STUDIES BASED ON A MALIGNANT TUMOR OF THE RABBIT.

VI. VARIATIONS IN GROWTH AND MALIGNANCY OF TRANSPLANTED TUMORS.

PART 2. FACTORS INFLUENCING THE RESULTS OF SERIAL TRANSPLANTATION.

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The results of serial transplantation of this tumor were presented in the first section of this paper (1) without reference to conditions that may have influenced the outcome. In taking up this aspect of the subject, we wish to state that, as transplantation progressed, there was unmistakable evidence of the development of a more vigorous growth and of a wider distribution of cells. But the most clearly defined effect of this change was to convert a slowly progressive condition into one that progressed much more rapidly. There was no corresponding gain in the incidence and distribution of metastases, nor was there an increase in the lethal effects of the tumor.

For several generations it did appear that the malignancy of the tumor was increasing, but, as transplantation continued, the results became extremely irregular, with the suggestion of a tendency to a rhythmical occurrence of periods of increasing and diminishing malignancy which occasionally reached very high or very low levels. Still, from the sixth to the twentieth generation there was a general decrease in malignancy in spite of the fact that the growth of the tumor was becoming more and more active, and the progress of the disease was more rapid.

This series of changes was conditioned upon serial transplantation and there is no doubt that adaptation to passage played an important
part in the production of the changes that occurred, but it is equally certain that the course of events was greatly influenced by other factors. Minor variations in the results might be accounted for on the basis of irregularities in technique, but some other explanation would have to be offered for the extreme variations in malignancy and the conflicting tendencies that developed. The technique employed and the general care of the animals varied very little, and there is nothing to indicate that factors of this kind had any material influence on the results. The three most likely sources of variation in these experiments concern the material used for inoculation, the animals used, and the time at which the experiments were carried out, and we will limit ourselves to a consideration of these three factors. It should be borne in mind that we are not dealing directly with the general problem of the effect that may be produced by intentional efforts to vary these three conditions but the effect that may be produced when an effort is made to preserve uniformity of conditions; that is, with unavoidable variations in experimental conditions.

Material Used for Inoculation.

The extent to which the results of serial transplantation may have been affected by inequalities in the growth capacity of the material used for the inoculation of the different groups of animals is extremely difficult to determine. Realizing that this factor might be of considerable importance, we gave the greatest care to the selection of material so that any inequalities that may have existed were due to circumstances that were beyond our control.

Comparisons of the results obtained with material derived from different sources may be made in nine of the twenty generations included in this series of experiments as shown in Text-figs. 1 to 4, Part 1 of this paper, but in most instances the groups of animals are too small to have more than a suggestive value or there were other differences in experimental conditions which introduce an element of uncertainty into an interpretation of the results. This applies especially to the factor of time. The best opportunities for studying the influence of the material used for inoculation are afforded by the third and nineteenth generations and by the seventh and eighth generations.
Both the third and the nineteenth generations contained two groups of animals (A and B); the groups were of equal size and were inoculated with freshly prepared cell emulsions, the second groups receiving the material 16 and 13 days respectively after the first. In the third generation, the primary tumors grew more actively in Group A than in Group B. Still, the incidence of metastases was the same with a slightly wider distribution in Group B so that, in the end, there was no material difference in the results obtained in the two groups of animals.

In the nineteenth generation, the tumor was decidedly more malignant in one group of animals than in the other. It will be noted, however, that there was a closer agreement between the two subdivisions of this generation than with either the preceding or following generations (Text-fig. 4 of Part 1). In fact, the malignancy of the tumor appears to have increased to a maximum level in the first half of the nineteenth generation and then to have diminished (Text-fig. 5 of Part 1). It is uncertain, therefore, whether the results obtained in this instance are referable to the material used for inoculation or to some other factor that may have influenced the growth of the tumor at this time, independent of the material used.

This possibility is strengthened somewhat by results obtained with material that had been subjected to the supposedly deleterious influence of freezing, thawing, and grinding in the frozen state before being used for inoculation. In the seventh generation, results obtained with material treated in this manner can be compared directly with those obtained with an emulsion of the fresh material. In the eighth generation, the comparison is less satisfactory, as only a few of the control animals (Group B) were held even as long as 3 or 4 weeks. We can, however, make an indirect comparison with the first ten animals of Group A, and this gives an additional advantage of indicating the character of the results that were obtained at nearly the same time with material derived from still another source.

Fortunately, we have in this instance one generation (the seventh) in which the tumor was relatively benign, to compare with another in which it was distinctly more malignant, but this appears to have made no appreciable difference in the results in so far as the influence of the material is concerned. In the seventh generation, the results were
practically identical in the two groups of animals, showing that the
treatment to which the material had been subjected had no appreciable
effect on the character of the disease produced. The differences that
appeared in the eighth generation were again comparatively slight.
Metastases developed in three of the five animals inoculated with
material that had been subjected to freezing, thawing, and grinding
as compared with metastases in all of those (five) of the control group
that were held as long as 3 weeks, and in six of the nine animals of
Group A. The character and extent of the growth that might have
developed in the animals of Group B are uncertain, but, in Group C,
the malignancy of the tumor was fully equal to that shown by the
animals of Group A (see Text-fig. 1, Series of November 1 and 9).

The evidence supplied by an analysis of the results obtained in these
few cases is by no means conclusive. It is sufficient, however, to make
it appear very unlikely that chance inequalities of the material used
in this particular series of experiments were a factor of any considerable
importance, and it is reasonably certain that the extreme variations
in malignancy could not be accounted for on this basis.

Animals.

In considering the animals used in different experiments as a factor
that may have influenced the results of transplantation, we are not
concerned with the general problem of animal resistance or with
individual peculiarities but with recognizable class distinctions as
determined by such conditions as age or breed, both of which have
been regarded as factors of considerable importance in the trans-
plantation of tumors of other animals. On account of the irregular
distribution of animals of different ages and breeds, it will be necessary
to approach this problem from the standpoint of a comparison of
results in general rather than by a systematic analysis of results from
generation to generation.

Age.—Nearly all of the animals used in this series of experiments
were classed as adults or young adults, so that the extremes of age
played no part in the determination of the results. It will be of
advantage, however, if we begin our consideration of the age factor
by a brief reference to certain peculiarities of the growth of the tumor
in young and in old animals as illustrated by the eighth generation (see Text-fig. 2 of Part 1).

Group A of the eighth generation contained five rabbits 2 to 3 months old, seven young adults, seven adults, and three old animals that gave positive results on inoculation and were observed for a period of 3 weeks or longer. The young animals of this group showed an unusually rapid growth of primary tumors, and all of them developed secondary lesions of some kind. Still, the growth of the primary tumors was of relatively short duration, and at the conclusion of the experiment healing was complete in four of the five animals and in the other the tumor was almost entirely necrotic. The systemic distribution of lesions was also peculiar in that the growth was confined to the spermatic cord of the inoculated testicle (one animal), the deep lymphatics (one animal), and the eyes and suprarenal glands. None of these rabbits developed extensive lesions; in fact, there was only one animal with more than a single focus of secondary tumor growth, and with two exceptions all of the tumors were either healed or in the process of resolution at the time of autopsy. There was one metastasis in a suprarenal gland and one in an eye that remained active for periods of 16 and 23 weeks respectively, but neither of these animals showed living tumor tissue elsewhere; they were in excellent physical condition and in spite of the persistence of the growth in these locations it is practically certain that they would have recovered.

The peculiar response observed in this instance appears to be characteristic of the reaction of young animals, as similar results have been obtained on several occasions. It seems, therefore, that while the tumor grows exceptionally well in young animals, the resistance of such animals is of an equally high order, and that in a large proportion of cases the tumor will be of a relatively benign character.

Our experience with old animals has been very limited. There were only five rabbits in this entire series of experiments that were classed as old, and only three of these showed well developed senile changes. These are listed as "old" in Generation VIII A. The two others are No. 6 of the same generation and No. 3 of Generation X C. The results shown by these animals agree, in general, with those obtained in more recent generations.
The impression gained from a study of a small group of old animals is that the growth of primary and secondary tumors is comparatively slow but tends to be persistent, with an absence of the vigorous reaction and clear definition of results that are seen in young animals. Primary tumors may reach a very large size and there is usually some extension of the growth to other parts of the body, chiefly along the lines of the regional lymphatics, but on, the whole, the disease rarely assumes highly malignant proportions. Still, both primary and secondary tumors remain active for long periods of time, leaving the end-result in doubt.

It would appear, therefore, that the situation presented by old animals is, in general, the reverse of that presented by young animals. The two agree in that neither is apt to develop a highly malignant type of disease, but in the one case this appears to be attributable to conditions that are unfavorable to the growth of the tumor rather than to an active opposition on the part of the host, while in the other an extremely active growth is more than counterbalanced by a prompt and vigorous reaction on the part of the animal.

The growth of the tumor in young adult and adult rabbits suggests a gradual transition with increasing age from the condition presented by the young animal to that of the old. Considering the four age groups, it may be said that both the energy of cell growth and the expression of resistance on the part of the animal diminished with increasing age, but with the beginning of this change resistance decreased for a time more rapidly than growth with the production of a reversal of the relationships obtaining at the two extremes of life. This change in relationships affected young adult and adult animals in such a way as to increase the malignancy of the tumor in both classes of animals, but the conditions presented differed somewhat in the two cases.

An analysis of the results obtained shows that, on the whole, the growth of the tumor was slightly more active in young adult than in adult animals, that the incidence of metastases was slightly higher (61 as compared with 50 per cent), and that there was a tendency to a more extensive distribution of lesions and an earlier termination of the disease. These differences are well illustrated by the results shown in Generation VIII A.
This general statement of the influence of the factor of age must be applied with caution. There is no doubt that the results of the experiments under consideration were influenced to some extent by this factor, but it is well to note that in some instances the adult animals of a group gave better results than the young adults, and that some of the lowest values were obtained in groups of animals composed chiefly of young adults.

Breed.—The factor of breed has also played some part in the transplantation of this tumor, but the extent to which it has influenced the results obtained in different series of experiments is uncertain.

The spontaneous tumor developed in an albino rabbit, but, contrary to what might have been expected, it was soon found that animals of this type were not as well adapted to the growth of the tumor as some others. This is clearly indicated by an analysis of the results obtained in the second and third generations. In this case, the gray, the brown, the black, and the Flemish rabbits showed a much higher incidence and a wider distribution of metastases than the albinos, but the results obtained with different types of animals varied somewhat from generation to generation and on several occasions albino rabbits gave results comparable with those of any other type of animal (Generations VI and VIII A). Nevertheless, if we consider the entire series of experiments, it will be found that the results were fairly consistent and that there were distinct differences in the susceptibility of different classes of animals. This can be shown in a very simple way by comparing the liability to metastasis which is not seriously affected by irregularities in the observation period.

If all the animals in which the results of inoculation were negative or doubtful and those that were observed less than 3 weeks are omitted, the liability to metastasis among animals of a type that was used in any considerable numbers was as follows:

<table>
<thead>
<tr>
<th>Breed</th>
<th>per cent</th>
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<tbody>
<tr>
<td>Black</td>
<td>80</td>
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<tr>
<td>Gray</td>
<td>73</td>
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<tr>
<td>Flemish</td>
<td>60</td>
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<tr>
<td>Brown</td>
<td>51</td>
</tr>
<tr>
<td>New Zealand</td>
<td>50</td>
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<td>Albino</td>
<td>50</td>
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The average for this entire group of animals (87 positive and 72 negative) was 54.7 per cent, while an additional group of 21 rabbits of miscellaneous types gave an incidence of metastases amounting to 66.6 per cent, or a combined figure of 56 per cent for the entire group of 180 rabbits.

We are not disposed to make any broad generalizations on the basis of the results obtained in this small series of animals, but it is quite evident that the liability to metastasis tends to vary with the color of the animal and that, in general, resistance to the growth of the tumor tends to increase from black to white so that, all other conditions being equal, the factor of color or breed,—of which color is one expression,—might be of very great importance.

There is no doubt that this factor had an appreciable effect on the results of the experiments under consideration; it also appears that, under the conditions that prevailed, the effect was in the main to equalize any differences that might have been produced by other factors. It so happened that in no instance was there an especially favorable or unfavorable grouping of animals and that the great majority of the animals used were of types that gave results close to the average. Animals that might have made a greater difference were not only used in small numbers but they were widely scattered. In addition, it will be seen that the extreme limits of variation in the results obtained for different generations (70 per cent) far exceeded the maximum variation (30 per cent) that might be attributed to any color or breed, and that both the maximum and minimum figures for the incidence of metastases were well outside of the figures for various breeds of animals.

It would appear, therefore, that while the chance grouping of animals must have affected the results of serial transplantation to some extent, any effect that might have been produced in this way would not provide a satisfactory basis for an explanation of the periodic occurrence of extremely malignant tumors or tumors of a benign character, nor would it be possible, on this basis, to account for the decided decrease in malignancy that occurred during the later generations.

Time.

We come now to a consideration of the time at which the experiments were carried out as a factor that may have had some influence
on the behavior of the tumor. In order to facilitate an analysis of the results from this point of view, we have plotted two sets of curves (Text-figs. 1 and 2) showing the relative incidence of secondary tumors in successive groups of animals, and the percentage distribution of the lesions, calculated first on the total number of animals in the group (relative distribution) and then on the basis of those that were positive (actual distribution). Both sets of curves follow the time order of inoculation, irrespective of generations. In Text-fig. 1 the results are recorded for each series of animals, while in Text-fig. 2 we have combined these into monthly averages.

In analyzing these curves, it is well to bear in mind that the incidence curves indicate merely liability to metastases, while the curves of distribution give a more accurate conception of the severity of the disease. In general, the two sets of curves run parallel; but at transition points there is a tendency for a change in the direction of one curve to precede that in the others.

If we consider the incidence of metastases, as shown in Text-fig. 1, it will be seen that the first series of animals, inoculated on March 10, 1921, gave a slightly higher value than did any of those immediately following. Still, the curve remained relatively high through April, May, and June. There was a slight upward tendency in July (record not included in text-figures), but the first decided increase came in the group of animals inoculated late in August, and while the September series also showed a high incidence of metastases the curve shows a progressive reduction for September and October.

The October 18 series (Generation VII, Text-fig. 2 of Part 1) is especially interesting in that it was composed of two groups of animals, one of which was inoculated with material that had been subjected to freezing, thawing, and grinding before inoculation. The incubation period in this group of animals was about 2 weeks longer than that of the other group, so that from the standpoint of the growing time of the tumor this group stands about midway between the regular series of October 18 and November 1. It is interesting to note, therefore, that the results (distribution as well as incidence) show a similar relationship. From October to November 9 there was a progressive increase in the incidence of metastases followed by a marked reduction, which, we may say, persisted through the series of January 23,
Fig. 1. Incidence and distribution of secondary tumors with relation to the time of inoculation. No data available for periods covered by broken lines. In this figure, F.T. indicates freezing and thawing.
TEXT-FIG. 2. Incidence and distribution of secondary tumors considered on the basis of monthly averages in the order of inoculation. No data available for periods covered by broken lines.
1922, as the only slight elevation in the curve probably has no significance.

Here again attention may be called to the fact that different values were obtained for the two groups of animals inoculated on November 9. In this instance, the delayed development of primary tumors in the animals inoculated with material that had been frozen, thawed, etc., was associated with a lower incidence of metastases, which places this group of animals in a position between that of the regular series of November 9 and that of November 22. The distribution curve in this case has little or no significance, as only one animal of the control series was held longer than 4 weeks.

No animals were inoculated in February, but, beginning in March, there was another period of increase which reached the maximum of 100 per cent on April 26. By June 7, the tendency to the occurrence of metastases was again extremely low. There was, however, a moderate but fairly sustained increase extending over the period from June 16 to August 15 and possibly on into late September.

In this connection, we should call attention to the fact that the series of September 25 and October 13 were the ones that gave such a high percentage of negative results on inoculation and that during July, August, and October many lesions showed a tendency to spontaneous resolution. This appears to be significant. At any rate, the two groups of animals inoculated during October showed a diminishing incidence of metastases which was followed by an even greater increase for November than was recorded for the same month of the preceding year. From the high point reached on November 10, the curve again dropped as it did in 1921.

It is not necessary to analyze the curves showing the distribution of metastases in the same way that we have analyzed that of incidence. It is obvious that, in the main, the three curves agree. There are, however, a few apparent discrepancies which will be referred to presently.

If we condense the results recorded in Text-fig. 1, so as to base our calculations on larger groups of animals, using the month as the unit of time and including in a given group all of the animals inoculated during that month, the main effect is to smooth out minor irregularities in the curves and to emphasize the occurrence of two periods of maximum
malignancy—one in the spring and the other in the fall—as is clearly shown by that portion of the curve in Text-fig. 2 which gives the results for the year 1922.

It will be noted that this effect is not so apparent during 1921; this, in itself, is significant. Transplantation began in March and relatively high values were obtained in the first three series of animals that were inoculated (March, April, and May). During this time, there was no material change in the proportion of animals that developed metastases. In fact, the incidence of metastases diminished, but in spite of this the severity of the disease increased, as indicated by the progressive increase in the distribution of secondary tumors. In June, there was a reversal of the direction of the two sets of curves (incidence and distribution), and this was followed by a further readjustment in August which brought out the first concerted movement of the three curves. In this instance, the distribution curves remained at a high level during September, while the incidence curve had already assumed a downward direction, but, from this point onward, the changes became regular and of a clearly defined character, and the conditions shown for 1922 were repeated with slight variations in 1923.

DISCUSSION AND CONCLUSIONS.

The interpretation of the facts that have been brought out with regard to the operation of various factors that may have influenced the growth and malignancy of this tumor is comparatively simple. It is at once apparent that, in its final analysis, the problem that confronts us is that of the interaction between host and pathogenic agent. We have to consider, on the one hand, the effects of passage on the energy of cell growth, and the viability of the cells at the time of inoculation, and, on the other, the suitability of various classes of animals for the growth of these cells and the capacity of the animal to oppose their growth or to dispose of cells that are introduced, as these several conditions may be affected by such factors as age or breed and by the existing state of the animal organism.

The changes that occurred in the course of transplantation were conditioned on passage, and there is no doubt that the energy of cell growth was greatly increased by adaptation to passage. The indications are that this change was especially rapid during the first five or
six generations, but there is evidence to show that as the rate of growth and invasiveness of the tumor increased, the incitement to reaction was also increased so that a state of virtual equilibrium between these forces was soon established. This state of equilibrium might have been permanent had it been possible to maintain all the factors in the equation at a constant level, but as transplantation continued the equilibrium was disturbed by unavoidable variations in the experimental conditions so that the subsequent course of events became extremely irregular, depending upon whether the conditions that obtained at any given time were more favorable to the growth of the tumor or to the maintenance of an effective defense on the part of the animal.

It is apparent, therefore, as has been pointed out, that there were two conflicting tendencies in operation. One of these clearly was a tendency to an increase in the energy of cell growth which has persisted in an effective form even to the present time; but the circumstances that favored opposition to the growth of the tumor are more difficult to define. We have considered the possibilities of variations in technique, chance inequalities of the material and of the animals used in different series of experiments, and the possible influence of the factors of time and the serial order of inoculation.

The results obtained cannot be explained as a manifestation of a simple equalization reaction in which each increase in the growth activity of the tumor called forth a corresponding increase in animal resistance. It is certain, however, that a reaction of this kind formed the basis for the changes that occurred. Irregularities in technique and chance variation in material and animals will account for some variation in results, but as one follows the succession of changes that occurred, he can hardly fail to be impressed by the suggestion of the operation of some obscure condition which tended to regulate the growth and malignancy of the tumor irrespective of the influence of other factors. This is most evident when we consider the results in serial order with respect to the time of inoculation. When viewed from this standpoint the entire series of changes assumes the form of an orderly succession of periods of increasing and diminishing malignancy with a distinct tendency to a seasonal distribution. This peculiar coincidence of effects is strongly suggestive of the action of some influence commonly referred to as meteorological.
It would appear, therefore, that the two main factors concerned in determining the results of transplantation were adaptation to passage and unavoidable variations in the conditions of season and weather that prevailed during the time these experiments were being carried out. It is possible to account for all of the variations in growth and malignancy that have been encountered in the course of routine transplantation as manifestations of the combined action of passage and meteorological conditions,—the one affecting the energy of cell growth and the other affecting animal economy. This aspect of the subject will be presented in detail in a subsequent paper.

SUMMARY.

The results of transplantation, as recorded in the first section of the paper, are analyzed from the standpoint of the influence of the factors of serial passage, the material used for inoculation, the age and breed of the animals, and the time or season at which the experiments were carried out.

The material used for the inoculation of different groups of animals appeared to have comparatively little effect on the ultimate results.

The character of the animals used was found to be a factor of more importance in that the tumor displayed definite peculiarities of growth and malignancy referable to age and to breed or color markings. Still, it seemed unlikely that any of these factors would account for the variations in growth and malignancy that had been observed.

After considering the various possibilities presented, it appeared that the principal factors concerned in determining the results of transplantation were adaptation to passage and variations in the conditions of season and weather that prevailed during the time the experiments were being carried out—the one affecting the energy of cell growth and the other affecting animal economy.

BIBLIOGRAPHY.