STUDIES BASED ON A MALIGNANT TUMOR OF THE RABBIT.

V. Metastases.

Part 2. Description of the lesions with especial reference to their occurrence and distribution.

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This paper continues the description of metastatic growths contained in Part 1. Consideration of this subject may be resumed with the lesions of the skin and subcutaneous tissues.

Skin and Subcutaneous Tissues.—Comparatively few animals showed metastases in the skin or subcutaneous tissues, but in view of the difficulty experienced in obtaining a growth in these tissues by direct inoculation, the fact that metastases occurred at all is of the greatest significance. With one exception, the lesions were confined to the trunk, the neck, and the proximal segments of the legs. They were most numerous over the anterior part of the body, especially over the back and in the region of the shoulder girdle, and in general followed the lines of blood vessels. In one instance, there were solitary lesions about 1 cm. in diameter symmetrically located on the shins.

As a rule, cutaneous metastases were small, shotty nodules of an opaque white or translucent appearance, although some of them were of a purplish red color. Occasionally, the lesions measured as much as 1 cm. in diameter, and the larger ones were usually soft and hemorrhagic in character.

The curve of incidence for cutaneous metastases (Text-fig. 1) differed very little from that of the hypophysis with an actual incidence

1 Part 1, Figs. 3 and 4 (the same animal).
2 Part 1, Fig. 3.
of metastases comparable to that of the skin (11.4 per cent). The majority of the lesions occurred somewhat earlier. In fact, cutaneous metastases were, as a rule, associated with cases of fulminating malignancy. There was only one instance in which a growth in the skin underwent spontaneous resolution, and there were two animals from early generations in which the disease pursued a chronic but fatal course. Otherwise, the extent of the cutaneous involvement was directly proportional to the severity of the disease and constituted the most reliable clinical index of malignancy. This might have been expected from the structure of the skin and from its known resistance to direct inoculation of the tumor cells.

![Text-Fig. 1. Relative incidence of skin metastases.](image)

From the data available, it appears that metastases occurred in the subcutaneous tissues under much the same conditions as in the skin, but on account of the possibility of confusing lesions of this class with metastases in subcutaneous lymph nodes, no attempt has been made to analyze them in detail.

Finally, the development of metastases in the skin and subcutaneous tissues furnishes what is perhaps the most conclusive evidence of the occurrence of marked alteration in animal resistance induced by the growth of the tumor itself.

Muscles.—Metastases occurred in the skeletal muscles with slightly greater frequency than in the skin. They were widely distributed but showed a distinct predilection for the muscles of the thigh, the face,
the thorax and shoulder girdle, and the abdominal walls. Other muscles involved were those of the lumbar region, the neck, and the anterior extremities in the order of frequency given. Metastases occurred also in the diaphragm, but in most instances it was impossible to distinguish between implantations and lesions which developed within the muscle itself.

Muscle metastases were usually few in number but were inclined to be rather large (1 to 2 cm. in diameter), soft, and hemorrhagic. They were usually associated with a well marked cellular reaction, but there was little tendency to encapsulation, and as far as is known, they did not undergo spontaneous healing.

In considering the incidence of muscle metastases with reference to time and the conditions under which they occurred, the factor of distribution, or the number of foci affected, has to be taken into account. As long as one is dealing with single or paired organs, distribution can be disregarded, but in the case of such organs as the muscles, the bones, and the lymph nodes, the number of foci affected becomes a matter of considerable importance.

Muscle metastases were confined to animals with relatively malignant tumors, but occurred with almost equal frequency at three widely separated periods, as shown in Text-fig. 2. That is, from the standpoint of animal incidence, lesions of this class were almost equally divided between slowly progressive and rapidly progressive cases of malignancy. If the curve of muscle metastasis were plotted upon this basis, however, it would be somewhat misleading in that the most extensive muscle involvement was encountered among animals with the most malignant tumors, while there were comparatively few lesions in those with slowly progressive tumors. These facts are brought out by correcting the curve of animal incidence according to the number of foci involved, which places the correct emphasis upon the factor of malignancy and at the same time brings the curve in harmony with those of other metastases whose incidence was determined by a similar group of factors.

**Bones.**—Bone metastases occurred with about the same frequency as metastases in the muscles or the thymus. In fifteen animals, the lesions were distributed as follows:
There were doubtless other metastases which were overlooked, since the bones could not always be examined with minute care. This list is sufficient, however, to indicate the general tendencies of distribution, the important features of which were the great preponderance of lesions in the axial skeleton and in bones with a comparatively direct blood supply. Moreover, there was a decided preference for cancellous bone, and even in the case of the long bones of the extremities, the growth was more abundant in the epiphyses than in the shaft.

There were a few instances in which metastases appeared to have developed beneath the periosteum in such locations as the mandibles,\(^2\) the distal ends of the ulna, and the external malleoli (not included in the above classification). As a rule, however, the growth arose within the bone, but not infrequently extended outward with the formation of prominent swellings over the surface, and in several instances large fungating masses developed about the teeth. The tumor also showed a tendency to localize in or to extend to the cranial sinuses, and there were two instances in which metastatic growths filled the middle ear on one or both sides.
The bone metastases that were found were rather large, extremely soft, and inclined to be hemorrhagic in character. They produced comparatively little bone destruction, which was probably due to rapid and invasive growth combined with short duration. There was a distinct reduction of bony tissue, however, and spontaneous fractures occurred in a few animals.

No apparent reaction occurred about the tumor mass within the bone, and there was comparatively little about that portion of the growth which extended into the surrounding tissues. In like manner, as far as is known, no bone metastases underwent spontaneous absorption.

Text-Fig. 3. Relative incidence and distribution of bone metastases.

From the standpoint of animal incidence, this group of lesions was again almost equally divided between cases of fulminating malignancy and animals with relatively malignant but slowly progressive tumors. The extent of the involvement, however, was much greater in the first group of animals, as shown in Text-fig. 3. In these respects, there was a close analogy between bone and muscle metastases, and there are points of resemblance between the two curves, but they do not coincide. The chief difference appears to lie in a more uniform distribution of bone metastases among four groups of animals, with a slightly larger proportion of cases occurring during the later periods of observation. In other words, there is evidence of a further shift of incidence from the first to the second half of the scale, while the relative extent of
involvement occurring at the two periods is still greatest in animals with rapidly progressive tumors.

_Lymph Nodes._—On account of the wide distribution of small masses of lymphoid tissue and the difficulty in determining the exact point of origin of metastases in such places as the subcutaneous tissues, the retroperitoneal tissues, and the mediastinum, it was impossible to observe the same degree of accuracy in recording the occurrence of metastases in lymph nodes that was possible elsewhere. Hence, in order to avoid placing undue emphasis upon this group of metastases, only those lesions were included which could be definitely localized in lymph nodes. When such a distinction could not be made, metastases were recorded as occurring in the tissues of the region concerned. In this way, the actual number of lymph node metastases was undoubtedly underestimated, and this caused a corresponding error of increase in the number of metastases referred to subcutaneous, retroperitoneal, and mediastinal tissues and to the thymus. Nevertheless, lymph node metastases ranked high in point of incidence.

The distribution and order of frequency of involvement of different groups of nodes were as follows:

<table>
<thead>
<tr>
<th>Node Type</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retroperitoneal</td>
<td>18</td>
</tr>
<tr>
<td>Mesenteric</td>
<td>10</td>
</tr>
<tr>
<td>Posterior cervical</td>
<td>8</td>
</tr>
<tr>
<td>Axillary</td>
<td>7</td>
</tr>
<tr>
<td>Inguinal</td>
<td>7</td>
</tr>
<tr>
<td>Mammary</td>
<td>6</td>
</tr>
<tr>
<td>Submaxillary</td>
<td>5</td>
</tr>
<tr>
<td>Peritracheal</td>
<td>5</td>
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<tr>
<td>Peribronchial</td>
<td>4</td>
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<tr>
<td>Posterior axillary</td>
<td>3</td>
</tr>
<tr>
<td>Anterior cervical</td>
<td>3</td>
</tr>
<tr>
<td>Flank</td>
<td>1</td>
</tr>
<tr>
<td>Auricular</td>
<td>1</td>
</tr>
</tbody>
</table>

The only groups of nodes of any considerable importance (size) which are not represented in this list are the main mesenteric mass and the nodes immediately connected with it, the deep cervicals, and the popliteals. This is of especial interest when it is recalled that these are the largest nodes in their respective regions. The absence of metastases from the popliteals is not so surprising, since to a very large
extent metastases were confined to the head, neck, and trunk, but they did occur in the skin, muscles, and bones of the posterior extremities and even in the loose areolar tissue of the popliteal space. It is more difficult to account for the absence of metastases from the mesenteric nodes and from the deep cervicals, except upon the basis of some structural or functional peculiarity, since all the neighboring nodes and tissues were affected with comparative frequency.

Lymph node metastases were usually rather large and of a grayish pink color. They were soft and frequently hemorrhagic but showed comparatively little necrosis, except in the case of retroperitoneal and mediastinal nodes. These nodes frequently contained large necrotic masses surrounded by a thick fibrous capsule, and it appeared that healing of metastases in the retroperitoneal lymphatics was not infrequent and that it probably occurred in the mediastinal nodes also. In these respects, there was a decided difference between the deep and superficial lymph nodes. In such nodes as the superficial cervicals, the axillaries, etc., there was practically no tissue reaction about the tumor cells, and no regressive changes occurred, except in one animal with a metastasis in the right inguinal node.

The curve of incidence for lymph node metastases was quite different from that of any group of lesions thus far considered. If all classes of nodes are included, as in Text-fig. 4, there is a high and relatively
constant level of incidence from the 3rd to the 9th weeks after inoculation with a second period of increased frequency of even greater extent occurring late in the course of the disease.

If this curve is divided on the basis of deep and superficial nodes (Text-fig. 4), a different picture is presented in that while both groups of nodes show a comparatively high incidence during the earlier periods of observation, those in the drainage area (deep nodes) of the primary tumor show a sustained elevation during the earlier weeks followed by a gradual decline, while the superficial nodes are uninvolved at the beginning but show a gradual rise with some fluctuations and a sharp drop at the 10th week, followed by a second period of increased frequency. This curve is not unlike that of bone and muscle metastases but still shows certain distinct differences.

If the curves for deep and superficial nodes are further modified upon the basis of the extent of involvement, or the number of nodes affected, the differences between the two groups of nodes become still greater, as shown in Text-fig. 5. Under these circumstances, the superficial nodes give a curve much like that of the lungs or the liver, showing a maximum involvement, which in this curve really means incidence, at the 6th week with a reduction during later periods, while the maximum involvement of the deep nodes occurs much later and is decidedly more marked during late stages of the disease.
The deep nodes of the abdominal and thoracic groups were involved in all classes of animals without especial reference to the severity of the disease, but in as far as incidence alone is concerned, the involvement was greater in slowly progressive cases than in fulminating cases of malignancy, while with the superficial nodes this condition was reversed. The significance of these relationships will be more apparent when we have considered the incidence of metastases in such locations as the retroperitoneal tissues and the mediastinum.

Retroperitoneal Tissues.—As has been intimated, it was impossible to draw a sharp line of distinction between metastases which were distributed along the course of lymphatics in the retroperitoneal tissues and those which were localized in well defined masses of lymphoid tissue. In describing this group of lesions, therefore, the main object is to bring out certain points of similarity and of difference between the two groups of metastases.

The lesions were alike in all respects and they occurred under much the same circumstances. If the curves of incidence are compared, however (Text-fig. 6), it will be seen that during the first 6 weeks, lymph node metastases were given a higher rating than those in retroperitoneal tissues. This indicates that the earlier lesions, being relatively small and not especially numerous, were more accurately localized than at other times. At the 12th week the same relations
obtained and may be attributed to the same causes. On the whole, it appears that the two curves tend to supplement each other. From point to point their direction is usually the same, but occasionally they assume opposite directions, as at the 6th, the 11th, the 12th, and the 14th weeks. Moreover, the curve of lymph node metastases gradually drops to a lower and lower level, while that of retroperitoneal metastases assumes a relatively greater importance in chronic or slowly progressive forms of disease. The significance of the two groups of lesions appears, therefore, to be essentially the same, the difference between them being largely one of degree.

A further relationship to be noted is that between the general distribution of metastatic growths, or the severity of disease, and the occurrence of this group of lesions. By comparing these two curves (Text-fig. 6), it will be seen that there is a decided lack of agreement, which suggests that the conditions which determine the occurrence of retroperitoneal metastases and metastases in general are not the same and that the conditions which favor the development of retroperitoneal metastases are of relatively greater importance during the late than during the early stages of the disease or are of greater importance in cases of relatively benign tumor growth than in those of fulminating malignancy.

Mediastinal Tissues.—Metastases in the mediastinum presented much the same difficulties of classification as retroperitoneal metastases. In some animals, the growth in the retroperitoneal tissues extended upward into the mediastinum with the formation of tumor nodules along the course of the lymphatics, in the lymph nodes and areolar tissue of the mediastinum, and in the thymus as well. In other instances, the lesions present appeared to be independent of any growth in the retroperitoneal tissues.

Exclusive of the thymic mass, mediastinal metastases occurred in twenty-nine animals, or in 25.4 per cent of those showing metastases. The lesions were usually few in number but rather large, varying from about 0.5 to 2.0 or 3.0 cm. in diameter, and were either attached to some neighboring structure or were freely movable in the loose areolar tissues.

\[1\] Part 1, Figs. 1 and 3.
The character of the growth also varied in individual animals depending upon the progress of the disease. In instances in which the disease was of a rapidly progressive or fulminating character, the growth was composed of a soft, grayish pink mass with a delicate covering of connective tissue, while in animals in which the disease pursued a more chronic course, the lesions were larger and inclined to be necrotic and were usually enclosed in a dense fibrous capsule.

As has been intimated, mediastinal metastases occurred under two entirely different sets of conditions. They were of frequent occurrence in cases of high malignancy but were even more frequent in animals with slowly progressive tumors and with comparatively few metastases elsewhere except in the retroperitoneal tissues. Represented graphically (Text-fig. 7), there were two periods during which mediastinal metastases occurred, one early and the other late, while the curve as a whole shows an upward rather than a downward trend from the 4th to the 14th weeks.

The curves for the two periods, however, are so different as to suggest that lesions represented by the two divisions may be referable to different causes. Moreover, the first part of the curve closely resembles corresponding parts of a number of the curves previously considered, while the second half is almost a duplication of the corresponding section of the curve of retroperitoneal metastases. The significance of this section is, therefore, quite obvious.
Thymus.—Metastases occurred in the thymic mass with about the same frequency as in the bones and the muscles, but it is uncertain whether any of these lesions could be regarded as having arisen within the substance of the thymus itself. Some of them were definitely located in small masses of lymphoid tissue included within the capsule of the thymus. These lesions were analogous in all respects to metastases in the superficial lymph nodes and occurred under exactly the same conditions; that is, in cases of fulminating malignancy.

In other animals, the thymus was replaced by a tumor mass which virtually filled the superior mediastinum and frequently gave rise to marked respiratory distress. These tumors were surrounded by a dense fibrous capsule and usually showed extensive necrosis but comparatively little absorption of the necrotic material; that is, there was no definite evidence of healing.

In only one instance were metastases found in a thymus that appeared to be normal. As in the case of the mediastinum, thymic metastases occurred in two types of disease, the one very acute and the other chronic, but in both instances, there was extreme atrophy of the thymus. Metastases were relatively infrequent among the animals killed within the first 10 weeks after inoculation (Text-fig. 7), while during the later stages of the disease they were almost as frequent as mediastinal metastases, and the curves of incidence for this period virtually coincide.

Perirenal Fat.—Scattered nodules of tumor growth occurred in the perirenal and lumbar fat of a considerable number of animals, and occasionally there was a striking tendency to localization of metastases in the lumbar fat in general, but especially in the perirenal tissues. The conditions under which this occurred are not entirely clear, but the feature of interest was the accumulation of large masses of tumor tissue in the form of multiple nodules which at times completely surrounded the kidneys while the kidneys themselves were unaffected. These animals showed few implantations and few metastases in other organs or tissues.

It appeared that the growth was the result of a rapid distribution of tumor cells by way of the lymphatics, and if this assumption is correct, the absence of renal involvement, or the involvement of other organs, is of especial significance.
Organs and Tissues in Which Metastases Were Infrequent.

In the preceding pages, a description has been given of metastases and the conditions under which they occurred in those organs and tissues which were most often affected. In contrast with these, there were several groups of organs which are of interest on account of the fact that they were rarely or never involved by metastatic growths. These include the gastrointestinal tract, the pancreas, the genitourinary organs, the central nervous system, the spleen, the thyroid and the parathyroids, and the pineal gland.

Gastrointestinal Tract.—There was only one instance of a clearly defined metastasis in the walls of the stomach, and even implantations on the serous surface were comparatively rare. In the one instance recorded, the growth presented the typical appearance of a peptic ulcer. The lesion was about 1 cm. in diameter with a depressed ulcer at the center surrounded by an elevated margin which was composed of tumor cells located chiefly in the submucosa.

The intestine was affected more often and was among the most frequent locations of implantation metastases. As a rule, however, the lesions in the intestine were small and were located either in the subserous or submucous tissues. In two instances, comparatively large tumors developed, and one of these led to an intussusception which caused the death of the animal.

Pancreas.—In view of the frequency of metastases in the omentum and mesentery (implantation), it is remarkable that there were only five instances in which secondary growths occurred in the pancreas or in that portion of the mesentery occupied by the pancreas.

Genitourinary Organs.—Omitting the kidneys, the genitourinary organs were rarely involved. There were comparatively few implantations on the bladder and only one metastasis of any considerable size in the bladder walls. This was situated in the trigone and formed a nodule about 8 mm. in diameter which projected from the inner surface. The prostate and seminal vesicles were affected only once by a growth which extended downward from the retroperitoneal tissues.

In like manner, metastases to the uninoculated testicle occurred in very few animals, and even extensions and implantations on the
cord and tunics of the uninoculated testicle were comparatively rare. In view of the ease with which successful inoculations were obtained in the testicle, the low incidence of metastatic growths in the uninoculated testicle is especially significant as an indication of the relative importance of so-called suitability of soil and of other factors in determining the location of secondary growths.

Central Nervous System.—There were five instances in which metastases were found in the meninges of the brain or cord. The origin of these tumors was not certain in all cases. Those in the cranial cavity appeared to have developed in the meninges, while those in the spinal canal arose either in the bones or in the surrounding tissues and entered the canal through the intervertebral foramina.

It is a striking fact that not a single case of brain or cord metastasis occurred. Here again we are confronted by the total absence of metastases from an organ which is highly susceptible to inoculation and in which primary tumors grow with the greatest ease.

Spleen.—Out of a total of 191 animals examined, there were four in which metastases occurred in the spleen as compared with thirty cases of metastasis to organs of a purely lymphoid character. The lesions were small and produced little or no local reaction, and all of them occurred in cases of fulminating malignancy. These findings are probably in accord with general experience, but they are nevertheless significant as an indication of the presence of conditions of some kind which prevent the occurrence of metastases in the spleen.

Thyroid and Parathyroids.—Metastases in the thyroid are of especial interest in comparison with the conditions presented by the suprarenals and the hypophysis as regards both the frequency of occurrence and the character and extent of the local reaction. There were thirty-seven animals with suprarenal metastases and thirteen with metastases in the hypophysis, while there were only two instances recorded in which visible metastases occurred in the external parathyroids and four in the thyroid. In two of these animals, it appeared, however, that the growth might have arisen from an attached or internal parathyroid rather than from the thyroid itself. There was one other animal with a metastasis in the trachea beneath the isthmus of the thyroid, but as far as could be determined, the thyroid itself was not involved.
These growths were comparatively small, the two largest measuring not more than 6 to 8 mm. in diameter, while the others were barely visible to the unaided eye. All of them occurred in animals with extremely malignant tumors, whereas suprarenal and hypophyseal metastases were of frequent occurrence in animals with slowly progressive tumors or with few metastases elsewhere.

The low incidence of gross metastases in the thyroid does not appear to be due to a failure of tumor cells to reach the thyroid. Numerous small collections of cells were found by microscopic examination, but in all instances, they were submerged beneath a massive lymphocytic and fibroblastic reaction which again was in striking contrast with the absence of such reactions in the suprarenals and hypophysis.

When it is recalled that gross metastases occurred in only a few animals with tumors of a most malignant character, indicative of an exceptionally low constitutional resistance, it would appear that the factors of constitutional and local resistance are of especial importance in determining the occurrence of thyroid metastases, and this probably applies to the parathyroids as well.

**DISCUSSION.**

The description which has been given of the metastases produced by this tumor will serve to convey a general conception of this feature of the disease. It will be seen that here again we are dealing with conditions which in many respects are closely analogous to those that obtain in man but with the added advantage of being able to produce an almost endless series of changes from a given tumor stock and of being able to interrupt the process at any point for the purpose of tracing the successive stages in its development. It is thus possible to link together cause and effect in a way that at best is extremely difficult from the study of human material.

Reviewing the facts presented, one is first impressed by the unusual possibilities of this tumor for the production of metastases, affecting, as they may, nearly all the organs or tissues of the body. Still, it will be seen that there is the greatest diversity in the results obtained in different animals under precisely the same experimental conditions. In the first place, a distinction may be drawn between animals
that develop metastases and those that do not, while a further
division may be made between animals that apparently recover after
metastases have developed and those in which the disease goes steadily
onward.

Moreover, among the animals of the last group, the character
and distribution of the lesions and the course of the disease vary
to such an extent in different animals that, if the two extremes
were compared, one could hardly recognize them as manifestations
of the same etiologic agent. In one group of animals, metastases
develop slowly and are confined largely to the region of the lymphatics
which lead upward from the primary tumor to the mediastinum,
while in the other, the development of metastases takes place with
incredible rapidity and few organs or tissues are spared. In the one
instance, the disease is slowly progressive, while in the other, it is
fulminating in character. Between these two extremes, one finds
various modifications and combinations of the two outstanding types
of metastatic involvement.

Finally, by a similar process of analysis, it may be seen that practi-
cally the same conditions obtain with reference to the development of
metastases in different organs of a given animal or in the homologous
organs of different animals.

At first sight, all of these differences may appear to be largely matters
of chance but upon closer examination, it will be seen that, in as far
as individual organs and tissues are concerned, there is a remarkable
consistency in their behavior and that such variations as occur in a
given organ or tissue bear a definite relation to the variations which
occur in other organs of the same animal. In brief, it will be seen
that the peculiarities displayed by individual animals are founded
upon constitutional differences which are most clearly expressed in
the behavior of the individual elements which form the more complex
animal organism.

If animal resistance is considered from this point of view, it is
obvious that the most direct method of approach to the problem of
resistance to tumor growth is by a careful investigation of conditions
which obtain in individual organs and tissues and the relations which
exist between one group of organs and another. From investigations
of this kind, it may be possible to reconstruct the whole.
This conception is applicable to the study of metastases, since they represent an expression of one form of interaction between tumor and host, and the data bearing upon the subject of metastasis will be taken up from this point of view in the next paper of the series.

SUMMARY AND CONCLUSIONS.

As a part of a general investigation of a malignant tumor of the rabbit, the phenomena of metastasis were studied in a group of 191 animals. The results of this investigation are presented from the standpoint of the relation of metastasis to animal resistance. The incidence and distribution of different classes of lesions are given and the peculiarities of the growth in different organs and tissues are described with especial reference to the time and circumstances of their occurrence and the relation of metastases in one organ or tissue to those of another.

It was found that while the picture presented by different animals varied greatly, there was a remarkable degree of uniformity in the character of the lesions in a given organ or tissue and in the circumstances under which metastases occurred in a given location. It was thus possible to establish a relationship between the distribution of metastases and the function of animal resistance.

In brief, the conclusion was reached that the peculiarities of metastatic involvement displayed by individual animals were not entirely attributable to chance distribution of tumor cells but that they were founded very largely upon constitutional differences and that the picture presented in any given instance represented an expression of the interaction between tumor and host, the character and force of which were indicated by the nature and distribution of the lesions or by the organs and tissues affected.