STUDIES BASED ON A MALIGNANT TUMOR OF THE RABBIT.

VI. VARIATIONS IN GROWTH AND MALIGNANCY OF TRANSPLANTED TUMORS.

PART 1. RESULTS OF TRANSPLANTATION FOR THE FIRST TWENTY GENERATIONS.

By WADE H. BROWN, M.D., LOUISE PEARCE, M.D., AND CHESTER M. VAN ALLEN, M.D.

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

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In previous papers of this series (1–5) we have reported the results of our study of a spontaneous tumor of the rabbit and of various methods of transplantation during the earlier generations, in so far as they pertain to the essential features of the growth of primary tumors, the course of the disease, and the occurrence and distribution of metastases.

It has been pointed out that the results of transplantation varied greatly, depending on the method employed, and that, while with some methods it was difficult to obtain even a primary growth, other methods gave an almost uniform series of takes, and that intratesticular inoculation produced a growth that was capable of metastasizing to almost all parts of the body. It was noted, however, that the tumors of individual animals of a given series varied from a small local growth of benign character to tumors that were sufficiently malignant to cause the death of the animal within a few weeks after inoculation. Reference was also made to the fact that similar variations in malignancy were shown by different series of animals and that in addition to such factors as the method of inoculation and inequalities in the resistance of individual animals, the malignancy of the tumor appeared to be influenced to some extent by such factors as continued passage, the age and the breed of the animal, the character of the material used, and the time at which the inoculations were made (season of the year). In other words, it appeared that the growth and malignancy of the
tumor might be affected intentionally or accidentally by numerous conditions and that before any attempt could be made to plan experiments, or to evaluate the results of experimental procedures whose object was to increase or diminish the malignancy of the tumor, it would be necessary to study the effects of those influences that were most likely to play a part in experiments of this kind.

A simple method of approaching this problem was afforded by an analysis of the results obtained in the course of routine transplantation, and for this purpose we have used material available from the first twenty generations of intratesticular transplants. The present paper serves a double purpose, therefore, in that it contains a report of the results of transplantation, as well as a discussion of unintentional and to some extent uncontrollable sources of variation in the growth and malignancy of the tumor. Part 1 of this paper will be devoted to a systematic presentation of the results of transplantation for the first twenty generations.¹ These results will be discussed in the second section of the paper from the standpoint of the influence of such factors as passage, the material used for inoculation, the age and the breed of the animal, and the time, or season of the year, at which the inoculations were made. Preliminary reports dealing with certain aspects of the subject of the growth and malignancy of the tumor have already appeared (6-10).

EXPERIMENTAL.

This report is based on the results obtained in 237 rabbits of the first twenty generations inoculated between March 10, 1921, and December 13, 1922, as recorded briefly in Text-figs. 1 to 4.

Experimental Conditions.—With few exceptions, the animals received a single unilateral testicular inoculation of a cell emulsion prepared by methods described in previous papers. The dose used varied from 0.2 to 0.3 cc. The rabbits in Group B of Generation XVI were inoculated in both testicles, with an interval of 10 days between the two inoculations. There were, also, two groups of animals that were inoculated with an emulsion that had been subjected to freezing and thawing, and grinding in the frozen state (Nos. VII B and VIII C).

Other irregularities in the experimental conditions that should be noted concern the question of castration before or after inoculation and the length of the period of observation. In a few instances, the testicle containing the tumor

¹ The tumor is now (May, 1924) in its thirty-third generation.
was removed soon after inoculation, but the general results appear to have been disturbed very little by such conditions. On the other hand, the removal of one testicle a week before the inoculation of Rabbits 11 to 15 inclusive of Generation VIII was associated with the occurrence of two cases of extreme malignancy.

During the first ten generations the observation period was very irregular. Some animals were held as long as 4 to 7 months, while others were killed as early as 2 to 3 weeks after inoculation. This was due, in part, to the relatively slow progress of the disease during the early generations and, in part, to an attempt to follow the course of events by postmortem examinations. As the work progressed these conditions were equalized to some extent and an effort was made to fix the period of observation according to the progress of the disease so as to give a time value to the results.

Method of Charting Results.—The experimental conditions and the results obtained in each series of animals are recorded graphically in Text-figs. 1 to 4 in the order of generations, which does not conform to the time order of inoculation. Three major divisions have been made on these charts. The first section (left) gives experimental data including the generation of the transfer, together with subdivisions of the generation when different emulsions were used, the date of the inoculation, and the age group and type of the animal. The second section of the charts contains the results of clinical observations and is divided into two parts. The first of these gives data pertaining to the primary tumor, while the second contains observations on the occurrence of metastases that could be recognized clinically.

The data in the first two sections of the charts are not as complete as might be desired owing to the fact that it was not possible to maintain detailed records of the clinical course of events in all cases, and in some instances in which the available data were not sufficiently definite it seemed best to omit them altogether.

The greater part of the data recorded in Text-figs. 1 to 4 refers to results of postmortem examinations. In the first column of this section we have noted any decided abnormality in the physical condition of the animal at the time of death or at the time the animal was killed, in so far as such information was available. The next two columns indicate what eventually happened to each animal. It will be noted that comparatively few deaths are recorded, due to the fact that in order to avoid loss from postmortem decomposition, animals were usually killed when it became apparent that death was imminent. But, from the data given, the probable fatalities and the approximate length of survival can be estimated in nearly all cases. The considerations that governed the time of killing other animals have already been mentioned.

The remaining sections of Text-figs. 1 to 4 record the state or fate of the primary tumor, or of a recurrent growth in instances in which the primary tumor had been excised, as regards size, the relative proportions of living and necrotic tissue, the degree of encapsulation, and the progress of healing, together with
Explanation of Text-Figs. 1 to 4.

<table>
<thead>
<tr>
<th>Rate of growth</th>
<th>Encapsulation</th>
</tr>
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<tbody>
<tr>
<td>Slow</td>
<td>Thin</td>
</tr>
<tr>
<td>Moderate</td>
<td>Slightly thick</td>
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<tr>
<td>Active</td>
<td>Moderately thick</td>
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<tr>
<td>Rapid</td>
<td>Heavy</td>
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<table>
<thead>
<tr>
<th>I</th>
<th>II</th>
<th>III</th>
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<tbody>
<tr>
<td>Active</td>
<td>Inactive or regressing</td>
<td>Healing</td>
</tr>
</tbody>
</table>

The system of symbols used in Text-figs. 1 to 4 to describe primary and secondary tumors.

**Age Groups.**—I indicates young; II, young adults; III, adults; IV, old.

**Types.**—A. indicates albino; Bd., brindle; Bg., Belgian; Bl., black; Br., brown; Bt., Dutch belt; F., Flemish; Fn., fawn; G., gray; Hm., Himalaya; M., Maltese; N.Z., New Zealand; T., tan; W., white (in color combination).

**Terminal Condition.**—C. indicates cachectic; M., moribund; P., paralysis; Pr., poor condition; Th., thin; W., weak.

**Disposition.**—D. indicates dead; Dis., discarded; K., killed; R., reinoculated.

Text-figs. 1 to 4 show the results of tumor growth for the first twenty generations.
data as to the number, kind, and distribution of secondary tumor growths divided on the basis of extensions or implantations and metastases to distant parts of the body.

The symbols and abbreviations used in these charts will require some explanation. In the first place, the animals were divided into four age groups on the basis of development since, in most instances, the age of the animal was unknown. These groups may be designated as young, young adult, adult, and old, which are indicated by the use of the Roman numerals I, II, III, and IV respectively. Group I includes animals that had not reached sexual maturity at the time of inoculation; Group II, animals that were sexually mature but had not attained full development; Group III, animals that were fully developed and in the prime of life; while Group IV includes animals in which senile changes were apparent.

A second classification of animals was made on the basis of breed or color markings. As far as possible, the breed or predominant cross is indicated on the chart by a system of abbreviations (see Explanation of Text-figs. 1 to 4). In other instances, the type of the animal is indicated by color markings or by a combination of the two designations. Thus, the abbreviation Bg. indicates a Belgian hare or an obvious cross of this breed as distinguished from common brown or gray-brown rabbits which are designated on the chart by the abbreviations Br. or G. Br. respectively. In the latter instance, it will be noted that the abbreviation used is a combination of the abbreviations for gray and for brown and will serve as an example of the system of combination that is used throughout.

The rate of growth of primary tumors is graded by the use of appropriate symbols as slow, moderate, active, or very active. As used here, these terms refer to conditions that prevailed during the first 3 or 4 weeks after the tumor became palpable. Rate of growth is not to be confused with the maximum size attained by the tumor nor even with the time within which the maximum size was attained, although in most instances, there is a close agreement between these two conditions. As a rule, estimations of the rate of growth were based on clinical observations rather than on actual measurements, and allowance must be made for an unavoidable change in standards of evaluation as the rate of growth increased with successive generations. This applies chiefly to an attempt to compare rate of growth at the beginning and towards the end of this series of experiments. The difference was greater than the symbols would indicate.

The size and state of both primary and secondary tumors as well as the degree of involvement of particular organs or tissues are represented by a system of circles. These symbols are divisible into three major groups, depending upon whether the tumor was in an active condition and composed largely or entirely of living tissue; whether it was inactive or regressing; or was largely necrotic and in process of resolution and absorption, or healing. Tumors that had completely healed are designated by the letter H.
Active tumors are represented by circles with a solid black center (Group I). These are the basic symbols of the system and they are subdivided into four sizes which represent the relative size of individual tumor nodules, the size increasing from 1 to 4. A further subdivision is made on the basis of the relative number of nodules or the extent of involvement of a particular organ or tissue which is indicated by the size or portion of the circle that is black, the number increasing from A to C. Thus, the symbols marked 4A, 4B, and 4C all indicate the presence of large nodules or of nodules that were large in proportion to the size of the organ, but in the first instance there would be only a few nodules or only a small portion of an organ would be occupied by tumor growth while in the last instance there would be a large number of isolated nodules or a large part of the organ would be taken up by the tumor. In like manner, the symbols 1, 2, 3, and 4 of any one group A, B, or C indicate an equal number of nodules or an equal degree of involvement, the only difference being the size of the nodules present. Thus, the extent of the growth indicated by 1C and 4C is the same, but in one case the growth would be composed of numerous small nodules while in the other there might be a single large mass filling the greater part of an organ, or a number of large nodules.

Two exceptions to this general rule are to be noted. In the case of primary tumors, the idea of numbers could be dispensed with, while it was necessary to make a greater allowance for possible size variations with relation to the size of the testicle so that the entire series of symbols included in Group I was employed to express this relationship. Here the increase in size runs from A to C and from 1 to 4 in each of these classes so that the smallest recognizable growth would be represented by the symbol marked A1 and the largest by C4 while any B tumor would be larger than any A tumor and C larger than B.

Another difficulty was encountered in the classification of metastases in such organs or tissues as the skin, subcutaneous tissues, muscles, and bones which were never involved to an extent equal to that of other organs. In order to meet this situation the highest values were assigned to the largest or most extensive growths that were encountered, while other lesions were graded with relation to these.

Tumors that were inactive or regressing are represented by the introduction of a white center into the same system of circles, while those that are in process of healing have the black center replaced by a second circle within the first or are represented by a single circle in the case of tumors corresponding to those of the C class in Group I.

An addition to this system is made in the case of autopsy records of primary or recurrent tumors. Here the proportions of living and necrotic tissue in large tumors (C4 tumors) that still retained some growth activity are shown by the portions of the circle that are represented in black and white respectively.

The degree of encapsulation of primary tumors is indicated by a series of black bars of varying widths which correspond with the relative thickness and density of the capsule.
The abbreviations used with reference to the terminal condition of animals have a special significance. In the first place, no attempt was made to record the condition of an animal unless it was obviously and decidedly abnormal. If the animal was extremely thin or emaciated but apparently strong and active it was recorded as thin (Th.); if, on the other hand, the animal was well nourished but extremely weak it was recorded Wk. When both conditions existed and were associated with marked pallor of the mucous membranes the animal was designated as cachectic (C.). The abbreviation Pr. (poor) was used to indicate a similar condition but less clearly defined as to weakness and pallor of mucous membranes; when there was complete prostration, and death appeared to be impending, the animal was classed as moribund (M.). Other signs and abbreviations require very little explanation. The question mark (?) wherever used indicates a possible or doubtful lesion or condition. The plus-minus sign (±) has a similar significance but is used only to indicate uncertainty as to the results of an inoculation; when used separately these signs (+ or −) have their usual significance.

Comparison of clinical and postmortem data will reveal some apparent discrepancies, especially so in the case of secondary growths in the cord of the inoculated testicle. These differences are due to the fact that the two evaluations were made independent of each other and that the size or extent of involvement, as determined by clinical examination, appeared at times to be greater than that found on postmortem examination.

For convenient reference, we have inserted a brief record of the primary and secondary growth that occurred in the animal with the spontaneous tumor at the top of Text-fig. 1. In interpreting this record, it is to be noted that the primary tumor was excised 18 days after it was first seen, that there was a recurrence, and that the excision of this growth was followed by a second recurrence.

Results of Continued Transplantation.

In presenting the results of continued transplantation, no attempt will be made to consider each of the twenty generations in detail. The records that have been provided make it possible for anyone to undertake comparisons of this kind. It will serve the purposes of this report equally well if we limit ourselves to a general consideration of those features of the results that indicate most clearly what happened during this period of transplantation, but the discussion will be based largely on data recorded in Text-figs. 1 to 4 and for convenience will follow the general order observed in these charts.

Morphology of the Tumor.—Frequent examinations of both primary and secondary tumors have failed, thus far, to show any differences in the gross or microscopic appearance of the tumor that cannot
be accounted for on the basis of varying conditions of cellular activity. The general morphology of the cells and the structure of the growth appear to have been unaffected by transplantation.

Percentage of Takes.—With one exception, failure to obtain a primary growth by intratesticular inoculation has occurred so rarely as to be of very little significance. It will be noted that in the majority of instances in which a negative or an uncertain result is recorded, the animal lived a very short time after inoculation or else there was some complicating condition, such as a secondary infection, to obscure the result. A good illustration of this point is supplied by the second animal in Generation VIII B. In this instance, there was apparently no growth in the testicle and the animal was regarded as negative until a fungating tumor mass was detected in the lower jaw nearly 4 months after inoculation.

The important point to be noted with reference to the percentage of takes is that the tumor grew in three of the first four rabbits inoculated from metastatic nodules in the liver of the original animal, and that thereafter, with the exception of one brief period in the fall of 1922 (Generations XVI and XVII), there was only an occasional instance in which a definite growth was not obtained. In the seventeenth generation the tumor failed to grow in four out of twelve rabbits. A second series of twelve rabbits was then inoculated with material taken from an earlier generation, constituting Series B of Generation XVI, with less than 50 per cent positive results (five out of twelve). Still, a second inoculation (left testicle) 10 days later gave positive results in all but one of the ten animals that were reinoculated.

This experience might be attributed to the use of poor or damaged material or to some error in technique, were it not for the fact that there is no other evidence to show that such results might be produced in this way, and that the high incidence of negative results harmonizes better with what is known concerning the general behavior of the tumor at this particular time.

On the other hand, the high percentage of negative results obtained in the sixteenth and seventeenth generations cannot be regarded as an effect of transplantation, since there has been no repetition of this condition. In general, the percentage of takes, even at the present
time, is essentially the same as during the early generations. Still, there has been a definite increase in the proportion of successful inoculations by the use of less favorable methods than the intratesticular route.

**Incubation Period.**—During the first two or three generations it required from 9 to 14 days to produce a palpable tumor in the testicle, but with successive transfers there was a gradual reduction of this time until the incubation period rarely exceeded 5 to 7 days and the initiation of the growth became merged with the reaction to inoculation so that no exact time could be fixed. Still, the incubation period has varied with different series and is by no means constant.

**Growth and Persistence of Primary Tumors.**—The most significant features of the primary tumor are the rate and character of the growth, the size attained, the degree of encapsulation, and the ultimate fate of the tumor. These conditions can best be considered in relation to one another.

On the whole, it can be said that none of these conditions exhibited a steadily progressive tendency in any given direction. During the second generation and the first part of the third, primary tumors attained a high rate of growth. But the rate of growth varied first in one direction and then in another, until it reached a very low level in the thirteenth generation and remained comparatively low until the nineteenth generation, when there was a decided change in the direction of an increased rate of growth.

In like manner, there were decided variations in the mode of growth of primary tumors and the size attained by the average tumor within a given period of time or the proportion of tumors that reached a large size within the first few weeks after inoculation. At the beginning of transplantation the tumor tended to form irregular nodular masses which required upwards of 7 to 10 weeks to attain their maximum size (Generations I and II). Later, the tumor grew more diffusely and reached a large size within 4 to 6 weeks, and eventually within 3 to 4 weeks. The tendency to a more vigorous growth was apparent as early as the second and third generations but was not maintained at a constant level. It will be noted, however, that toward the end of this series of inoculations (Generations XIX and XX), all tumors were of comparatively large size and attained their maximum growth.
within a period of 2 to 4 weeks, which is in striking contrast with the results obtained in the first two generations.

If we consider the state of the primary tumor at the time of autopsy, we will find additional evidence of the occurrence of changes in the growth activity of tumor cells. In this instance, we have to consider the survival of tumor cells and the resolution and healing of primary tumors. Among the animals of the earlier generations there was a greater tendency to the persistence of primary tumors with a larger proportion of living tissue than among those held for corresponding periods of time in later generations. This may be seen by comparing the results given for Generations II and VI with those for the last three generations. There was no instance of complete healing of a primary tumor in the first or second generation, although eleven of the thirteen rabbits were held for a minimum of 8 weeks, while five of them were kept under observation for upwards of 6 to 7 months. Two animals showed tumors in process of healing at the expiration of 24 weeks but one of these never developed a large tumor, while the other had been castrated and the growth present represented a recurrence (Generation II, Nos. 1 and 6).

From the third generation onward, complete healing of primary tumors occurred in a variable proportion of animals of nearly all the generations in which the average period of observation exceeded 4 weeks and not infrequently reached as high an incidence as 40 to 50 per cent among animals that were held as long as 12 to 16 weeks. The time of healing is not given in the charts and the period of observation was so irregular that an exact comparison of the results for different generations cannot be made. Nevertheless, it may be said that while the proportion of tumors that healed within a specified period of time varied greatly, there was a decided tendency for resolution and healing to occur earlier as transplantation was continued. Similar conditions obtained with reference to encapsulation.

In so far as the growth and persistence of the primary tumor are concerned, it would appear, therefore, that continued transplantation produced two effects: an increased rate of growth and an increased extent of growth which were in turn counterbalanced by a shortening of the period of active growth and an increase in the rate and extent of the local reaction on the part of the animal, all of which had the effect
of hastening the course of events following inoculation whether they tended towards recovery or the extension of the growth to other parts of the body. This will be brought out more clearly by a consideration of the effects of transplantation on the occurrence and distribution of secondary tumors.

Incidence and Percentage Distribution of Secondary Tumors.—The phenomena of metastasis or of secondary tumor growth give a far better conception of the behavior of this neoplasm than can be gained from a study of the primary tumors. In order to simplify analysis of this phase of the subject the data contained in Text-figs. 1 to 4 have been reduced to a series of curves (Text-fig. 5) which show the proportion of animals of each generation in which secondary tumors were observed clinically or at autopsy and the distribution of these lesions. These curves will be referred to as the curves of incidence and distribution. The distribution curves, as here given, are based on the number of organs or tissues in which tumors were found as compared with a theoretical possible distribution which was determined by the actual location of secondary tumors during the first twenty generations. The first of these curves (relative distribution) gives values calculated on the basis of all animals of the group, while the second curve (actual distribution) includes only those in which secondary tumors were present.

In making the calculations for the curves of incidence and distribution we eliminated all animals that were held less than 2 weeks and all of those that were missing or lost. Healed tumors were taken into account but doubtful or uncertain lesions were regarded as negative.

It is to be noted that the curve of incidence indicates merely the liability to the occurrence of secondary growths among the animals of a given group, while the distribution curves give some conception of the actual prevalence of metastases and the extent of the growth in these animals and is, therefore, a more accurate indicator of the malignancy of the tumor. Still, the fact should be emphasized that there are conditions under which neither of these curves furnishes an accurate index of malignancy. This is especially true when conditions are such as to favor an initial development of metastases and the subsequent occurrence of resolution and healing. Under these circum-
stances, high values might be given by both curves when in reality none of the animals was likely to die. This is clearly illustrated by Generation XIV.

By reference to Text-figs. 1 to 4, it will be seen that secondary tumors occurred in a large proportion of animals even at the beginning of transplantation. In fact, of the four rabbits comprising the first generation, a growth was obtained in three, and all of them developed secondary tumors. It will be noted, however, that none of these animals developed very extensive metastases even though they were held for periods of 14 and 26 weeks. From this point onward, the course of events was very irregular and can be followed best by combined use of Text-figs. 1 to 4 and Text-fig. 5. For a time, it appeared that metastases were becoming more frequent and that the disease was assuming more malignant proportions. There was a slight reduction in the number of animals that showed metastases but this was compensated for by an increased distribution.

The high point in this direction was reached with the fifth and sixth generations, but, in the seventh, the incidence and distribution of metastases dropped unexpectedly to a much lower level. With the next generation there was some recovery, but again the incidence and distribution of metastases diminished, reaching a point lower than any previously recorded and did not return to a condition approximating that of the fifth and sixth generations until the twelfth generation was reached.

The twelfth generation was represented by only three rabbits but all of them succumbed to a very malignant type of disease and the pictures presented by them were so nearly alike that there could be little or no doubt as to the true malignancy of the tumor at this time. Still, this accession of malignancy was only temporary. From the thirteenth to the eighteenth generation inclusive, the incidence figures were lower than at any time except the ninth, tenth, and eleventh generations. The distribution figures were also much lower than they had been but were less affected on the whole than those for animal incidence.

The nineteenth generation brought a decided increase in the number of animals showing metastases and there was an appreciable increase in the distribution of the lesions, but the last generation of the
series showed a reduction in malignancy to a point lower than any that had been reached since the beginning of transplantation. Of the five animals comprising the twentieth generation, three developed metastases, but the growth was confined to the eyes of one animal and to the left suprarenal of the others. As was pointed out in a previous paper (5), the suprarenals, the hypophysis, and the eyes are the most poorly defended organs of the body, and metastases may develop in these organs with no probability of being able to extend to other parts of the body.

This brings us to a consideration of the character and location of secondary growths as influenced by transplantation.

Character and Location of Secondary Tumors.—By reference to Text-figs. 1 to 4, it will be seen that the character and location of secondary tumors changed fully as much during the first twenty generations as the incidence and percentage distribution of the lesions. At the beginning of transplantation, and for several generations thereafter, a large proportion of the secondary tumors belonged to the class designated as extensions and implantations. The quantitative relationships that obtained between lesions of this class and metastases to distant organs are shown in Text-fig. 6.

This figure shows the relative distribution of secondary tumors of all classes, which is indicated by the total height of the columns, and, in addition, the proportion of the lesions classed as extensions and implantations and metastases to distant organs without regard to the possible distribution in either case. It will be seen that at the beginning of transplantation, extensions and implantations were more frequent than metastases to distant organs. These relationships changed a number of times, but, during the first seven generations, the tendency was to increase the original disproportion between these two classes of lesions so that the most prominent feature of the disease during this period was the occurrence of implantations and the extension of the growth upward along the lines of the lymphatics from the region of the primary tumor. Gradually, this condition changed so that in later generations a greater proportion of the lesions occurred in organs or tissues far removed from the primary growth.

This change in the character and location of secondary tumors is clearly shown by a comparison of the conditions that prevailed dur-
ing the two periods of maximum malignancy in the fifth and sixth generations and in the twelfth generation. In the first instance, the great increase in secondary tumors was due largely to extensions and implantations, while in the twelfth generation it was attributable to a more widespread distribution of lesions. Only once was there a return to the condition that existed in the seventh generation. This was in the sixteenth generation when the malignancy of the tumor had reached an extremely low level.

**Text-Fig. 6.** Relative distribution of secondary tumors (twenty generations), showing the varying relations between implantations and extensions and metastases to distant organs.

A similar change in the behavior of the tumor might be brought out by an analysis of the frequency and extent of involvement of different organs, but this aspect of the subject will be reserved for future consideration. It is sufficient to say that such a change has taken place, as can be seen by noting the gradual shift of metastases, as recorded in Text-figs. 1 to 4, from the first three columns including lungs, liver, and kidneys to a more general and more frequent involvement of organs placed further to the right.
Resolution and Healing of Secondary Tumors.—It will be noted (Text-figs. 1 to 4) that comparatively few secondary tumors are recorded as in process of resolution and healing and that fewer still are recorded as healed. Lesions of these classes have, however, a special significance and for that reason they will be considered briefly.

In the first generation three lesions are recorded as largely necrotic and apparently undergoing resolution at the expiration of 26 weeks. From the second to the fourth generation inclusive only one lesion was seen that appeared to be regressing, although most of these animals were held for long periods of time.

Resolution and healing were first noted as conspicuous features of the disease among the animals of the fifth generation inoculated late in August, 1921. All of the animals in this group developed secondary growths, but, of the six animals in the series, three showed lesions that were largely necrotic and in process of resolution at the end of 8 weeks, while another showed a number of healed metastases in the kidneys 18 weeks after inoculation. The indications were that four of the six animals would have recovered.

In the next generation, there were fewer animals with retrogressing lesions, but, again, in the seventh and eighth generations the condition became more frequent, being associated here with some prolongation in the period of observation. From the ninth to the thirteenth generation, few instances of retrogression were seen, although there were a number of animals with secondary tumors that were held for a period of time comparable to that of the fifth and seventh generations.

In the fourteenth generation (July, 1922) necrosis and resolution were again a conspicuous condition in that at the end of 10 to 14 weeks all of the animals (four) that developed secondary tumors showed this condition as an outstanding feature of every lesion present. This tendency was carried over into the next two generations, occurring in two of the four animals of the fifteenth and three of the four in the sixteenth generation in which secondary tumors occurred. It then diminished in frequency until the nineteenth generation.

The tendency or liability to undergo resolution and healing has an important bearing on the estimations of malignancy based on the incidence and distribution of secondary tumors as stated above, but it has an additional significance in the present connection as evidence of a tendency to periodic changes in the behavior of the tumor.
Duration and Termination of Disease.—The final points to be considered in this series of experiments concern the duration and the mode of termination of the disease, neither of which can be stated with absolute accuracy, as it was impracticable to extend the period of observation indefinitely. From the data available, it can be said, however, that, at the beginning of transplantation, the expectancy of life appeared to be relatively good. In the first generation, one animal survived 26 weeks and another 14 weeks, with no definite indication as to what the outcome would be, but with a strong probability that they might recover. The third animal of this group lived 26 weeks, but would have died as a result of a growth involving the bones of the lower jaw and interfering with mastication.

In the second generation, there was no death attributable to the tumor, but one animal showed pronounced weakness at the end of 10 weeks and was killed. Again, there were three rabbits that survived for periods of 24 and 29 weeks. One of these virtually recovered within this period of time, a second was in good physical condition but showed several actively growing tumors, while the third (No. 9) became weak and emaciated and probably would not have lived more than a few days.

In the third generation, there were two animals that died from intercurrent infections, four recovered completely, and there were four deaths from the tumor. These deaths occurred 8, 11, 19, and 23 weeks after inoculation. The other animal of this group (B 5) was killed at the end of 8 weeks, but probably would have died within a short time, as there were extensive metastases in various parts of the body.

This establishes a fair basis for consideration of the duration of the disease and the probable proportion of deaths or recoveries, both of which varied greatly with succeeding generations. As early as the fourth generation there were two deaths within 6 to 7 weeks after inoculation. In later generations, some animals with malignant tumors lived as long as 14 to 16 weeks before their condition became critical, but the probable length of life of such animals rarely exceeded 7 to 10 weeks. Eventually this period was reduced still further so that in the nineteenth generation there was one death at 5 weeks and
another at 4; more recently, deaths have been recorded as early as 3 weeks after inoculation and have been not uncommon at 4 and 5 weeks. This increased rate of progress of the disease was not limited to animals with highly malignant tumors, but, as transplantation continued, it became a characteristic feature of the disease in all classes of animals. The critical period in the growth of the tumor was greatly diminished, so that, in the great majority of instances, death or complete recovery occurred or was determined within 4 to 6 weeks after inoculation as compared with a much longer and rather indefinite period of time during the first three generations.

The effect on the death rate was distinctly different. The probable mortality for the first three generations may be estimated roughly at from 40 to 60 per cent. It is certain that higher points were reached in the fifth, sixth, and twelfth generations. On the other hand, it is doubtful whether any of the animals of the seventh, fourteenth, sixteenth, or twentieth generation would have died. It is certain, therefore, that, with the exception of three generations, there was no definite increase in the probable mortality and it would appear that in most instances the tendency was in the opposite direction. Still, this is not to be interpreted as evidence of an actual reduction in growth capacity of the tumor cells, as the results of more recent generations show that the tumor may be fully as malignant as at any time in the past.

DISCUSSION.

The influence of various factors that may have contributed to the production of the results here reported will be considered in the second section of this paper, so that in concluding this report, a brief summary of the outstanding features of the results is all that is necessary.

In the first place, the experiments that have been carried out show that we are dealing with a tumor that may be transplanted indefinitely, and that, thus far, transplantation has produced no material change in the morphology of the tumor and no fundamental alteration in its biological properties. Still, it cannot be said that the biological properties of the tumor cells have remained unaltered. The incubation period, the rate of growth of primary tumors, the time required
to attain a maximum size, and the persistence of primary tumors were all affected in a manner that indicated a decided increase in the rate of cell growth and the progress of the disease towards death or recovery. The same was true of the occurrence of edema in association with primary tumors and of metastases that could be recognized clinically. In addition, the character and location of secondary tumors changed in a way that suggested an increased distribution of tumor cells and an increased capacity for growth in all parts of the body. Still, with the exception of a few generations, there was no corresponding increase in the occurrence or percentage distribution of metastases, and, while the progress of the disease was greatly hastened, the death rate remained almost unaffected or was actually diminished.

Finally, it is important to note that none of the changes recorded was of a steadily progressive character. At times it appeared that the malignancy of the tumor had been greatly increased by transplantation, but almost immediately there was a change to a relatively benign condition, so that at the conclusion of this series of experiments the outstanding features of the results were: first, an unmistakable increase in the growth activity of the tumor cells which was associated with a corresponding reduction in the duration of the disease; second, a diminished incidence and distribution of secondary tumors with a corresponding reduction in mortality.

This paradoxical situation is a very significant feature of the results of transplantation and points clearly to the operation of some factor other than continued passage as the decisive influence in determining the end-results. This aspect of the subject will be considered in the second section of this paper.

SUMMARY.

A report is given of the results obtained by intratesticular inoculation of a malignant tumor of the rabbit based on a study of the first twenty generations.

The subject is presented from the standpoint of variations in growth and malignancy as they occurred with continued transplantation. Essential features of the experimental conditions and of clinical and postmortem observations are condensed and recorded graphically.
On analyzing the results of these experiments, it was found that many changes had occurred in the behavior of the tumor during transplantation, but that the changes were of a very irregular character and as a rule did not proceed constantly in any given direction. Moreover, the evidence as to the effect of transplantation on the growth and malignancy of the tumor was contradictory in that there was a great deal of evidence to show that there had been a decided increase in the activity and capacity for growth on the part of the tumor cells. But, with the exception of a few generations, there was an apparent reduction in the incidence and percentage distribution of secondary tumors, while the death rate was unaltered or actually diminished.

It was believed that this paradoxical situation afforded a basis for an explanation of the results that had been obtained by transplantation.

BIBLIOGRAPHY.