THE NATURE OF THE REACTION OF THE TISSUES OF SUSCEPTIBLE AND NON-SUSCEPTIBLE MICE TO AN INOCULABLE TUMOR.*

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It has been observed repeatedly that in mice there exist racial differences in susceptibility to inoculable tumors. Such differences in susceptibility have been noted in the case of mice inoculated with an adenocarcinoma which originated spontaneously in a Japanese waltzing mouse (Tyzzer, E. E., p. 519). Implants of this tumor have been observed to grow in ninety-eight per cent of Japanese waltzing mice inoculated, while common mice have proved in every case non-susceptible. Furthermore, as a result of cross-breeding susceptible Japanese waltzing mice with common mice, it has been found that hybrids of the second filial generation are in all cases non-susceptible to the tumor.

The present investigation consists of a study of the histological basis of this difference in susceptibility. Japanese waltzing mice have been used throughout as the susceptible animals, and hybrids of the second generation as the non-susceptible. Series of susceptible and non-susceptible mice have been inoculated, killed at certain periods after inoculation, and the tissue taken for histological study.

With regard to the inoculability of mouse tumors in general it was shown by Jensen,¹ — by the examination of the "early stages" in the development of transplanted tumors,— that the parenchyma of the new tumor is derived entirely from the cells of the parent growth. Following this, Bashford and Murray,² working with Jensen's tumor, showed that the connective tissue and vascular supply of the successfully implanted cancer are derived solely from the tissues of the new host. They find that the injected mass is surrounded by a fibrinous exudate within two hours after inoculation,

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and that within the first fifteen hours there are present aggre-
gations of polymorphonuclear leucocytes which have pene-
trated the interstices of the tumor tissue, especially those
portions which are necrotic. At this time (fifteen hours) they
describe the beginning of proliferation by the amitotic division
of the surrounding connective tissue cells of the host. This
process continues until the tissue around the injected mate-
rial becomes very cellular. The fibroblasts thus formed by
direct or amitotic division subsequently undergo division by
mitosis and penetrate the tumor mass. Concomitant with
this their preparations show a retraction and gradual
degeneration of the introduced stroma. By the end of four
days they note the commencement of the growth of capil-
laries into the tumor mass; and this continues until finally
the growth has acquired a new blood supply which, like the
connective tissue elements of the stroma, is derived entirely
from the tissues of the new host.

While the present investigation was in progress a histologi-
cal study of mouse tumors by Russell appeared. This
included a comparison of the early stages of inoculated
carcinomata in normal mice and in mice rendered resistant
by the previous injection of mouse blood, mouse embryo
emulsion, or normal mouse tissues. Injections of this sort
had already been shown to render such mice immune to
tumors subsequently inoculated,—Bashford, Cramer, and
Murray, and Shöne. In addition to these, mice which
had once proved resistant to an inoculation with the tumor
were also used for comparison with susceptible mice, and the
results were found to be similar to those observed in mice
artificially immunized. This fact led Russell to conclude that
the nature of the immunity was identical in both. He reports
in detail his observations upon the early stages of an inoc-
ulable adenocarcinoma of mammary origin which gave an
average of about thirty per cent of successful implantations.
A study was also made of the early stages in the development
of other inoculable tumors, including the Jensen tumor. The
changes were essentially similar in all the tumors used.
Russell's principal finding was a complete absence of stroma
formation in the resistant mice. He observed in these animals that the implanted parenchyma excited no formation of fibrous or vascular stroma on the part of the tissues of the host, and soon became necrotic, with the exception of those cells at the periphery of the masses which lay in contact with the tissues of the host. This epithelium, he noted, continued its growth for several days, and usually formed a small cyst, but was finally destroyed apparently by the late ingrowth of fibrous tissue. A similar study of these tumors, inoculated into rats, showed, on the other hand, a perfect "stroma reaction" on the part of the tissues of the rat, a vigorous growth for about nine days, and then a simultaneous degeneration of all living tumor cells, with a subsequent complete absorption of the growth. Thus from his observations it appears that in mice the failure of growth is due to absence of stroma formation on the part of the host's tissues, while in rats the ultimate destruction of the tumor occurs after the growth has received a stroma, and is the result of a simultaneous degeneration of all the introduced parenchyma.

In the following experiments the changes resulting from the inoculation of the Japanese waltzing tumor into susceptible and into non-susceptible, or refractory mice, have been the object of study. This tumor originated as a primary adenocarcinoma in a Japanese waltzing mouse, and has been described in a previous paper by Tyzzer. In its present state of growth it consists of solid masses of epithelial cells with a relatively small amount of stroma. It extends by the continuous invasion of its capsule by processes of tumor tissue, and metastases occur relatively late. Its rate of growth in Japanese mice is relatively slow, so that the average weight at the end of five weeks is not over one gram.

The most striking peculiarity of this tumor is that it has been grown successfully in the first generation of the hybrids, obtained by cross-breeding Japanese and common mice, as well as in Japanese waltzing mice; while, on the other hand, all other mice inoculated, including hybrids of the second generation (i.e., the offspring of hybrids of the first generation bred together), have constantly proved resistant to it.
In Japanese waltzing mice ninety-eight per cent of the inoculations have been successful. For the purposes of this experiment, therefore, series of Japanese waltzing mice and hybrids of the second generation were inoculated for comparative study.

The technic employed in the inoculations is as follows: A well developed tumor in a Japanese waltzing mouse is removed under aseptic precautions (the animal having been killed by chloroform), and placed in a sterile Petri dish. Then a small bit from the periphery of the tumor, which shows by its translucency that it is not necrotic, is taken and finely divided with a pair of fine scissors, and is then drawn up into the end of a small trochar. All instruments used are previously sterilized by boiling, and the trochars are sterilized before each inoculation. The point of the trochar is then inserted under the skin of the mouse to be inoculated in the posterior portion of the back where the hair has already been clipped. It is then carried forward beneath the skin of the right side to the region of the axilla, the contents are expelled by pushing home the plunger, the instrument is withdrawn, and the tissues around the site of the inoculation are then gently rubbed together a few times between thumb and finger. In this way a large number of mice may be inoculated from the same tumor.

In the first experiment a series of twelve young Japanese waltzing mice and twelve young hybrids of the second generation were inoculated, by the method just described, with small portions of a "Japanese" tumor of six weeks' growth. One Japanese waltzing mouse and one hybrid of the second generation were killed at the following periods after inoculation: twenty, thirty, forty-eight hours, three, four, five, seven, ten, fifteen, twenty, and twenty-eight days. The skin of the right side was in each case carefully dissected back and the tumor material exposed. This was inspected, removed by cutting off the skin flap, and placed immediately in Zenker's fluid.

Later a second series of mice was inoculated from another
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"Japanese" tumor, slightly larger doses being injected. This consisted of six Japanese waltzing mice and twelve hybrids of the second generation. In this series one "Japanese" and two hybrids were killed at the end of each of the following periods: seven, eight, nine, ten, eleven, and twelve days.

The results are as follows: In both the Japanese mice and the hybrids the central portion of the introduced tumor becomes necrotic, but the cells at the periphery of the mass continue their growth, and mitotic division is frequent. The relative amount of necrotic tissue varies in different individuals. The introduced stroma, on the other hand, degenerates and becomes involved in the central mass of necrotic tissue, so that at the end of four days it is no longer distinguishable. The injected tumor material is surrounded by an inflammatory exudate which probably represents a reaction to the trauma of inoculation. This is sero-fibrinous or fibrinopurulent, and contains many large cells of endothelial origin which become phagocytic near the tumor mass. In some cases the tumor mass is to a large extent surrounded by a space containing serum and lined by a layer of fibrin. All the early stages—from twenty to forty-eight hours inclusive—show large numbers of polynuclear leucocytes and large endothelial phagocytic cells lying in the surrounding tissues, filling the space immediately around the tumor, and in many cases penetrating throughout the interstices of the tumor mass. After forty-eight hours, however, this exudate begins to be resorbed, and at the end of four days there remain in the surrounding tissues merely a trace of the fibrin and a few scattered inflammatory cells: polynuclear leucocytes, eosinophiles and large endothelial cells.

Meanwhile proliferative changes occur in the connective tissue of the host. In the twenty-four-hour specimens many of the connective tissue nuclei are large and vesicular, and have their chromatin gathered into one or two large masses. Proliferation of this tissue has actually begun at this stage as occasionally a cell may be found in mitosis. From this time on, the process continues until the tumor is surrounded by an area of very cellular connective tissue. Into this zone
of fibroblasts the proliferating tumor cells begin to push their way, while at the same time the proliferating connective tissue cells invade, though apparently much more slowly, the interstices of the original tumor mass. This condition is seen in the specimens at the end of three days both in the case of the susceptible and of the non-susceptible animals.

At this time also (three days) the neighboring blood vessels have become much dilated, and their endothelium is swollen (mitotic division of endothelial cells may occasionally be found at this time). The next specimens, however (four days), show the beginning of capillary extension. Endothelial processes, some of which have acquired a lumen and contain red blood corpuscles, are found in the zone of young connective tissue and some of them have penetrated between the outgrowing masses of tumor cells. This process continues, so that in the tumors of five days' growth the proliferating connective tissue and blood vessels form a new stroma for the rapidly proliferating tumor epithelium. The same is true of the seven day tumors, of which the one from the Japanese mouse shows the more necrosis, but has acquired its new stroma, and is very rapidly growing in some portions. The specimen from the hybrid (seven days) is a well established and vascularized tumor—a perfect reproduction of the parent growth.

This series shows, therefore, that the tumor by acquiring a new stroma from the tissues of the host becomes established in both susceptible and non-susceptible mice. The process is similar to that described by Bashford and Murray² as occurring in the Jensen tumor when inoculated into susceptible animals. Definite evidence of amitotic division of connective tissue as described by Bashford and Murray² was not found, although certain appearances were suggestive of it. Its main points may be summarized as follows:

(1.) A continuous growth of those tumor cells which are situated near the periphery of the injected mass and are therefore in a position to receive nutriment; and a necrosis of the cells in the central portion of the mass furthest from the supply of nutrition.
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(2.) A reaction to trauma—an inflammatory exudate varying in amount, which is for the most part resorbed by the fourth day.

(3.) A proliferation of the surrounding connective tissue forming a zone or layer of fibroblasts about the tumor, which has begun twenty-four hours after inoculation.

(4.) An extension of the neighboring capillaries into this zone.

(5.) An extension of the tumor into the very cellular and vascular capsule thus formed and a concomitant penetration of the original area of the tumor mass by the proliferating connective tissue and growing blood vessels. In this way the tumor epithelium acquires a new stroma.

After it had thus been shown that the "Japanese" tumor becomes well established and begins vigorous growth in mice known to be resistant to it, it remained to trace the fate of these small tumor nodules, and determine if possible the factors which bring about their ultimate destruction. The study of the remainder of the first series of implants (i.e., those taken after ten, fifteen, twenty, and twenty-eight days' growth) showed that while in the Japanese mice the tumor had continued its growth, in the hybrids it had in every case undergone necrosis. The ten-day specimen showed a distinct nodule made up of necrotic cells and surrounded by a layer or zone of connective tissue and cellular exudate, but no living tumor tissue was present. At fifteen days this mass had been partially absorbed, at twenty days it was barely visible, and at twenty-eight no trace of it could be found. In view of these facts the second series (twelve hybrids of the second generation and six Japanese waltzing mice) were inoculated and taken for study after the following periods of growth: Japanese, seven, eight, nine, ten, eleven, and twelve days; hybrids, seven, eight, nine, ten, eleven, twelve, and fourteen days.

The histological study showed that in all the Japanese mice in this series the tumor was continuing its growth. Processes of tumor cells were seen in every case to be pushing their way into the zone of growing connective tissue about the tumor.
Many of the specimens, however, showed a relatively large amount of central necrosis. Probably this was due to the use of slightly larger doses of tumor material in this series, with the result that in each case there was a larger mass of tumor tissue which was deprived of nutrition after implantation. Notwithstanding these large areas of necrosis, in none of them was there to be noted any appreciable inflammatory reaction on the part of the surrounding tissues. All the nodules had received a new stroma from the host, and in every case the blood supply of the actively growing portions of the nodules was seen to be intact. One of these tumors (eleven-day specimen) was only very slightly larger than the mass originally injected, but most of them had at least doubled their original size. The twelve-day specimen had reached the size of a small pea.

Examination of the tumors from the hybrids, on the other hand, disclosed the following conditions.—In most of the cases (taken after seven days) the tumor tissue was reduced to a thin layer of cells (some of which were still dividing) surrounding a relatively large area of necrosis. One tumor, however (a ten day specimen), was growing vigorously, and had reached at least three times the diameter of the original injection, but was infiltrated with leucocytes. Both specimens of seven days' growth and one of nine days showed a fairly large amount of living tumor tissue. On the other hand, in three hybrids killed ten, eleven, and fourteen days after inoculation all trace of living parenchyma had disappeared. The whole series, then, apparently presents various degrees of the same process, i.e., the destruction of the tumor.

A very important factor in this process seems to be the accumulation of cellular exudate in or about the tumor mass. This is seen throughout the series in case of the hybrids, and is not present in any of the tumors from Japanese mice. It consists of polynuclear neutrophiles, eosinophiles, lymphoid and plasma cells. In some cases one type of cell predominates, in others another. Immature leucocytes and cells resembling those in the germinative centers of lymph nodes are also to be seen in the exudate. Even the
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largest and most flourishing nodule in the series of resistant animals (the ten-day specimen mentioned above) is infiltrated throughout with lymphoid and plasma cells. The blood supply of this tumor has not yet been interfered with, but these cells are distributed throughout the entire growth. This tumor is exceptional in that it shows practically no necrosis.

Apparently following or associated with this cellular infiltration there is evident in most of the other specimens from the resistant mice an excessive production of collagen fibrils on the part of the connective tissue cells of the new-formed stroma (see Fig. 9). This begins about the edge of the central necrotic area. It results in a widening of the interstices of the tumor with a consequent crowding together of the tumor cells and thus an impairment of their nutrition. So we have two processes, a cell infiltration and an increased formation of connective tissue fibrils, each of which may be considered a part of a distinct inflammatory reaction, and either or both of which is present to a marked degree in every case in this series of non-susceptible mice.

The parenchyma of those tumors in which the process of degeneration is apparently well advanced shows no tendency whatever to invade the surrounding tissues. Although the presence of occasional mitoses shows that it is still in a state of proliferation, there are evident no new outgrowths of tumor epithelium. The explanation of this condition seems to lie in the fact that the nutrition and probably the blood supply of this tissue is insufficient. In no tumor where peripheral extension is practically at a standstill, and central necrosis has considerably advanced, is it possible to discover any blood vessels which have remained patent and are in close relation to the tumor cells.

The following table shows the nature and the degree of the inflammatory reaction and the condition of the tumors in each of the non-susceptible mice of this series.
<table>
<thead>
<tr>
<th>Mouse Number</th>
<th>Days of Growth</th>
<th>Inflammatory Cellular Infiltration</th>
<th>Reaction, Fibrous Change of Connective Tissue</th>
<th>Condition of Tumor Tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>2459 A</td>
<td>7</td>
<td>+</td>
<td>-</td>
<td>Some growth peripherally. Mitoses relatively few. Necrosis relatively large.</td>
</tr>
<tr>
<td>2469 B</td>
<td>7</td>
<td>+</td>
<td>+</td>
<td>Mitoses few. Peripheral extension slight. Necrosis relatively large.</td>
</tr>
<tr>
<td>2461 A</td>
<td>8</td>
<td>+</td>
<td>+</td>
<td>Tumor tissue in a thin layer around large central necrosis. Mitoses very few. No peripheral extension.</td>
</tr>
<tr>
<td>2471 B</td>
<td>8</td>
<td>+ +</td>
<td>Slight.</td>
<td>Tumor tissue in a thin layer around large central necrosis. Mitoses very few. No peripheral extension.</td>
</tr>
<tr>
<td>2475 C</td>
<td>9</td>
<td>+</td>
<td>+ +</td>
<td>Actively growing. Large central necrotic mass.</td>
</tr>
<tr>
<td>2465 A</td>
<td>10</td>
<td>+</td>
<td>-</td>
<td>Well developed and actively growing. Very little central necrosis.</td>
</tr>
<tr>
<td>2477 C</td>
<td>10</td>
<td>+</td>
<td>+</td>
<td>Tumor tissue practically gone, a few faintly staining cells remaining. No mitoses.</td>
</tr>
<tr>
<td>2479 C</td>
<td>11</td>
<td>Slight.</td>
<td>+ +</td>
<td>Thin broken ring of tumor tissue seen in section around large necrotic area.</td>
</tr>
<tr>
<td>2473 B</td>
<td>11</td>
<td>Slight.</td>
<td>+ +</td>
<td>Tumor tissue gone.</td>
</tr>
<tr>
<td>2467 B</td>
<td>12</td>
<td>+ +</td>
<td>+</td>
<td>Thin layer of tumor tissue remaining. No peripheral extension.</td>
</tr>
<tr>
<td>2302 D</td>
<td>14</td>
<td>+</td>
<td>+ +</td>
<td>Thin layer of tumor tissue remaining. No peripheral extension.</td>
</tr>
<tr>
<td>2304 D</td>
<td>14</td>
<td>+</td>
<td>+ +</td>
<td>Tumor tissue gone.</td>
</tr>
</tbody>
</table>

(Note.—The mice of this series were from four different litters. Those from the same litter are designated by the same letter following their number in the first column.)

From the data at hand we are thus justified in concluding that the process of destruction of the tumor in the non-susceptible mice is somewhat as follows: After a period of growth on the part of the tumor, the duration of which
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varies with different individuals, there appears in the surrounding tissues an aggregation of exudative cells. It is probable that this reaction may take place in some cases before the tumor has become well established and as a continuation of the original reaction to trauma. On the other hand, there is in most cases a vigorous growth on the part of the tumor for from seven to ten days before the appearance of this second cellular exudate about the tumor. The cells of this exudate lie in the neighboring areolar tissue, and infiltrate the zone of connective tissue immediately about the tumor. Apparently as a result of this process the nutrition of the latter suffers. The cells furthest from the periphery degenerate, and thus the area of centrally situated necrotic tissue becomes increased. Later the exudate in many cases actually separates the tumor cells from the connective tissue of the host. This destruction of the relation of tumor cells to host's tissue apparently renders the former unable to continue their growth. Associated with this impairment of nutrition there occurs the previously mentioned overproduction of collagen fibrils on the part of the connective tissue of the stroma. This takes place at the edge of the central necrosis, separates the tumor cells of this region into thin strands, and still further impairs their nutrition. Thus the area of central necrosis enlarges.

At the same time peripheral extension of the tumor becomes diminished. This seems to be due to a general impairment of the nutrition of the whole nodule, caused by the factors just mentioned. Not only may the exudate which collects in the region of the tumor separate it from its capsule, and thus deprive it of its nutrition, but the entire nodule may eventually become infiltrated also with exudate. Thus the tumor cells become reduced to a thin layer surrounding a large necrotic mass. As the cells of this layer are still subject to the same conditions which have brought about the degeneration of the rest of the nodule, the process continues. The tumor epithelium no longer sends outgrowths into the surrounding connective tissue, although the presence of a few mitoses shows that growth is not entirely
at a standstill. They stain rather more faintly than the cells of a healthy tumor, and are not so closely packed together. Finally they all become completely degenerated, and there is left simply a mass of necrotic material. This sometimes contains a number of leucocytes, and usually shows large areas of very fibrous connective tissue, the result of the changes in the new stroma which have been mentioned above. Surrounding this is a zone of fairly dense connective tissue and around this there is more or less cellular exudate. Finally this exudate and also the necrotic material become absorbed.

With regard to theoretical considerations it is evident that the facts here presented neither support nor tend to disprove the hypothesis of Ehrlich in which he advanced the idea that in resistant animals the failure of the growth of the tumor is due to the lack of some specific substance ("X substance") which stimulates the tumor cells to proliferate. In these experiments there is no evidence as to the presence or absence of such a substance. They show, on the other hand, a reaction on the part of the host which consists of a more or less mechanical interference with the nutrition of the tumor. The failure of the tumor to grow in insusceptible mice is in this way satisfactorily accounted for without considering the possible presence of a hypothetical "X substance."

The experiments of Russell lead him to consider that in mice naturally immune or artificially rendered so by injections (as described above) the tumor degenerates because it receives no stroma. In his opinion either the tissues of the host fail to react to the stimulus of the newly implanted cancer cells or these cells fail to exert their usual influence. He concludes, however, that it is probable that the latter condition obtains, i.e., the absence of a typical "fibroblastic and angioplastic stroma reaction" is due to suppression of the chemiotactic properties of the tumor cells. In the case of the tumor inoculated into animals of a different species (e.g., rats), on the other hand, the sudden disappearance of healthy growth after some days is due, he believes, to the
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formation of specific cytolysins or cytotoxins in the body of the host.

In the present experiments it must be remembered that we are dealing with animals of very closely related varieties capable of interbreeding freely; and that the non-susceptibility of one of these varieties is as absolute as is that of a different species. It is clear that in this case there is no failure of stroma reaction and that immunity in the resistant animals is developed only after several days of vigorous growth on the part of the tumor. In fact the stroma reaction eventually surpasses that of susceptible mice. The destruction of the growth which then ensues, however, is apparently dependent upon an interference with its supply of nutrition. It has been suggested that the inflammatory changes observed may be secondary to a specific serum reaction which reduces the growing nodule to the status of misplaced normal epithelium, and that the process is then similar to the retrograde changes seen in the destruction of such tissue. While the possibility of such a condition cannot be denied, the fact that the necrosis begins at the centers of the nodules, progresses slowly toward the periphery, and is always associated with a definite interference with nutrition, renders it more probable that the immediate cause of the degeneration of the tumor is the appearance of the inflammatory reaction in and around the growth, which cuts off its nutrition. In rats, on the other hand, Russell observed that the necrosis was at first more pronounced, if anything, at the periphery of the nodule and was thus due in all probability to the presence of specific substances in the serum of the rat. In the present instance we have, at all events, a specific immunity developed in a variety of mice very closely related to that in which the tumor originated and grows. Whether or not this immunity is fundamentally of the same nature as that developed in animals of a different species, it is at present impossible to state with certainty. It is at any rate a definite immunity, which develops after an interval of at least seven days from the time of inoculation, and manifests itself
in the appearance of an inflammatory reaction which mechanically interferes with the nutrition of the tumor.

SUMMARY.

In susceptible Japanese waltzing mice inoculated with the "Japanese" tumor, the tumor receives a new fibrous and vascular stroma from the tissues of the host.

In the non-susceptible hybrids of the second generation (the offspring of hybrids obtained by mating Japanese and common mice) the tumor receives a new stroma and blood supply in the same manner as in Japanese mice.

After a short period of active growth (usually about a week) the tumor in the non-susceptible mice is surrounded by an inflammatory exudate which impairs its nutrition.

Apparently as a part of this inflammatory reaction, in many of the non-susceptible mice there occurs an over-production of fibrils on the part of the more centrally located portions of the new stroma of the tumor.

No such inflammatory reaction is seen in case of the tumors of a corresponding age in Japanese mice.

Probably as a result of impairment of nutrition, due to the factors already mentioned, peripheral extension of the nodule (in the resistant mice) ceases, central necrosis at the same time advances, and ultimately all the tumor tissue undergoes necrosis and absorption.

In the non-susceptible mice there is developed, therefore, an active immunity the mechanism of which is apparently an inflammatory reaction which interferes with the nutrition of the tumor.

REFERENCES.

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6. Tyzzer, E. E. A series of twenty spontaneous tumors in mice with the accompanying pathological changes and the results of the inoculation of certain of these tumors into normal mice. Journal of Medical Research, 1907, xvii, 137.


DESCRIPTION OF PLATES.

PLATE LVI.

Fig. 1. — Tumor in Japanese mouse after twenty-four hours' growth. Reaction to trauma. Tumor mass surrounded by a space containing serum and lined by a layer of fibrin.

Fig. 2. — Tumor in Japanese mouse five days after implantation. Formation of new stroma. Outgrowth of tumor cells. Ingrowth of connective tissue and blood vessels (compare with Fig. 3).

Fig. 3. — Tumor in hybrid of second generation five days after implantation. Formation of new stroma (condition same as that shown in Fig. 2).

Fig. 4. — Tumor in Japanese mouse after seven days. Tumor well established. It has acquired its new stroma and is invading the surrounding tissues (compare with Fig. 5).

PLATE LVII.

Fig. 5. — Tumor in hybrid of second generation after seven days. Condition of tumor similar to that shown in Fig. 4. Note, however, the beginning cellular infiltration of the tissues surrounding the tumor.

Fig. 6. — Tumor in Japanese mouse after nine days. Tumor well established and actively growing. Note absence of inflammatory reaction (compare with Figs. 7 and 5).

Fig. 7. — Tumor in hybrid of second generation after nine days. Tumor well established and actively growing. Note marked inflammatory reaction about tumor.

PLATE LVIII.

Fig. 8. — Tumor in Japanese mouse after ten days. Tumor especially well developed.

Fig. 9. — Tumor in hybrid of the second generation after eleven days. Mallory's connective tissue stain. Note the darkly stained areas of connective tissue fibrils. The living parenchyma of this tumor is reduced to a narrow layer surrounding the relatively large central area which is necrotic and which is darkly stained in this specimen. The parenchyma
is not well shown, but is for the most part separated into thin strands by the masses of fibrils formed by the connective tissue of the new stroma. Moderate infiltration of surrounding tissues.

Fig. 10. — Tumor in hybrid of the second generation after twelve days. The inflammatory reaction in this case has taken the form of a marked cellular infiltration. Peripheral extension of the tumor has ceased, central necrosis has advanced, and the living tumor cells are reduced to a relatively thin layer. The tumor epithelium is to a large extent cut off from surrounding connective tissue.
Inoculable tumor.
Inoculable tumor.