

THE HEMOLYTIC REACTIONS OF THE BLOOD IN DOGS
AFFECTED WITH TRANSPLANTABLE
LYMPHOSARCOMA.*

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During the course of the present year I reported a series of experiments¹ on the hemolytic properties of extracts from organs and from tumors of dogs. I showed at that time that two sorts of hemolytic substances could be extracted from tumors. One of these, which was present in undegenerated tumors, was thermolabile, and hemolytic only when activated by a substance extracted from the red blood cells. This first substance is comparable to a similar hemolytic amboceptor present in certain normal organs, e. g., kidney. At about the same time and independently it was shown by Friedmann² that a hemolytic substance could be extracted from the pancreas, which destroys the red blood cells of the same species, when complemented by some substance (probably of the lecithin group) present in the serum of certain other species. The second type of hemolysin is present only in necrotic tumors; it acts as a simple hemolysin, needing no complement. I ventured to suggest in my first paper that it is the second group of substances which probably passes into the blood, thus contributing to the anemia and the cachexia of malignant tumors.

Since that time I have had the opportunity, thanks to the kindness of Dr. S. P. Beebe, of prosecuting this investigation along somewhat different lines. I have had at my disposal dogs inoculated with the same strain of tumor previously studied, in every stage of its growth. In some the tumors were actively growing, in others they were regressing, while still others were overcoming their tumors as the result of a passive immunity conferred by transfusion. Some of the dogs were in good condition; others were cachectic to the last degree. At the same time I have had a large number of dogs not affected with these tumors, for comparison; some of the latter have been healthy, some cachectic.

It was the object of the present research to study the serum of dogs which had been inoculated with tumors, and to determine whether it possessed any of the hemolytic power characteristic of extracts made

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1. Jour. Med. Research, Boston, 1907, xvi, 287.

2. Deutsche med. Wochschr., Leipzig u. Wien, 1907, xxxiii, 585.

from the tumors themselves. In withdrawing blood from these animals and separating it into serum and corpuscles for further study, the following observation was made: It was generally found that the serum obtained from animals with tumors was stained with hemoglobin, whereas that obtained from animals not having tumors was of the normal straw color. In other words, during the process of separating out in the icebox, the serum of tumor animals had destroyed a sufficient number of its own corpuscles to give it the characteristic tinge of hemoglobin. At the same time, emulsions of corpuscles from the same animals in salt solution remained unchanged, demonstrating that the hemolysis was due not to the lowered vitality of the corpuscles, but to the hemolytic quality in the serum.

The next step was to determine the effects of definite amounts of serum both of normal and of tumor dogs on their own and on other corpuscles. For this purpose, twenty-five dogs in all were selected, of which eleven had tumors and fourteen had no tumor history. All of these dogs were treated in the same fashion. The femoral artery was laid bare under morphin anesthesia, and from twenty to thirty cubic centimeters of blood was obtained from it. The major portion of the blood was allowed to coagulate for the purpose of obtaining serum, while the rest of it was shaken up with glass beads, centrifuged, and made up into a 1 or 2 per cent. suspension in normal salt. The sera, and the corpuscles thus obtained on one day from a large number of dogs, were tested on one another on the following day. One cubic centimeter of serum was always added to an equal quantity of red cell emulsion, incubated for a few hours and transferred to the icebox, where it was allowed to remain over night; the results were noted the following morning. In this manner, 226 separate serum-corpuscle combinations were examined.

A record of one of the experiments will indicate better than any amount of description the type of result which was regularly obtained. The description of the dogs made use of in the experiment, and indicated by numbers in the table, is as follows:

Dog 455.—Four tumors growing slowly for six weeks. Condition good.

Dog 509.—Four tumors growing for two weeks very rapidly. Thin.

Dog 400.—Spontaneous recovery from tumors, which were three in number, and were very rapidly absorbed six weeks before the experiment. Very cachectic animal.

Dog 244.—Tumor almost completely absorbed, owing to repeated transfusions. Dog very cachectic.

Dog 458.—Spontaneous recovery from tumors. In fine condition.

Dogs 516, 517 and 518.—Normal.

Dog 520.—Cachectic. No tumor history.

The following table shows the comparative hemolytic effects of serum from normal and tumor dogs on corpuscles. One c.c. of serum from each animal was mixed with 1 c.c. of corpuscles of each animal, in emulsion. The record of each serum reads along the horizontal line.

CORPUSCLES.

	1-455	2-509	3-458	4-517	5-518	6-516	7-520
Serum I 455	—	—	—	v. sl.	v. sl.	v. sl.	v. sl.
Serum II-509	—	—	—	sl.	sl.	sl.	sl.
Serum III-460	+	+	+	++	++	++	++
Serum IV-244	+	++	+	+++	+++	+++	+++
Serum V 458	—	—	—	—	sl.	v. sl.	sl.
Serum VI-518	—	—	—	—	—	—	—
Serum VII-520	—	—	—	—	—	—	—

NOTE: — denotes negative reaction; v. sl. denotes very slight reaction; sl. denotes slight reaction; + denotes moderate reaction; ++ denotes strong reaction; +++ denotes complete reaction.

If the table be interpreted in the light of the preceding data, the following conclusions may be drawn from it:

1. The serum of all the tumor dogs is distinctly more hemolytic than is the serum of non-tumor dogs. Of the tumor animals, the two cachectic examples (460 and 244) are more actively hemolytic than are the other three.

2. There is a distinct difference in the resistance of the various corpuscles to the destructive activity of the sera. The corpuscles of the tumor animals are always more refractory than are the normal; they break down less rapidly and less completely.

These two phenomena, the hemolytic activity of the sera and the resistance of the corpuscles of tumor animals as compared with other dogs, dominate the entire series of experiments.

The cause of this difference has seemed to me to be a matter of some importance. It might conceivably be due to a difference in the tonicity or content in electrolytes of the serum, resulting in some obscure manner from the growth of the tumor. In order to determine this matter, each set of corpuscles was in every experiment subjected to the action, not only of a normal salt solution, but also to one of a 0.4 per cent. and one of a 1.6 per cent. strength. It would seem that the degree of anisotonicity represented by these solutions must far exceed that of any serum obtained from a living animal. Nevertheless, the activity of the resulting hemolysis was in no way comparable to that produced by the sera. The general condition of the animals can not be held

responsible, inasmuch as some of the controls were much more emaciated and cachectic than some of the tumor dogs. The hemolytic power of the serum resides, therefore, in some other factor, at present undetermined—possibly the same toxins as were extracted from the tumors in the experiments which I previously reported.

The apparent coincidence of poor general condition and marked hemolytic activity is quite striking, and might at first sight impose as a "*propter hoc*." It has, however, been pointed out that dogs in worse general condition, but without tumors, show less activity in hemolysis. On the other hand, it is perfectly justifiable to interpret the "poor condition" as a resultant of the toxic character of the circulating serum, which manifests itself in the test tube by hemolysing the contained red cells. Two exceptions occurred to the general rules above described. Dog 125, whose tumors had regressed owing to three successive transfusions, in good condition, possessed a serum of slight hemolytic power and corpuscles of slight resistance. Whether this was due to the predominance of normal blood in his vessels, I leave undecided. The second case, Dog 638, normal, has a markedly hemolytic serum, but non-resistant corpuscles. This dog had proved naturally immune to a sarcoma implantation. It may also seem contradictory that dogs which had recovered from their tumors, either spontaneously or as the result of transfusion, should still retain the hemolytic character of their serum to an excessive degree. The fact is, however, in accord with what is known of the clinical absorption of tumors. If, owing to an excessive use of x-rays or the mixed toxins, a tumor breaks down too rapidly, its absorption may lead to extreme intoxication of the organism, and even to death. Thus the disappearance of the tumor goes hand in hand with the circulation of the toxic products which have been absorbed. How long an animal may retain these products in his circulation is still to be determined.

The bearing of these experiments on the problem of the anemia accompanying malignant new growths is manifest. They demonstrate that the growth of tumors causes the development of toxic qualities in the blood serum, the exact nature of which has not been determined. It is suspected, however, to be due to the absorption of hemolytic substances previously demonstrated in the tumors themselves.

The bearing of these experiments on the practice of transfusion in curing the tumors of animals, or of men, is important. As is well known, the operation has been performed a number of times in dogs with some success. It is quite evident, however, that to mix the blood of dogs, either of which is hemolytic for the other, would entail the destruction

of a very considerable number of corpuscles, and so would, in part, defeat the very object of the transfusion. There can be no question that the test-tube reactions may be taken as a very good indicator of what would happen were the same bloods to be mixed *in vivo* by transfusion. Of this fact we have had indubitable evidence in the hemolysis resulting after transfusing the blood of one dog into the vessels of another whose serum had been shown to destroy the corpuscles of the former in test-tube experiments. (These experiments have not yet been published.) It is clear, therefore, that every transfusion should be logically preceded by test-tube reactions, and that only those animals should eventually be selected whose serum and corpuscles are mutually tolerant.

On the bearing of the experiments on the subject of human disease I must, for the present, forbear to enter. A considerable number of observations already completed go far to indicate that the results in dogs illustrate conditions which have a parallel in the blood of human cancer cases. On this important point, however, much information must be collected before final conclusions can be drawn.

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