ON THE CAUSE OF THE LOCALIZATION OF SECONDARY TUMORS AT POINTS OF INJURY.*

By F. S. JONES, V.M.D., and PEYTON ROUS, M.D.

(*From the Laboratories of The Rockefeller Institute for Medical Research.*)

Plates 18 to 20.

The localization of secondary tumors at points of injury has been so often remarked upon that it is unnecessary to cite specific instances. The cause for the phenomenon is unknown. Lubarsch[1] has shown that mouse tumors may be made to localize secondarily in the liver, about splinters implanted in this organ, but he did not attempt to explain the finding.

There are a number of observations which make it evident that the development of tumor metastases from tumor emboli is conditional upon a special set of circumstances. Schmidt[2] has shown that a large proportion of tumor cells cast off into the blood stream may die without giving rise to metastases. He found that the pulmonary arterioles of patients with visceral cancer often contain many tumor emboli that are dead or dying. The fact has been repeatedly noted that rats and mice inoculated intravenously with tumor fragments seldom develop growths in the lungs despite the fact that the same material causes tumors when implanted subcutaneously. So too in these animals the intraperitoneal inoculation of active tumor material yields comparatively few takes.

It has seemed to us possible to study some of the factors concerned in the secondary localization of tumors by means of experiments involving tumor localization on the lining of the peritoneal cavity. According to Schmidt, the intima of the blood vessels constitutes the essential barrier to invasion of the tissue by the cells of tumor emboli. The peritoneal lining presents much the same structural

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[1] Lubarsch, O., *Med. Klinik*, 1912, viii, 1651.

[2] Schmidt, M. B., Die Verbreitungswege der Karzinome und die Beziehung generalisierter Sarkome zu den leukämischen Neubildungen, Jena, 1903.

features as the intima of the blood vessels, namely, a single layer of flattened cells covering a connective tissue. When tumor fragments are injected into the blood stream they are often surrounded by a thrombus and furthermore are so widely scattered that their fate is difficult to follow. Both these difficulties are avoided by the use of the peritoneal cavity.

The first question to present itself is that of the nature of the forces which prevent, or at least hinder, the take of tumor fragments inoculated intraperitoneally. Is there an immunity reaction in which the fluids of the cavity are concerned, or merely a resistance offered by an intact serosa? To test the point we have injured the peritoneum of mice by mechanical means, afterwards inoculating a tumor. For the purpose finely ground diatomaceous earth (*Kieselguhr*) has been used and an adenocarcinoma known in our laboratory as Mouse Tumor 33. Mouse Tumor 33 grows in discrete masses, seldom infiltrating or becoming diffuse (figure 1). Because of this it was especially suitable for the work.

Experiment 1.—Fifteen mice were injected intraperitoneally with 0.25 c.c. of finely ground, sterile *Kieselguhr* suspended in Ringer's solution, and three days later with 0.1 c.c. of a suspension of very small fragments of mouse carcinoma in Ringer's solution. Control animals were injected with the tumor suspension only.

At the end of two weeks nine of the mice receiving both *Kieselguhr* and carcinoma remained. They were killed and carefully autopsied. Seven had tumors in the subcutaneous tissue and on the peritoneum where the injecting needle had been thrust through. In all of these, nodules were found scattered throughout the peritoneal cavity, and in several the liver, spleen, and kidneys were involved. The remaining two animals were negative.

Eight controls survived the two weeks. Six had tumors along the track of the inoculating needle similar to those in the experimental animals, but in only one had dissemination taken place in the peritoneal cavity.

This experiment and others similar show that an acute injury to the peritoneum, mechanically caused, renders it more suitable for the lodgment and growth of mouse tumor. Microscopic examination of the nodules on the parietal peritoneum, in the mesentery, and on the surface of the liver and spleen of the experimental animals has revealed an interesting condition. The particles of *Kieselguhr* are not distributed evenly but lie in aggregates here and there in the midst of a layer of newly formed and very cellular connective tissue

covered with endothelium. The tumors are in general definitely localized to these areas (figure 2). Many small discrete clumps of neoplastic cells are to be noted lying embedded in the reactive tissue and covered with endothelium. In the case of the larger, more diffuse tumors the association with the *Kieselguhr* is also evident (figure 3).

Although the results are clear cut they do not enable one to conclude whether it is damage to the connective tissue or to the endothelium that renders the peritoneal surface susceptible. For the inoculations were made at a time when the *Kieselguhr* had but just cut its way through the endothelium. In a later series of experiments two weeks were allowed to elapse between the *Kieselguhr* inoculation and the injection of the tumor, in order that the endothelium might have opportunity for complete repair. Sections show that after this time the *Kieselguhr* is enclosed in small discrete accumulations of quiescent connective tissue, completely covered by endothelium.

Experiment 2.—Ten mice were injected intraperitoneally with 0.25 c.c. of finely ground *Kieselguhr* suspended in Ringer's fluid, followed two weeks later by 0.1 c.c. of a suspension of mouse carcinoma. Ten control animals received the tumor suspension only.

Five of the mice that received the *Kieselguhr* and carcinoma were alive two weeks after the injection of the latter. They were killed and examined at this time. In four, tumors had developed along the track of the injecting needle while in the remaining one the tumor had failed to take. In all four susceptible animals tumor nodules were found throughout the peritoneal cavity.

Autopsy of the seven surviving controls revealed the following: Four had growths along the track of the needle and in three the tumor had failed to take. In one of the susceptible animals a nodule was present in the mesentery directly opposite the point of injection. In the others the peritoneal lining was normal.

This experiment and others of the same sort show that an injured peritoneal lining remains favorable to tumor implantations after the endothelium has repaired itself completely. Experiments in which lycopodium spores were used as the foreign body have given identical results. Unlike the *Kieselguhr* the spores do not penetrate but are rapidly surrounded by endothelial cells and later encapsulated by connective tissue (Marchand). As a rule quite a number of them are found lying together in a web of newly formed connective tissue. The little nodules so composed are covered with endothelium (figure 4). They offer a most favorable locus for tumor im-

plantation (figure 5). We feel justified in concluding that it is the derangement of the connective tissue, rather than of the endothelium which renders an injured peritoneal lining favorable to the lodgment and growth of tumor fragments.

The damage caused by *Kieselguhr* or by lycopodium is punctate in character, but it is wide-spread and might conceivably alter the ability of the peritoneum to elaborate immune substances, or to form the medium of their passage. It has seemed necessary, therefore, to perform experiments involving a relatively insignificant and sharply localized damage to the peritoneum. One or several small, sterile glass rods, rounded at the ends, were introduced into the peritoneal cavity of mice through a trocar and followed later by a tumor suspension.

Experiment 3.—Two or three glass rods about 1 mm. in diameter and 8 mm. long were introduced into the peritoneal cavity of each of ten mice. Two weeks later the animals were inoculated intraperitoneally with 0.1 c.c. of a fine suspension of mouse carcinoma. Ten control animals also received the tumor material at this time.

Six of the experimental animals were alive after two weeks. Four had a tumor in the track of the inoculating needle. In all four, intraperitoneal tumors were found situated next to the glass rods and there only (figure 6). The remaining two mice were negative.

Three of the controls survived two weeks. Two had tumors in the injection track and the other was negative. In one of the susceptible animals a few tiny, discrete tumor nodules were found on the mesentery.

The injury caused by a smooth, glass rod where it lies in contact with the peritoneum renders this latter favorable to tumor implantation. An alternative explanation, that the localization of the neoplasm was due to an accumulation of tumor fragments in a dead space about the rods, fails, because there was no dead space, the rods being closely enveloped in mesentery.

Evidently the resistance manifested by a healthy peritoneum to the lodgment and growth of tumor fragments is not due to a general immunity reaction, but is referable to the physical characters of the lining membrane.

In the light of our results, one may ask whether the factor of injury may not play a part, heretofore unrecognized, in the peritoneal dissemination of certain visceral tumors of human beings. It is true that some growths are so malignant that fragments sown on an in-

tact serosa can successfully lodge and grow. This has been noted of certain rat and mouse tumors as well. But at the other end of the scale there are visceral growths which fail to localize on the peritoneal lining, although fragments of them must be distributed to it. With tumors of intermediate malignancy may it not be that the first fragments that are cast off die, and, causing inflammation, render the peritoneal lining more susceptible for future implants? To test the point mice were inoculated intraperitoneally with bits of killed tumor and later with particles of the living growth.

Experiment 4.—Fifteen mice were injected intraperitoneally with 1.0 c.c. of coarse particles of killed mouse carcinoma suspended in Ringer's fluid. The suspension had been heated in the water bath at 55° C. for 15 minutes, a temperature sufficient to kill the cells of the tumor. Three days later each animal received a second inoculation of 0.06 c.c. of a suspension of living tumor fragments. Fifteen control mice were also inoculated at this time.

Seven of the experimental animals were alive two weeks later. In all, tumors had developed in the inoculation track. In six, there were nodules throughout the abdominal cavity. In the remaining animal two discrete nodules were found in the mesentery.

Ten controls survived. Four were completely negative as regards tumor. Six showed tumors in the injection track. Three of these had one or two tiny, sharply circumscribed growths in the mesentery, and a fourth showed many disseminated growths. In the other two the peritoneal lining was healthy looking.

Jobling[3] has shown that the intraperitoneal injection of a suspension of rat carcinoma killed by heat may increase the susceptibility of animals for later subcutaneous implantations of the same tumor. The results of the present experiment might be referred to a similar hypersusceptibility, were it not that in other of our experiments with identical intraperitoneal findings there is no evidence for this, tumors developing along the track of the injecting needle in about the same proportion of control animals and those injected with the killed suspension. Furthermore, special tests have shown that killed suspensions of Mouse Tumor 33 do not induce hypersusceptibility.

Since dead tumor fragments in contact with the peritoneal lining render this latter more suitable for the lodgment and growth of tumor cells, it seems probable that the peritoneal dissemination of some human tumors may indeed come about through the death of the first tumor fragments cast off, and the reaction thus caused.

[3] Jobling, J. W., *Monographs of The Rockefeller Institute for Medical Research*, 1910, No. 1, 52.

In what way does an injury to the peritoneal lining, or, more precisely, to the subendothelial connective tissue favor tumor localization and growth? The observations of Schmidt,[4] already mentioned, offer a suggestion. Schmidt found that tumor cells which had lodged in the pulmonary arterioles were unable to penetrate the vascular endothelium directly, although they might proliferate and ramify within the lumen of the vessel. Whether they ultimately invaded the surrounding tissue depended upon whether they were supplied with a supporting stroma by the subendothelial connective tissue. Now, in the case of the peritoneal lining we have found that the reactive changes caused by an injury to the subendothelial connective tissue greatly favor the lodgment and growth of bits of tumor. It seems possible that the stroma for a tumor fragment might be elaborated with especial ease by a connective tissue in course of proliferation as the result of an injury. As bearing on the point, we have compared the growth *in vitro* of connective tissue reacting to the presence of a foreign body with the growth of normal connective tissue from the same region. Implantations were made into chicken plasma of bits of tissue from about glass rods embedded for various periods in the breast muscle of fowls. Fowls were chosen because they are extremely resistant to local infection and because their plasma can be readily handled.

Experiment 5.—Eight sterile, smooth glass rods about 1.5 by 8 mm. were inserted on successive days by means of a trocar into the pectoral muscles of each of three fowls. The rods were marked for identification. When they had been in place for 1, 2, 3, 4, 6, 8, 10, and 12 days, respectively, the fowls were killed and many small pieces of tissue from about the rods were implanted in chicken plasma and incubated at 41° C. No infection had occurred. Pieces of normal connective tissue, of fascia, and of muscle from near by were also implanted and incubated. The results were striking. No growth occurred from the control fragments or from those about rods that had been in place only one day. There was marked and very prompt emigration of large, rounded ameboid cells from the fragments that had been next to the rods for two or three days. In a few instances there was a definite growth of fibroblasts as well. The pieces removed from about rods that had been in place 4, 5, 6, and 8 days showed a profuse connective tissue growth which began after only a few hours of incubation. In fact, the rapidity and amount of this growth compared very favorably with that of some sarcomata and of embryonic tissue. By the twelfth day the tissue encapsulating the rods had become quiescent and little growth was obtained from it.

[4] Schmidt, M. B., *loc. cit.*

The experiment shows conclusively that connective tissue reacting to the injury caused by the presence of a foreign body has a proliferative energy greater than the normal. Furthermore, its growth in plasma takes place without that latent period which Carrel has described for normal adult connective tissue.[5] The rounded cells that emigrated from the tissue which had been two and three days in contact with the glass rods were doubtless wandering cells attracted by the foreign body; and the true growth that took place obviously came about by the proliferation of the many fibroblasts present in the reactive tissue. From such results it seems highly probable that connective tissue, reacting to an injury, is in a condition to elaborate the stroma for a tumor more rapidly and abundantly than normal tissue.

Altogether, the findings seem to us to indicate that the secondary localization of tumors at points of injury is referable to the presence at such points of a very cellular connective tissue which may come more readily than the normal to the support and nourishment of the tumor cells. A number of facts in the literature may be taken to support this view. To mention only two of them, Loeb and Sweek[6] have described epitheliomata of which the sluggish course was apparently referable to the resistance offered by an inert connective tissue; and Levin[7] has shown that the Flexner-Jobling rat tumor, inoculated into the normal testicle of rats and into testicles previously injected with *Scharlach R* and ether water, will grow only in the latter. Levin ascribes this finding to some chemical influence inducing a "precancerous state" in the testicle. It would seem more likely that it is referable to the presence of a highly labile connective tissue capable of immediate and active proliferation in support of the tumor. The rapid spread of tumor tissue in a wound is explicable on the same basis.

SUMMARY.

The cause of the frequent localization of secondary tumors at points of injury is not known. Our work deals with this problem.

[5] Loeb has found that regenerating kidney grows better than the healthy organ *in vitro* (Loeb, L., *Anat. Rec.*, 1912, vi, 109).

[6] Loeb, L., and Sweek, W. O., *Jour. Med. Research*, 1913, xxviii, 235.

[7] Levin, I., *Jour. Exper. Med.*, 1912, xv, 163.

For the experiments the peritoneal cavity has been employed as offering relatively uncomplicated conditions, and the fate of mouse tumor brought into contact with a peritoneal lining injured in various ways has been studied.

The injection of a suspension of mouse tumor into a healthy peritoneal cavity has little success as a rule compared with a similar injection into the subcutaneous tissue. We have found that the resistance of the peritoneal lining thus indicated can be largely if not completely abolished by the preliminary injection of a mechanical irritant (*Kieselguhr,* lycopodium). That the change thus brought about is independent of general immunity phenomena is shown by the fact that a local injury renders susceptible the part of the peritoneum immediately affected and that part only. Special tests show that the factor important in rendering the peritoneum more susceptible is the injury to the subendothelial connective tissue. Susceptibility persists after the endothelium has regenerated over the reacting connective tissue.

Schmidt has found that the cells of tumor emboli in the pulmonary arterioles are able to penetrate the endothelium of the vessel only after they have been provided with a stroma from the subendothelial connective tissue. Our findings are easily explained on the basis thus suggested. A connective tissue highly cellular and perhaps still proliferating as the result of injury may well elaborate the stroma for a tumor more rapidly than normal connective tissue. Tests of growth *in vitro* support this idea. Connective tissue reacting to an injury grows profusely and almost immediately when incubated in plasma, whereas normal tissue from the same region shows usually no growth whatever.

Dead tumor fragments in contact with the peritoneum cause a change favorable to the lodgment and growth of later tumor fragments. It seems not improbable that the peritoneal dissemination of certain human neoplasms may be accomplished indirectly through the death of the first tumor fragments cast off.

Our observations have been purposely confined to the effects of injury on the peritoneal lining ; but they seem to afford the basis for a generalization. The secondary localization of tumors at points of injury may be attributed with good reason to the presence at such

points of an active connective tissue capable of elaborating a stroma rapidly and abundantly. For it is the proliferation of the subendothelial connective tissue to form a supporting stroma that determines the fate of free tumor cells, whether these lie on the peritoneum or within a vessel.

EXPLANATION OF PLATES.

PLATE 18.

FIG. 1. A nodule of Mouse Tumor 33 on the serous coat of the intestine, showing the discrete, uninvasive character of the growth. The nodule is largely necrotic.

FIG. 2. A portion of the parietal peritoneum and abdominal muscle of a mouse receiving an injection of *Kieselguhr* and three days later one of mouse tumor. The animal was killed two weeks after the second injection. On the peritoneal surface are two nodules of reactive tissue containing *Kieselguhr* and a third such nodule in which the tumor has localized.

PLATE 19.

FIG. 3. A higher magnification of a portion of the tumor nodule shown in the preceding figure. The *Kieselguhr* is indicated by the arrows.

FIG. 4. Lycopodium spores on the surface of the spleen. They lie grouped together, are embedded in connective tissue, and covered by endothelium.

PLATE 20.

FIG. 5. Portion of a tumor associated with the reactive tissue about lycopodium spores. To be compared with figure 4. Part of the abdominal muscle is shown.

FIG. 6. Viscera of a mouse receiving an intraperitoneal injection of tumor fragments two weeks after the introduction into the peritoneal cavity of three small glass rods. There are discrete tumors (*a* and *b*) in the vicinity of the rods but none elsewhere.

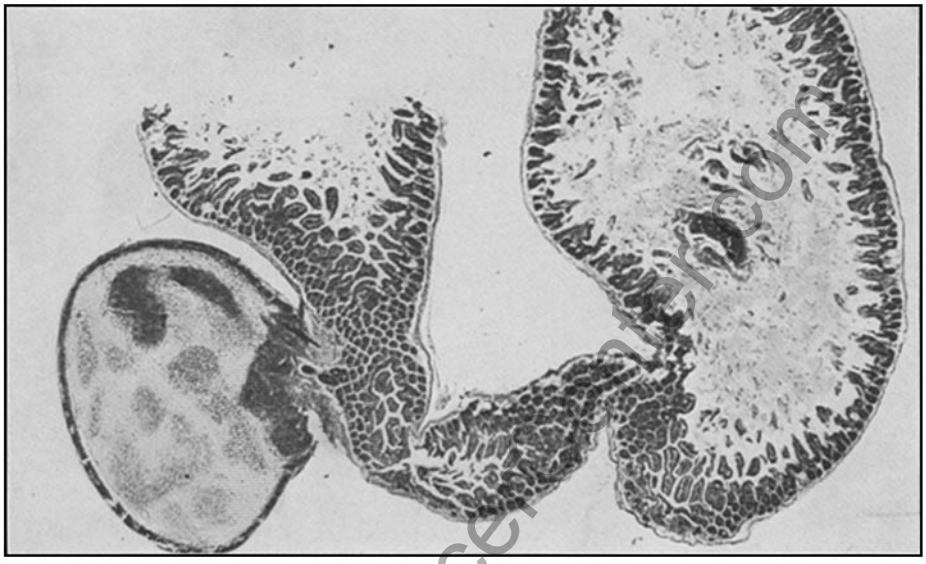

FIG. 1.

FIG. 2.

(Jones and Rous: Localization of Secondary Tumors.)

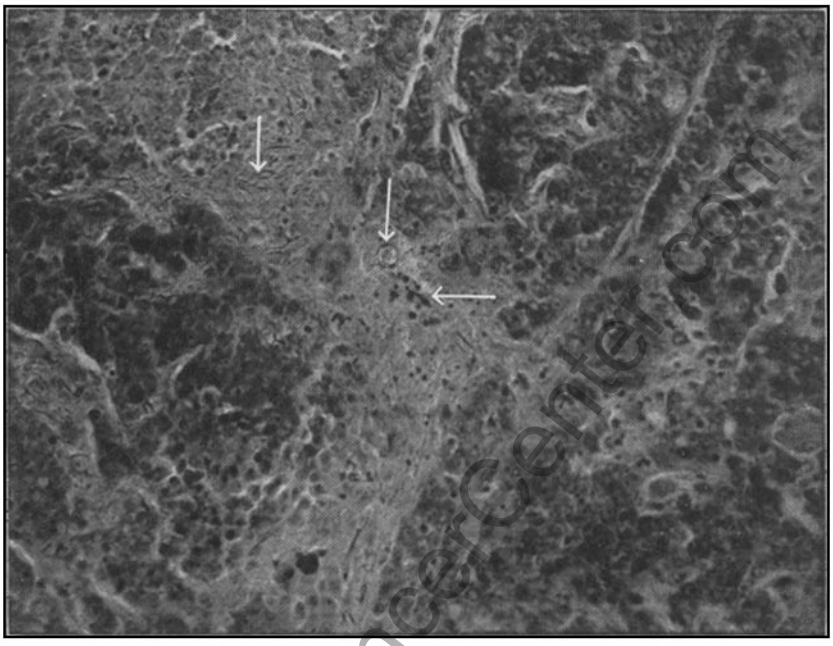

FIG. 3.

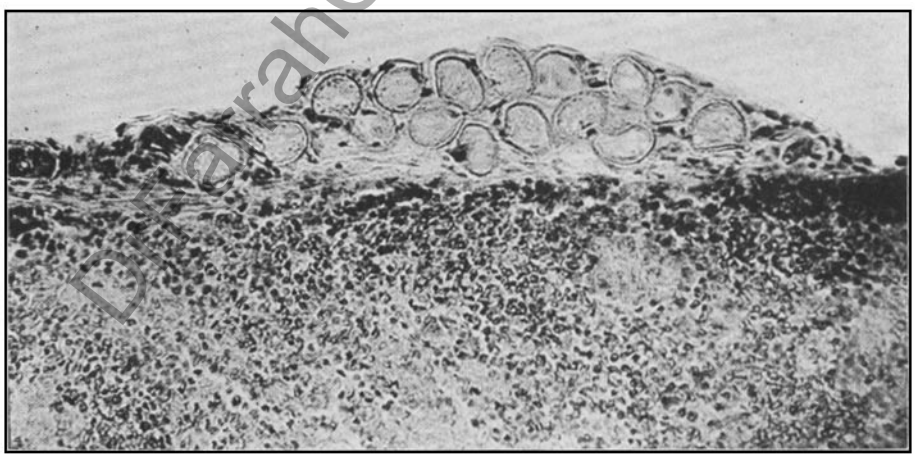

FIG. 4.

(Jones and Rous: Localization of Secondary Tumors.)

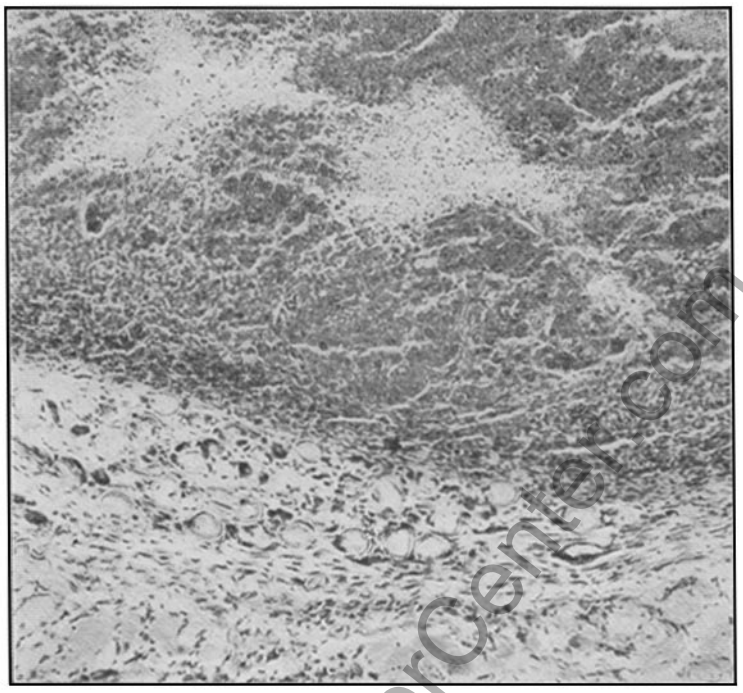

FIG. 5.

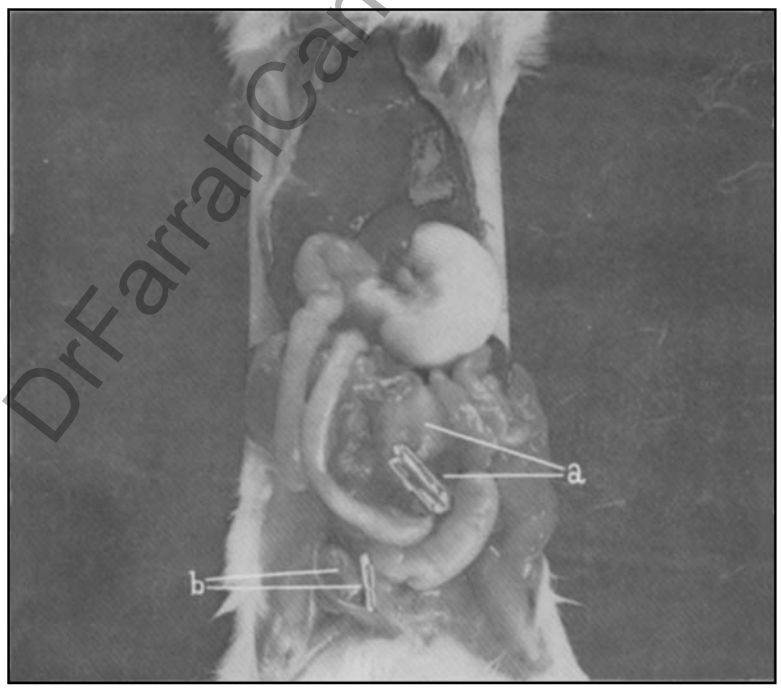

FIG. 6.

(Jones and Rous: Localization of Secondary Tumors.)