investigated the action of the tumor agent when injected intravenously in chickens, and found that tumors seldom resulted, but when they did develop it was nearly always in the functioning ovary.

The above observations on chicken tumors suggested that the induced inflammatory reactions might prove favorable to the localization of the causative agents on intravenous injection with as a result the subsequent development of tumors. It was therefore determined to attempt the experiment.

Method.

The method selected was the injection into the pectoral muscles of various irritating substances such as Scharlach R, kieselguhr, tar and hashed chicken embryo tissue, followed after a period by the intravenous injection of fresh tumor filtrate. The pectoral region was selected as the site, partly for convenience, and partly because metastases have never been reported there, even after intravenous injection of the tumor agent. Chicken Sarcoma 1 of the series described from The Rockefeller Institute was chosen for use in these experiments, and only adult Plymouth Rock and Rhode Island red hens were used. These birds are now equally susceptible to this tumor.

Experiments with Scharlach R.

First Experiment.—Six hens received 1 cc. of a saturated solution of Scharlach R in olive oil in each breast and this injection was repeated after 7 days. There resulted a certain amount of inflammatory reaction which could be made out on palpation. Three weeks after the last injection of Scharlach R each fowl received 5 cc. of fresh tumor filtrate into the wing vein. This filtrate was prepared in the usual way by grinding about 25 gm. of the tumor material with sand and about 400 cc. of Ringer's solution, centrifuging to remove the larger particles and filtering through a Berkefeld V filter.

Four weeks later the fowls were killed and thoroughly examined. The pectoral muscles showed a marked inflammatory reaction around the Scharlach R which had become localized and was lying in pockets shut off by well formed connective tissue. Only in one bird out of the five was a tumor present in the breast tissue, this being in contact with the reaction tissue. That this failure to localize in the pectoral tissue was not due to general resistance of the chickens or to the low potency of the filtrate was clearly demonstrated by the fact that four of the five animals showed formations of tumors in other locations, namely lungs, spleen, ovaries, liver and in the wing at the site of the filtrate injection.

In only one fowl out of six employed in this experiment a tumor developed in the breast muscle in the region infiltrated with Scharlach R. In view of this fact the procedure was slightly altered, the period between the second injection of Scharlach R and the intravenous injection of tissue filtrate being shortened.

Second Experiment.—Five hens were injected into the breast muscle with the same solution of Scharlach R as before. Four of these received 1 cc. on each side, while the last received only 0.5 cc. After an interval of 7 days these injections were repeated. A week later, the intravenous injection of 5 cc. of fresh tumor filtrate prepared as described above was made into a wing vein. The animals were killed 4 weeks later, and the breast tissue was examined.

As before, the solution of Scharlach R spread through the pectoral tissue and became only slightly encapsulated by fine connective tissue. In all these birds, small tumor nodules were found in the breast muscles, lying amongst the Scharlach R (Fig. 1). This was later confirmed by microscopical examination. The smaller amount of the dye received by one of the hens did not appear to affect the result, which was the same in all the injected fowls.

Experiments with Tar.

Following the same plan as before, three fowls were injected with 1 cc. of a solution of purified tar in benzene. This was repeated after a week. Seven days later they were given an intravenous injection of 5 cc. of tumor filtrate. When the birds were examined at a later date, they all showed tumor formation in the breast muscles in the region of the tar injections (Fig. 2). Evidently tar resembles Scharlach R in its localizing effect.

Experiments with Kieselguhr.

The reaction produced by kieselguhr in the tissues is a peculiar one, and has been studied by Podwyssozki (7) and others. It consists very largely of giant cells, probably of the foreign body type, with little of the ordinary connective tissue (Fig. 3). It seemed of interest to attempt localization with this substance. Accordingly five hens were inoculated with a mixture of kieselguhr in Ringer's solution, 1 cc. being injected into each breast. This was repeated in a week, and then 7 days after the second injection, the tumor filtrate was passed into a wing vein. One of the birds died early in the experiment and was discarded. Of the remainder, only one out of the four developed a tumor of the breast muscle. All showed tumor localizations in other parts of the body.

This experiment was carried out under conditions very similar to those of the second experiment with Scharlach R and the one with tar. The same filtrate was used for the three. The differing results would suggest that the nature of the tissue reaction is of importance in determining the degree of localization.

Experiments with Embryonic Tissue.

In these experiments the local change was induced with fresh chick embryo tissue.

First Experiment.—7 to 10 day embryos were hashed up in Ringer's solution, and 1 cc. injected into the breast muscle of each of five hens. Only one injection of embryo tissue was given to each bird. When, after 10 days, well formed embryomas had appeared, an injection of 5 cc. of fresh tumor filtrate was given into a wing vein. After 21 days had elapsed, a time presumably sufficient for localiza-

tion to have taken place in or around the embryo nodules, the birds were killed and examined. Of the five used, only one, showed such a localization of the growth. That the filtrate was active and the birds not resistant was shown by the fact that all five developed tumors in other regions. Furthermore the filtrate was the same as that used for the second Scharlach R experiment where localization took place in the areas of induced reaction in all the fowls injected.

In view of the results obtained in the second Scharlach R experiment, it was decided to reduce the time between the injection of embryo tissue and the injection of the filtrate.

Second Experiment.—Four hens were injected into the pectoral muscles with 1 cc. of tissue. Two of these were then injected intravenously with 5 cc. of tumor filtrate 3 days after receiving the embryo tissue, and the other two received their injections 5 days after the embryo hash. The results were as follows:

(a) The chickens injected with filtrate 3 days after the introduction of the embryonic tissues had numerous tumor nodules in and around the embryoma (Fig. 4), all being in contact with the embryonic tissue. These findings were verified by microscopic examination.

(b) The chickens which received the filtrate 5 days after the embryonic tissue injection showed macroscopically no evidence of tumor localization but microscopic examination disclosed in one fowl a few scattered areas of sarcomatous transformation in the embryonic tissue.

The same filtrate was used in both of these groups and its potency was shown by the fact that all of the fowls developed tumors in other parts of the body.

While the first and second experiments were carried out with different filtrates it seems justifiable to compare the results, since in both the filtrate was very active as shown by the fact that all of the fowls developed tumors elsewhere than in the breast. The findings suggest that the reaction induced by the injection of embryo tissue is only active as a localizing factor in its early stages.

DISCUSSION.

Localization of the agent of Chicken Tumor 1, resulting in tumor formation has been accomplished by inducing the development of reaction tissue in the pectoral muscles prior to the intravenous injections of fresh tumor filtrate.

The irritants successfully used for the purpose differ somewhat in their effect, those in one group, Scharlach R, tar and embryo tissue,

eliciting an ordinary subacute reaction of the fibroblastic type, while kieselguhr gives rise to the development of a peculiar tissue consisting largely of giant cells. In the experiment with irritants of the first type, the tumor localization occurred most frequently in the early stages of the reaction when the reaction cells were young and actively growing. As the reaction tissue became older less localization in it took place.

While the number of fowls injected with kieselguhr was relatively few, the small proportion of positive results obtained with this substance has some significance, since the filtrate employed was the same which caused tumors in all of the fowls that had been injected with Scharlach R and tar. It may be concluded tentatively that the peculiar reaction tissue elicited by kieselguhr is less favorable to the localization of the tumor-producing agent than is that induced by other substances. It is of interest to note that kieselguhr is less active in localizing vaccine virus after intravenous injection than is the case with a variety of other substances (10).

Earlier work by Murphy and Rous (11) has shown that in order to produce Chicken Tumor 1 in the embryo the agent must be brought into contact with mesodermal tissue. It is reasonable to suppose that young and actively growing connective tissue will be more susceptible than older tissues to the influence of the tumor agent. This supposition is supported by the above experiments. They would also suggest that it is the reaction to injury rather than the injury as such which renders a tissue susceptible to the tumor-producing activity of the agent.

SUMMARY.

A localization out of the blood stream of the agent causing a chicken tumor, with the subsequent development of the growth can be brought about in the breast muscle, by inducing in this tissue a reaction by such substances as Scharlach R, tar, embryonic tissue and kieselguhr. Localization in the reaction tissue elicited by kieselguhr takes place relatively infrequently. The earlier stages of the reaction induced by these substances localize the tumor agent more regularly than the later stages of the reaction.

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EXPLANATION OF PLATES.

PLATE 19.

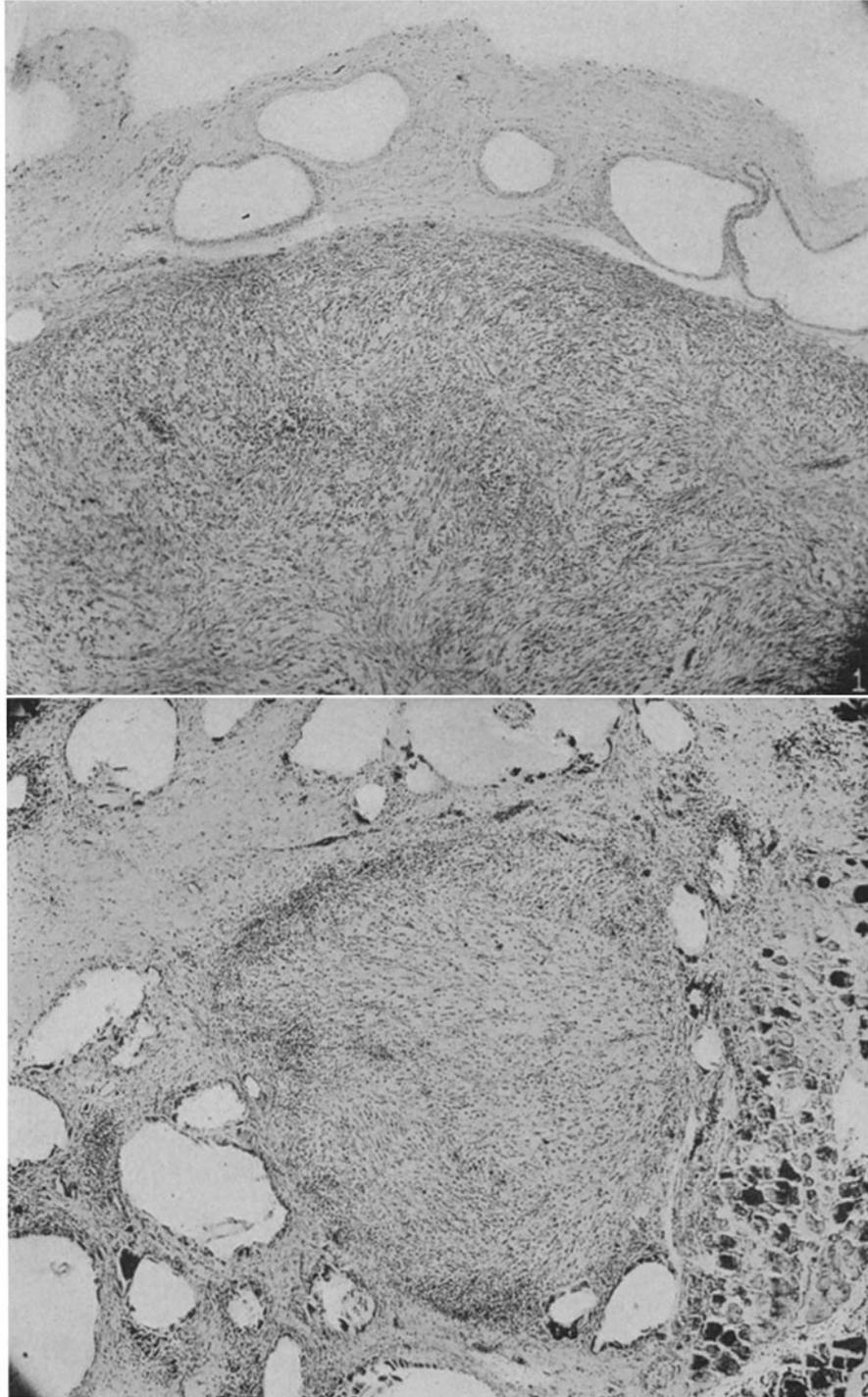
FIG. 1. Localization of Chicken Tumor 1 in a region injected with Scharlach R in olive oil. Clear spaces which contained the Scharlach R are surrounded by young connective tissue, and lying alongside is a mass of tumor tissue.

FIG. 2. Localization of chicken tumor in a region injected with tar. Clear spaces indicate position of tar, and in between these is much young connective tissue. In the center is the tumor nodule. The dark spots are more or less damaged muscle fibers.

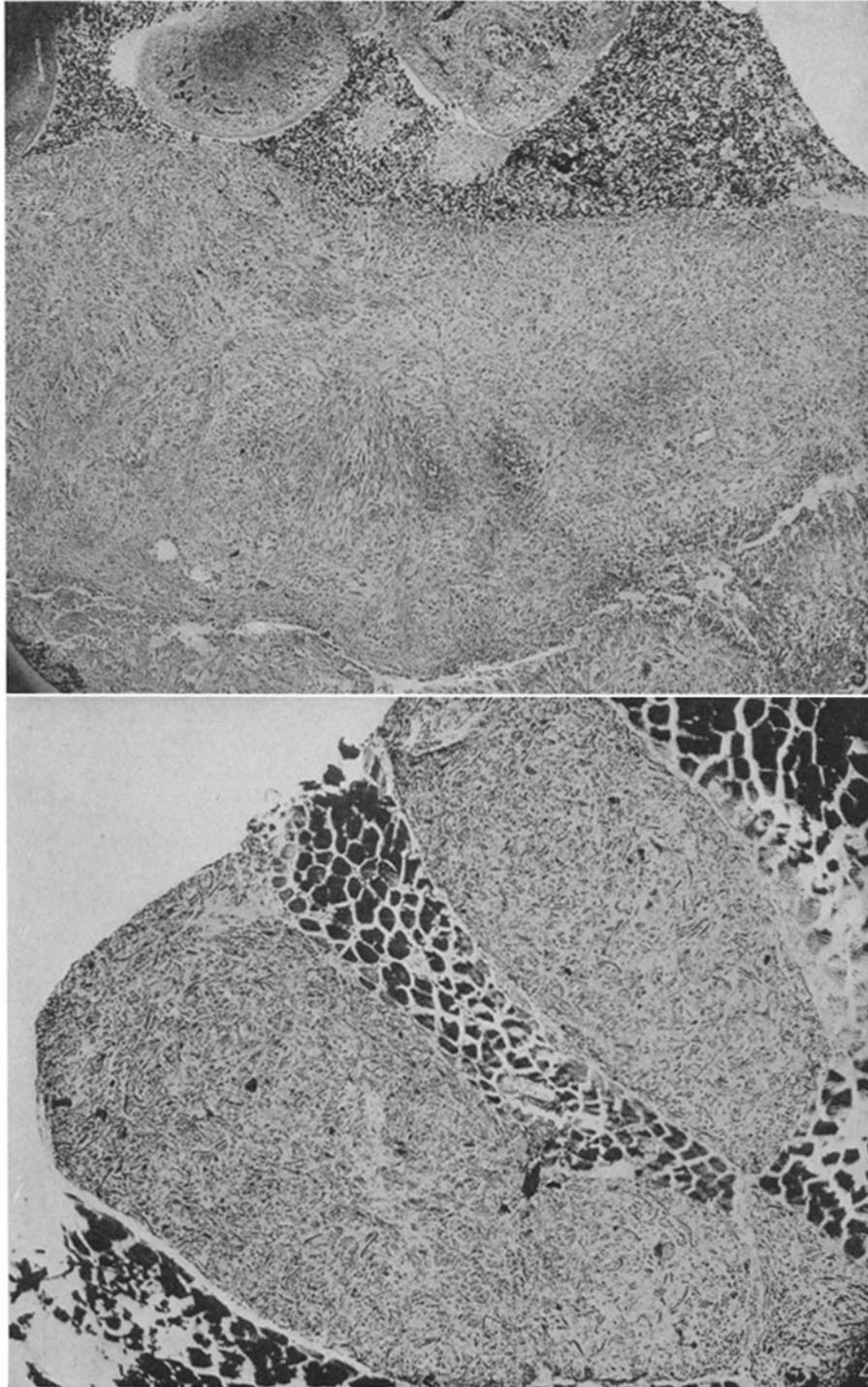
PLATE 20.

FIG. 3. Area of kieselguhr reaction in chicken muscle. This is composed mainly of giant cells with very little connective tissue. There is no localization of the tumor agent.

FIG. 4. Localization in a region of embryoma formation. A well formed tumor nodule is seen above, and below is a mixture of embryonic tissues, composing the embryoma.



(Mackenzie and Sturm: Localization of chicken tumor agent.)



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