A STUDY OF THE BLOOD IN RATS RECOVERED FROM IMPLANTED SARCOMA.*

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The studies reported in this paper were made in the attempt to determine whether the factor of immunity can be demonstrated in the blood of animals which have recovered from implanted tumors. Two series of experiments were carried out, each from a different standpoint.

THE PASSIVE TRANSFERRENCE OF IMMUNITY.

In the first series large amounts of immune blood plasma were injected into animals with growing tumors, and also into normal animals subsequently implanted with tumors, in order to determine the effect upon the tumor process. Similar experiments were reported in 1905 by Gaylord, Clowes, and Baeslack.1 Their results,

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1 Gaylord, H., Clowes, G. H. A., and Baeslack, F. W., Med. News, 1905, lxxxvi, 91. They state: “The blood-serum of mice which had recovered spontaneously from tumors possessed a power, when injected into mice infected with growing tumors, of inhibiting the growth of large tumors and causing the retrogression of smaller tumors, leaving the animal possessed of an immunity which prevents recurrence of the growth. The degree of immunity in the mice thus far tested varies within considerable limits, the most marked illustration of its activity being found in one mouse whose blood-serum injected in a single dose of .2 c.c. caused the rapid retrogression and entire disappearance of two tumors in one animal and one in another, all of which were as large as peas, in the space of three days. The same serum injected into a mouse with a tumor the size of a small cherry (about two grams) caused a noticeable reduction in size of the tumor, which remained stationary for ten days, when an operation for the removal of a portion of it resulted in a return of activity to the growth. The latter part of this experiment will be dealt with in our final publication.

“All of these experiments were controlled with mice inoculated at the same time, the tumors of which were smaller than those of the mice treated with the
however, have not been confirmed by any extensive series of experiments. Lewin, indeed, obtained some cures in both rats and mice by this method, using the serum of immune animals approximately fourteen days after a negative inoculation in the latter. He is under the impression that the "serum of such animals does actually contain immune substances."

Before proceeding to my experiments it may be well to state that no method hitherto employed has effectively demonstrated the presence of immune substances in the serum. In this general statement are comprehended not only the results of a large number of test-tube determinations, but also the results obtained by Lambert and Hanes by means of artificial cultivation. Although individual observers have occasionally published data that seem to indicate the presence of specific reaction products in the blood of animals either suffering from or recovered from tumors, there has never been a general confirmation of these views. The results obtained by the biological method, employed by Gaylord and his co-workers, and partially confirmed by Lewin, would therefore be valuable, as indicating the existence of immune substances, even though they are not demonstrable by the present methods of test-tube analysis.

In the following experiments a rat sarcoma was employed, which under favorable circumstances has a record of 80 to 100 per cent. of takes. After an average period of growth ranging from immune serum. These control mice received doses of normal mouse serum equal in volume to the doses of immune serum referred to above. In every case the tumors in the control mice developed rapidly and led in the course of three or four weeks to the death of the animal. In spite of the fact that the control tumors were invariably smaller than those used for the immune serum at the commencement of each experiment, in the course of a week or ten days the control tumors were found to be larger, and up to the date of making this announcement, while several control mice have died of their tumors, not a single mouse treated with immune serum has so far succumbed. In those cases in which the tumor was too large or the immune serum too weak to effect a cure, the marked retardation in the development of the tumor was always associated with a diminution in the cachectic symptoms invariably exhibited by the tumor mice in the last stages."


3 The sarcoma was obtained many years ago from the Cancer Laboratory, in Buffalo, and has since then been under constant observation in our laboratory.
two to four weeks, a considerable number of the takes begin to retrogress, and either completely disappear or are reduced to small and indolent masses. The exact percentage of retrogression varies considerably in different series, and does not seem to depend upon any demonstrable factor. In every case, therefore, it is necessary to control an experimental series by an observation of at least an equal number of controls, both sets of animals having been inoculated at the same time, from the same tumor, and by the same method. The immune serum was invariably obtained by the following procedure. A large number of inoculated rats were constantly kept in stock. From this group a number of animals were selected whose tumors had undergone total retrogression within a period not greater than ten days. The animals were bled to death by section of the thorax, and the blood was defibrinated and centrifuged. Spontaneously recovered animals were used in preference to those that had not taken the inoculation (nullers), for the reason that the former were found, over a long series of experiments, to be almost uniformly refractory to a second inoculation, which is not the case with the latter. Recovered animals are, therefore, distinctly immune, whereas nullers are not necessarily so.

The method of immunization by previous injection of killed tumor or other tissue was discarded, in view of the fact that animals so treated become immune only in an uncertain, often small, proportion of the cases.

In the first series of animals that was studied in this connection, twenty-four young white rats were included. These were divided into two equal lots of twelve each. Of the first lot, six rats received an intravenous injection into the jugular vein of one cubic centimeter of plasma, obtained from recovered rats. One cubic centimeter represents the average amount of plasma which it was possible to obtain from one rat. The other six received two cubic centimeters of the same plasma intraperitoneally. On the day following this injection, all the animals received a subcutaneous inoculation, by the trocar method, of rat sarcoma. After ten days the animals were examined. Of the treated series, nine, and of the untreated series, ten showed growths. It was not possible at this time, in view of the minute size of the tumors, to determine any
difference quantitatively. On the eighteenth day the animals were again examined. Of the treated series ten remained alive, of which again nine showed tumors. Of the untreated series the total number remained alive, and ten showed tumors. There were no quantitative differences discernible in the average growth of the tumors. On the thirtieth day there had been retrogression in all except four of the treated series, and in all except three of the untreated series. At this point the observation of both series was discontinued. The conclusion was drawn that the preliminary inoculation of immune serum does not protect rats against subsequent implantation of tumors, and does not noticeably influence the rate of growth, nor the percentage of retrogressions in such tumors.

In another series of animals the conditions of the experiment were varied by making, in addition to the preliminary treatment with immune serum on the day preceding the tumor inoculation, a subsequent injection of serum five days thereafter. It seemed possible that the second injection, being made at a critical period in the history of the graft, might conceivably exercise a more potent influence than the earlier injection. It does not seem necessary to give the details of this second experiment. In brief, fourteen controls were used, and eight treated animals. The percentage of takes and of recoveries in the two series differed by so little that it was evident that the treatment had produced no therapeutic effect.

In spite of the fact that it had not proved possible materially to influence the process of tumor implantation by means of the injection of immune serum, it seemed possible that the rate of tumor growth, or of retrogression, might conceivably show the influence of repeated treatments of this nature. Theoretically, it is possible that immune substances may be ineffective in preventing successful implantation, whereas they may be of importance in controlling growth.

In order to determine this point rats were selected whose tumors had shown progressive growth, and had reached a size measuring not less than 1.5 centimeters in diameter. These rats, in favorable series, offer a fair prospect of continued growth without retrogression. It is necessary, however, to correct the results obtained
in a treated series, by the observation of a considerable number of exact controls planted from the same tumor at the same time.

Fourteen animals were selected for treatment. All the animals had been inoculated twenty-five days previously. There were 100 per cent. of takes, and on the day on which observation began all of the tumors were actively growing and showed no ulceration. The rate of growth of each tumor was plotted by a second observation made ten days later, or on the thirty-fifth day after inoculation. Upon that day each of the animals received an injection of immune plasma. In five, 0.5 of a cubic centimeter was injected intravenously. In the other nine, one cubic centimeter was injected intravenously. In all the animals three further injections, at intervals of two days, each of one cubic centimeter of plasma from immune rats, were made intraperitoneally. The size of the tumors was again plotted seven days after the beginning of treatment, and again fourteen days thereafter. All but two of the animals survived the treatment and were in good condition. In all the twelve that survived, except two, there had been continuous striking increase in the growth of the tumor, which at this stage is unusually active. In the two exceptions there had been retrogression which, in one, had been almost complete. Comparison with the controls brought out the fact that the percentage of retrogressions in this series was greater by 12 per cent. than in the treated animals. In the rest of the series the rate of growth was approximately the same as in the ten of the first series described above. It does not seem justifiable to infer that the injection of the immune serum improved the growth in the first series. The treatment, however, seems to have been entirely ineffective in controlling tumor growth.

The conclusion is therefore drawn that the repeated injection of plasma, derived from immune rats, failed to influence favorably the growth of a certain strain of rat sarcoma. Therefore, as far as the results of this series of experiments justify generalization, one may conclude that the blood of recovered rats does not carry the factor of immunity present in these animals.

PASSIVE SENSITIZATION.

One of the best methods at present available for the determination of immune substances in serum is that afforded by passive
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anaphylaxis. If a rabbit is immunized by repeated injections of horse serum, the presence of immune substances may be demonstrated in the serum of the rabbit by the following method: normal guinea pigs are given an intraperitoneal injection of the rabbit serum, and on the next day an intravenous injection of horse serum. If the rabbit was immunized the guinea pigs become anaphylactic. Thus, guinea pigs treated with 0.05 of a cubic centimeter of immune rabbit serum may be killed by 0.01 of a cubic centimeter of horse serum.

It seemed possible that by the use of this method immune substances might be revealed in the blood of rats immune to tumor implantation, even though other methods, either biological or serological, had failed to reveal such substances. In order to determine this fact the following experiment was performed.

A large number of rats that had recently recovered from implanted tumors were bled to death on the same day and the plasma thus obtained was pooled. Quantities of this plasma, ranging in amount from 0.5 to four cubic centimeters, were injected intraperitoneally into six guinea pigs. Two days later each of these guinea pigs received an intravenous injection of an extract of the actively growing tumor, freshly removed, in normal salt solution; in each instance, one cubic centimeter of the extract was injected. None of the guinea pigs manifested more than slight discomfort. The fatal dose of this extract was not determined. Two and a half cubic centimeters, injected intravenously into normal guinea pigs, of approximately the same size as those that had received the preliminary intraperitoneal injection, produced marked prostration and weakness. All the controls, however, recovered.

In view of the fact that 0.005 of a cubic centimeter, or less, of a dose of foreign proteid, such as is ordinarily non-toxic in doses of 200 or 300 times that amount, is apt to produce fatal results in a sensitized guinea pig, it seems safe to conclude that the above experiment proves that guinea pigs cannot be sensitized by means of the blood of recovered rats.

In the second series of experiments, recovered rats received a series of subcutaneous inoculations of tumor extract, in order, if possible, to heighten their immunity. The animals were then bled.
Blood in Rats Recovered from Implanted Sarcoma.

and the plasma was injected into guinea pigs, as in the previous series. In this series again, however, there was complete failure to demonstrate sensitization.

The conclusion is therefore drawn that it is impossible, by means of the passive sensitization of guinea pigs, to demonstrate the presence of immune substances in the blood of rats recovered from an implanted sarcoma, or in the blood of rats recovered from this tumor and subsequently injected therewith.