

## STUDIES BASED ON A MALIGNANT TUMOR OF THE RABBIT.

### III. INTRATESTICULAR TRANSPLANTATION AND CLINICAL COURSE OF THE DISEASE.

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When a satisfactory method had been devised for the transplantation of the tumor which forms the subject of this series of papers (1), a systematic study of the tumor was undertaken, using the method of correlation of clinical and pathological phenomena. The immediate object of these investigations was to obtain a general conception of the behavior of the tumor as an agency for the production of disease, with a view to utilizing this knowledge as a basis for future work. It had been found (1) that under favorable conditions, the tumor was capable of producing a very malignant disease, while under other conditions, it was difficult to obtain even a primary growth. It appeared, therefore, that by the use of a variety of methods of transplantation, one might obtain results which would permit an estimation of the relative importance of the several groups of factors which were concerned in determining the results of transplantation and would give some insight into their mode of operation.

These investigations necessitated a long series of experiments which extended over a period of nearly 2 years. During this time, the tumor was passed through more than twenty generations; inoculations were made into the testicles, the skin and subcutaneous tissues, the muscles, the anterior chamber of the eye, the brain, the peritoneal cavity, and the blood stream. All these animals were studied clinically and pathologically as individual subjects of disease. Thus far, only male rabbits have been used; they have been carefully selected and have been kept in individual cages, and given the best care and attention. With this material, we have been able to study phe-

nomena of tumor growth and of animal resistance, and to correlate these with clinical and pathological effects under favorable and unfavorable conditions of transplantation.

In reporting the results of these investigations, it seems advisable to begin with a description of the growth obtained by various methods of transplantation and the clinical effects observed in tumor-bearing animals, in order to provide a general perspective of the disease to which individual phases of the subject may be related. The present paper will deal, therefore, with the results obtained by testicular inoculation, while the results of other methods will be presented in the next paper of the series (2). In view of the complexity of the conditions which influence the results of transplantation in one way or another, statistical data will be omitted as far as possible until such time as they can be introduced and analyzed to advantage. At present, any attempt to deal with the subject from this standpoint would only lead to confusion unless accompanied by a great deal of explanatory material which would carry us beyond the limits which have been assigned to these papers.

#### *Intratesticular Transplantation.*

##### *Methods and Material.*

The description of the results obtained by intratesticular transplantation is based upon the study of a large series of rabbits. These animals were inoculated with tissue fragments or with a cell emulsion prepared according to the method described in a previous paper (1). With few exceptions, they were inoculated in only one testicle, which was usually the right, so that there was only one primary focus of tumor growth.

##### *Results.*

*Percentage of Tumor Growths.*—Intratesticular inoculation of finely minced tumor tissue or of a homogeneous cell emulsion prepared from actively growing tumors yielded a definite growth in nearly all animals. Occasionally, the growth was slight, and, in rare instances, no growth could be recognized by ordinary methods of clinical examination, but the percentage of positive results was rarely less than 90 and as a rule was 100 per cent.

*Incubation.*—Inoculation was usually followed by a slight diffuse swelling of the testicle which disappeared within 24 to 48 hours, leaving a small area of thickening or induration at the point where the tissue was discharged. This in turn diminished or disappeared within 3 to 5 days and was followed immediately by the development of one or more circumscribed nodules or by a diffuse enlargement of the testicle with ill defined areas of thickening. These changes marked the appearance of a definite tumor growth. During the earlier generations, this period varied from 5 to 10 days, but with later generations, the time has been appreciably shortened and now rarely exceeds 5 days.

*Growth Characteristics.*—The growth of the tumor was usually periodic in character. As far as could be determined by palpation, there was a steady increase in size for a period of 2 to 3 weeks before any interruption occurred. By this time, the tumors in the majority of animals had reached a size of from 1 to 3 cm. in diameter. Occasionally, complete regression occurred after this first cycle of growth. This was especially true of slowly growing tumors in young or adult animals.

In a second group of animals with unusually active tumor growths, there was apt to be a sudden development of edema in the scrotum and adjacent tissues, followed by rapid regression, the tumor softening and diminishing in size. This phenomenon of crisis, as it may be termed, was frequently followed by complete resolution of the primary tumor, but many of these animals subsequently developed secondary growths. In other instances, the primary tumor was only temporarily affected.

The course of events in a third and comparatively large group of animals differed from that of the second in that the edema was less marked, and while growth was arrested, there was comparatively little regression, or even though there was an extensive edema, little or no regression took place.

Finally, there was a fourth group of animals in which growth of the primary tumor was active or of moderate degree but progressed with only slight or brief interruption at this period.

Small or feebly growing tumors, which formed only a small proportion of the whole, not infrequently began to regress at about the end

of the 2nd week, but usually the first critical period in the growth of primary tumors occurred at or near the end of the 3rd week after inoculation and was remarkably uniform in this respect. This was not peculiar to testicular tumors but was observed after all forms of inoculation and is worthy of especial emphasis as a feature of the behavior of the transplanted tumor.

The interruption of growth which occurred at this time was usually of short duration and rarely extended beyond the 4th week. Growth was then renewed with greater or less activity. In many instances, the primary tumor increased actively, involving the entire testicle and extending up the cord into the abdominal cavity in the form of a continuous mass or as discrete nodules which might be few in number and comparatively large, or small and very numerous. It sometimes happened that the testicle was drawn into the abdominal cavity and growth continued in this location. In other instances, a testicular mass developed which involved tunics and scrotum and not infrequently reached a size of 5 to 7 cm. in length by 2 to 3 cm. in diameter.<sup>1</sup>

In a smaller proportion of the animals, the growth which occurred after the interruption of the 3rd or 4th week was less rapid than before or was more circumscribed in character. In some instances, the primary tumor remained comparatively small (1 to 3 cm. in diameter), while in others, it reached a very large size but was confined within the scrotum.

After the 4th or 5th week, periodic changes in the activity of primary tumors were less evident. By this time, the animals of a series had become separated into three fairly distinct groups. The first included animals in which growth appeared to have reached its limits, and the tumors were either quiescent or definitely regressing; the second group was composed of animals with slowly but irregularly growing tumors; while the third was composed of animals with actively growing tumors. With few exceptions, however, what took place was still of an intermittent character, resolution being interrupted from time to time by periods of quiescence or even by a slight renewal of growth, while the growth of more active tumors was

<sup>1</sup> See Pearce and Brown (1), Figs. 1 to 4.

likewise interrupted by periods of quiescence or slight regression. The exceptions to this rule occurred with tumors of an unusually malignant character and less often in instances of unusually rapid resolution in which either process took place with no appreciable interruption.

*Metastases.*—Metastases developed in the majority of animals, and there were few organs or tissues in which secondary growths were not found, but a detailed report of this feature of the transplanted tumor will be reserved for a future communication. At present, it is sufficient to say that extension of the growth through the inguinal canal and the formation of implantation metastases in the abdominal cavity occurred as early as the 3rd week after inoculation, and metastases by lymph or blood vessels to distant parts of the body made their appearance anywhere from the 3rd to the 5th week.

*Termination.*—The ultimate fate of the tumor or of the tumor-bearing animal was variable and depended upon a number of factors to be discussed in a subsequent paper. As has been intimated, the growth was suppressed very quickly in some instances; while in others, resolution and apparently complete recovery took place after the tumor had grown for several weeks or months, and this sometimes occurred even after extensive metastases had developed. More often, however, the occurrence of metastases was eventually followed by death. In some instances, death was due to mechanical causes, such as intestinal obstruction, or pressure on the spinal cord, or to mechanical interference with nutrition as in the case of metastases affecting the jaws and especially the region of the lower incisors, but a considerable proportion of the animals showed none of these complications.

Finally, there was a third group of animals in which a condition of equilibrium became established, and for months there was no decided change in one direction or the other, leaving the outcome uncertain even after prolonged observation.

The probable mortality in different series of animals cannot be estimated with accuracy owing to the fact that few series were held intact for more than 2 to 3 months and that it is impracticable to prolong the period of observation sufficiently to obtain a definite end-result. In general, it may be said, however, that within a period of 2 to 3 months, the mortality varied from nil to 100 per cent, depending

upon a number of factors, such as the composition of the series as regards the age and breed of the animals used, the state of the inoculating material, and especially the season at which the experiments were carried out. Under ordinary conditions, from 20 to 30 per cent of the animals apparently recovered during an observation period of 2 to 3 months, while 20 to 40 per cent died, leaving from 40 to 60 per cent in which the outcome was not determined. These proportions are significant as indicating a resistance distribution which is relatively constant for different groups of rabbits. That is, in any group of five rabbits, there are usually one of relatively high resistance, one of distinctly low resistance, and three which are intermediate and comparable to one another.

*Duration.*—For the same reasons as those given above, the duration of tumor growth or the length of survival of tumor-bearing animals can be stated only in general terms. The growth of the tumor in surviving animals varied between 10 days to 2 weeks and an indefinite period of more than 6 months. Few animals showed complete suppression of tumor growth before the end of the 2nd or 3rd week. In most instances, final regression began between 6 weeks and 2 months after inoculation. That is to say, the turning point was reached at about this time, and one was able to forecast the probable outcome with a considerable degree of accuracy. Resolution and absorption required a longer time, and in the case of large tumors were rarely complete before the end of the 3rd month, and frequently the time was much longer.

Death from the tumor usually occurred between 7 and 12 weeks after inoculation, the period of greatest frequency being from 7 to 10 weeks. Occasionally, the course of the disease was more prolonged, and the animal survived 4 to 6 months after inoculation. More often, the disease was fulminating in character, and death occurred within 6 to 7 weeks.

*Relation between Growth and Malignancy.*—In concluding the section dealing with phenomena of tumor growth, it should be made clear that no constant relation was observed between the rate of growth or size of primary tumors and the malignancy of the tumor as indicated by the occurrence of metastases or by the course of the disease. In fact, it appeared that the relationship was more often a

reverse than a direct one. That is, tumors which grew most actively during the first 2 to 3 weeks were usually suppressed and rarely gave rise to extensive metastases or caused the death of the animal. In other instances, large primary tumors developed which were confined within the tunics and the scrotum and produced no serious consequences. The most malignant tumors, on the other hand, were characterized by a moderate but steady rate of growth with invasion of the tunics and extension up the cord into the abdominal cavity. Similar conditions obtained with reference to metastatic tumors. The most distinctive feature of the two classes of tumors was, therefore, the manner in which the animal responded to the presence of the tumor rather than the rate or size of the growth.

*Immunity.*—The subject of immunity of tumor animals was not investigated extensively, but it may be said that, with rare exceptions, animals that had once been inoculated with negative results were insusceptible to a second inoculation. In like manner, animals that had apparently recovered could not be reinoculated successfully by any of the usual routes. The duration of this resistance is not known, but reinoculations made as late as 6 months after recovery still gave negative results.

#### *Symptomatology of Tumor Animals.*

In describing the symptomatic effects produced by this tumor, we shall confine ourselves almost entirely to such conditions as can be detected by ordinary methods of observation supplemented by records of weight.

The first recognizable change which occurred in tumor animals was of a local character and consisted of an enlargement of the regional lymph nodes which appeared almost immediately after inoculation and gradually increased for upwards of 7 to 10 days. This reaction in the regional lymphatics was usually followed by similar changes in other groups of nodes. As a rule, the initial swelling diminished towards the end of the 2nd week, leaving the nodes still somewhat enlarged and distinctly indurated. This condition persisted with but little change until the turning point in the disease was reached. At that time, the nodes showed either a further increase in size or underwent a decided atrophy. Thus, if the growth was progressive,

further enlargement of the lymph nodes usually occurred, but when resolution took place, the nodes diminished in size and in some instances became extremely small and indurated. In this respect, there was a striking contrast between the conditions presented by animals in which the disease pursued a rapidly fatal course and those which survived for a longer time. In acutely fatal cases, the lymph nodes were usually smaller than normal, whereas in the more chronic cases, they tended to be greatly enlarged and were succulent or edematous in character. During the late stages of the disease, therefore, either a hypertrophy or atrophy of the superficial lymph nodes might occur, depending upon the course of the disease.

During the first few weeks after inoculation, there were no decided changes in the general physical condition of animals with actively growing tumors. They usually gained in weight, and at times, the increase was somewhat greater than that of normal animals under similar conditions, and was usually greater than that of animals in which the growth of the tumor was slight or of short duration. In fact, these animals not infrequently showed an actual loss in weight.

The first evidence of a nutritional disturbance appeared as an accompaniment of the phenomenon of suppression of tumor growth and consisted of a cessation of the normal increase in weight or a sudden loss in weight which in extreme cases amounted to several hundred grams. This condition might be transient or might last for from 10 days to 2 weeks, and was frequently associated with some change in disposition. In the milder cases, this usually took the form of a nervous excitation, while in other instances, there was a decided apathy with dryness of the skin, roughening of the coat, and loss of appetite which doubtless accounted for some part of the loss in weight and the prolongation of this condition.

These changes in physical condition appeared to be influenced largely by the extent of the tumor growth and by the suddenness with which the reversal of this process took place. With slight or transient growths, there might be no appreciable alteration in the physical condition of the animal. If, on the other hand, the growth had reached a considerable size and regression began abruptly, the change was usually marked. In some animals with large primary tumors or with metastases to internal organs, in which the change



from progression to regression took place slowly, the loss in weight might be barely perceptible, while the change in disposition was very decided. In some instances, there was no more than an apathy, while in others, there was a marked nervousness which was frequently accompanied by a pronounced exophthalmos. This latter condition was also noted in other classes of animals. It was frequently transient, but at times persisted for weeks, or disappeared and recurred from time to time with successive periods of progression and regression of the tumor. It was not permanent, however, but disappeared as the tumor was absorbed.

As absorption of the tumor progressed, the general condition of the animal tended to improve. Animals that had made a prompt recovery showed a decided improvement in their general appearance, with a substantial increase in weight, and remained in excellent physical condition for months. In cases in which recovery was slow and at times uncertain, there was evident impairment of physical condition and the animals were subject to intercurrent infections and their powers of resistance to such infections were diminished as contrasted with those of the first group.

When the growth was not arrested, the disease followed either an acute or chronic course, terminating in death, and the changes observed in the physical condition of the animal varied accordingly. Those animals in which the tumor grew most actively usually remained in excellent condition until shortly before death. If death occurred suddenly as a result of accidental interruption of some essential process, there might be no obvious deterioration in the physical condition of the animal, depending upon the progress which the disease had made at the time of such an occurrence. In the absence of accidental complications, the animal began to lose weight and strength, the conjunctivæ and mucous membranes became pale, movements were sluggish, the skin was relaxed and was thin and delicate, while the coat was dry and roughened, the eyes were sunken, the lids drooped, and towards the end, there was not infrequently an excessive flow of lacrimal and mucous secretions.

The pulse rate of these animals was so variable that no estimation of its character can be given. The temperature during the early periods of tumor growth varied very little from the normal, but as the

disease progressed, there was an apparent tendency to an elevation of temperature which was relatively slight in most animals. Occasionally, however, the temperature was subnormal, and a decided elevation followed by a fall below normal immediately preceding death was noted in several instances.

The conditions described made their appearance anywhere from a few days to 2 or 3 weeks before death and increased with greater or less rapidity according to the progress of the disease. It appeared that these symptoms were rarely if ever the result of tumor growth *per se*. In fulminating cases of malignancy, metastases were nearly always present in one or both suprarenals, and they were frequently found in the pituitary body, hence it is difficult to determine the part played by tumor growth and that which may be ascribed to accidental involvement of organs such as these.

In this connection, mention should be made of the occurrence of clinical conditions simulating Addison's disease—minus the element of pigmentation—and of genital atrophy from metastases in the suprarenals and pituitary body respectively, and the occasional occurrence of a peculiar skeletal overgrowth for which as yet no cause can be ascribed. The conditions which suggested the Addison syndrome occurred in their most typical form in animals which did not show extensive tumor metastases, the presence of metastases in the suprarenals being indicated by an extreme weakness and pallor of mucous membranes which were out of proportion to other evidences of tumor involvement. The picture was so characteristic as to lead one to believe that a close approximation of the human disease might be produced experimentally by inoculations made directly into the suprarenal glands.

In like manner, the presence of a metastasis in the pituitary body was diagnosed on several occasions by the occurrence of an extreme atrophy of the uninoculated testicle which under ordinary circumstances retained its normal size and condition or underwent an hypertrophy. The other element of the Fröhlich syndrome (adiposity) was rarely seen in these animals, due to the presence of metastases in other organs or to the acute course of the disease, but in one instance, the complete picture of marked accumulation of fat and extreme atrophy of the testicle occurred in typical form.

The picture presented by animals in which the disease pursued a chronic course extending over some 4 to 6 months was quite different from that of the acutely fatal affection. The change from a condition of well-being to one of extreme cachexia occurred insidiously, and at the end, the picture was complicated by the presence of some inter-current infection. There was a gradual loss of weight and of strength; the animal became apathetic and the movements were sluggish; the hair was dry and roughened and the eyes sunken as in the preceding case, but the skin of these animals was also dry and thick, or of a parchment-like texture, and was covered by fine scales, and frequently there was a decided thinning out of the hair. In addition, areas of mucoid infiltration were frequently present in the skin and subcutaneous tissues of the genital region, and as the end approached, they occasionally appeared elsewhere. These animals maintained a firm hold upon life in the presence of extensive tumor metastases, and death was not infrequently due to some form of bacterial infection.

It is obvious that conditions other than those described were present in all classes of tumor-bearing animals, but as yet there are many aspects of the disease which we have not been able to investigate in a systematic way. The blood, for example, showed distinct changes, the best known features of which were an anemia, a mononucleosis, and some change in coagulability. Under certain circumstances, the coagulation time was diminished, but in cases of high malignancy, it was apt to be increased and instances were noted in which the blood either failed to coagulate after prolonged standing or formed a very imperfect clot.

#### CONCLUSION.

Since the paper which follows this will deal with the results obtained by other methods of inoculation, the discussion of the transplanted tumor and its effects upon the animal organism may be conveniently deferred until the report dealing with these subjects has been completed. From what has been said, it is apparent, however, that when transplanted by the method described, the tumor under consideration is capable of producing a highly malignant disease. But even under these conditions, the results of transplantation are

extremely variable, which indicates that the factor of animal resistance is of equal if not greater importance than that of the capacity for growth possessed by the tumor cells.

#### SUMMARY.

The results obtained by intratesticular inoculation of a malignant tumor of the rabbit are presented in the form of a general summary covering the phenomena of growth and spontaneous regression of the tumor and the clinical evidence of disease in tumor animals.

Under ordinary circumstances, growth is obtained by this method of inoculation in practically all animals, and the majority of them show metastases.

The ultimate fate of tumor animals is variable. Spontaneous regression is of frequent occurrence, and apparent recovery may take place even after extensive metastases have developed. More often, the disease terminates fatally. The course of the disease may be fulminating in character with death occurring 6 to 7 weeks after inoculation, or it may be prolonged over a period of more than 6 months, but death usually occurs between 7 weeks and 3 months after inoculation.

The symptomatology varies with the course of tumor growth but is in general that of a debilitating disease of an acute or chronic character in which the picture is frequently complicated by symptoms referable to mechanical causes or to one or more of the glands of internal secretion. The most prominent of these are due to the presence of metastases in the suprarenals or pituitary body, in which case symptoms of Addison's disease or diseases of the pituitary may be superimposed upon those attributable to the presence of extensive tumor growths. In the absence of such growths, however, the symptoms of suprarenal or pituitary involvement may dominate the picture.

#### BIBLIOGRAPHY.

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