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A STUDY OF THE CONCENTRATION OF THE ANTIBODIES IN THE BODY FLUIDS OF NORMAL AND IMMUNE ANIMALS.*

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THE presence of antibodies of various kinds in serum has long been known, and the concentration in that fluid has been carefully studied. The presence of antibodies in the various other body fluids has not been so carefully investigated, nor has sufficient allowance been made for individual variations in animals of the same species. While the authors were associated with Dr. Carlson in his work on lymph formation, he suggested that a careful comparison between the concentration of the antibodies in the various body fluids of the same animal might be of considerable importance in determining the differences between the lymph and serum, and in that way throw light on some of the problems of lymph formation, and possibly also on the point of origin of antibodies. When the work was begun, we intended to collect lymph from the different organs, but the practical difficulties encountered in introducing cannulae into the delicate lymphatics of such organs as the spleen was so great, that the project was temporarily abandoned, and the work has been confined to a comparison between serum, lymph from the cervical lymphatics, lymph from the thoracic duct, pericardial fluid, cerebrospinal fluid, and aqueous humor. Thus far work has been done on the hemolysins, hemagglutinins, agglutinins for the typhoid bacillus, the protein precipitins, and the opsonins, bacterial and erythrocytic. No work has yet been undertaken on the bacteriolysins. We have not enough data to enable us to draw any broad conclusions, and we will content ourselves with presenting what we believe to be the facts under the various conditions studied.

Literature.—The first studies that we were able to find on the relative concentration of antibodies in the various body fluids were those of Pegano,[36] who found the concentration of hemolysins of the thoracic lymph in dogs lower than that of the serum. Falloise [11] and later Batelli[4] confirmed the work of Pegano. Hughes and Carlson[8] working on normal dogs, horses, and cats found the concentration of hemolysins for

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rabbit corpuscles in the body fluids to form a descending series: serum, thoracic lymph, neck lymph, pericardial fluid, aqueous humor. No lysins were found in the cerebrospinal fluid. Straus and Wolf[40] studied the hemolytic power against rabbit corpuscles of the cerebrospinal fluid, edema fluid, pleural and pericardial transudates, and blister fluid, and attempted to correlate the hemolytic strength with the protein content. Marshall and Morgenroth[25] found anti-complement and anti-amboceptor in a pathological exudate—an ascites fluid. Hedinger[15] studied the hemolytic power of non-inflammatory exudates like those arising from cirrhosis of the liver and heart failure, and found that they were not so hemolytic as the serum. The inflammatory exudates arising from cases of tuberculosis and carcinoma were not so strongly hemolytic as non-inflammatory exudates. He failed to find hemolysins in the fluid from an ovarian cyst, or in the cerebrospinal fluid in two cases of tuberculosis. Marshall[24] found that pleural and ascites fluids were more strongly hemolytic than the serum from an infant. But no conclusions can be drawn from this comparison in regard to the comparative hemolytic power of serum and other body fluids in the same individual. He found a multiplicity of amboceptors and complements in the fluids that he studied. Grollo [4] could find no amboceptors for rabbit corpuscles in transudates, but found them in exudates, altho in the latter complement is often lacking. He suggests this method as a means of diagnosis between transudates and exudates. Lüdke[22] confirmed the findings of Marshall in regard to the hemolytic strength of transudates and exudates. Granström[13] found wide variations in the hemolytic content of transudates and exudates, and could establish no characteristics essential for either. The hemolysins did not run parallel with those of the blood. Isolysins are found less frequently in transudates and exudates than in the blood. Hemolysins were not found in the cerebrospinal fluid. Isolysins and heterolysins were found independent of the albumen content, number of the leukocytes, and the osmotic pressure of the fluids tested. Terleschi[4] found precipitins in both transudates and exudates, less frequently in the latter than in the former. Mioni[30] found amboceptor but no complement for guinea-pig corpuscles in the pericardial fluid of the ox. Bard[2] claims to have found hemolysins in the cerebrospinal fluid of patients, and found that they were increased during various diseases. Massaglia[26] could not confirm the work of Bard. His results in both healthy and diseased individuals were negative. The presence of antibodies for syphilitic material in the cerebrospinal fluid has been shown by various investigators, among them Morgenroth and Stertz,[31] and Wassermann and Plaut.[42] Gatti [3] could demonstrate no hemolysins in the aqueous humor of the ox. Levaditi [3] showed that there is normally no opsonin in the aqueous humor; but if the fluid of the anterior chamber of the eye of an immune animal is withdrawn, the newly formed aqueous humor will contain opsonin. Böhme[6] investigated the opsonin content of pleural, peritoneal, and abscess fluids. He found that usually in such cases the opsonin content of the fluid was reduced for the infecting organism, but remained unchanged for other bacteria. He could find no opsonin in normal cerebrospinal fluid, but found them there after an inflammation had been set up in the dura. He could not develop opsonins in the cerebrospinal fluid by repeated puncture as Levaditi had done by drawing off the aqueous humor. He believes that there is a relation between the protein content and the opsonin action of a fluid.

Methods.—The plan of study adopted was to determine first the concentration of the antibodies in the body fluids of normal cats and dogs; then the concentration in actively immunized animals; and, finally, to study the passage of the antibodies from

the blood into the other body fluids in animals passively immunized by the withdrawal of large quantities of blood, and the injection of a corresponding amount of warm, defibrinated blood from an actively immunized animal.

The body fluids were secured under as nearly aseptic conditions as possible. The animal was anesthetized with ether, and kept in a state of complete anesthesia, by the administration of the vapor through a trachea cannula or through a tube introduced through the larynx. The neck lymphatics were then isolated, and small, sterile, glass cannulae provided with sterile, rubber tubing were inserted. If there was no free flow of lymph, the neck was gently massaged. The lymph was never allowed to come in contact with the air of the room, for as soon as it filled the cannula and a part of the rubber tubing, it was drawn off by means of a fine, sterile Pasteur pipette, and placed in a dry, sterile test tube plugged as for bacteriological work. The lymph was allowed to coagulate spontaneously in the test tube, and was then defibrinated, and the delicate coagulum removed.

The thoracic duct was tied off at the same time as the isolation of the neck lymphatics so that the lymph formed during the experiment was retained in the duct. Usually the lymph from this duct was not collected until the animal had been bled to death, altho sometimes it was collected simultaneously with the neck lymph. The routine method was to draw the lymph by means of a Pasteur pipette provided with a bulb, so that the fluid never came in contact with the air at all. This fluid was also defibrinated.

The pericardial fluid was never collected until after the death of the animal by very complete bleeding from the arteries and veins of the neck. The thorax was opened by removing the sternum, a small hole was cut into the pericardium, and the fluid was removed by means of a sterile Pasteur pipette.

We found it a good plan in our experiments to suspend the animal by the jaws for a few minutes before attempting to withdraw the cerebrospinal fluid. This drained away the blood from the head and made admixture of this fluid with blood less likely. Our method was to open the dura between the first and the second cervical vertebrae, and then remove the fluid by means of the Pasteur pipette. The end of the pipette must be well rounded in the flame, otherwise rupture of the delicate blood vessels of the meninges is likely to follow.

The method of collecting the aqueous humor was simple and easy. It consisted in thrusting a sharp pointed Pasteur pipette into the anterior chamber of the eye through the cornea, and allowing the aqueous humor to flow into it, largely by the tension within the eyeball. A little suction sufficed to remove the last drop of the fluid.

The serum was secured from blood drawn when the animal was bled to death, and in most cases was freed from the corpuscles at once.

Careful notes were made in regard to the condition of the fluids, and in most cases where there was any admixture of blood, the fluid was discarded.

HEMOLYSIS AND HEMAGGLUTININS.

Methods.—The study of the hemolysins and hemagglutinins in normal* animals was made on dogs only. The animals utilized were brought in from various parts of the city. Most of the tests were made with rabbit corpuscles in 5 per cent suspension in 0.9 per cent NaCl solution. In some cases rat and horse corpuscles were used.

*The term "normal" means animals which had not previously been immunized by us. We had no way of knowing what their history had been previous to coming to the laboratory.

Our methods were the following: Quantities of the various body fluids of the animal to be tested varying between 0.1 c.c. and 0.0001 c.c. were placed in a series of eight dry, sterile test tubes plugged as for bacteriological work. In order to make the necessary measurements with a pipette graded to $\frac{1}{100}$ of a c.c., dilutions of the body fluids $\frac{1}{10}$ and $\frac{1}{100}$ were made. To the fluid in each tube enough sterile 0.9 per cent NaCl solution was added to make the total volume up to 0.4 c.c. To this was then added 0.2 c.c. of a 5 per cent suspension of the corpuscles to be tested. In this way we got dilutions of the fluid varying between 1:6 and 1:6,144. All of the fluids from the same animal were prepared, the tubes were placed in a block containing a suitable number of holes, and adjusted to the sliding platform of a shaker in an incubator warmed to 37° C. The shaker was run by water power, and the motion was rapid enough to secure constant, thorough agitation, but not violent enough to injure the corpuscles. The routine technic was to keep the tubes in the shaker for an hour and in the ice-box from 12 to 20 hours to permit sedimentation of the corpuscles before the final reading.

In determining the amount of hemolysis in the final reading, the following method was employed: A measured sample of the corpuscle suspension in the test was sedimented in the centrifuge, and the supernatant liquid drawn off with a pipette. The corpuscles were then laked by adding distilled water to restore the original volume. This sample contained three times as much hemoglobin as the hemolytic tests, because 0.2 c.c. of the corpuscles were added to 0.4 c.c. of the fluid tested. Therefore, the above sample was diluted to three times its volume with water. This, then, would give exactly the same concentration of hemoglobin as in any tube in the test, provided that the hemolysis was complete, and is termed 100 per cent for this sample of corpuscles. By further dilution tubes containing 90 per cent, 80 per cent, etc., were prepared. No attempt was made to estimate closer than 10 per cent. A new scale was made for each sample of corpuscles.

The agglutinins were read from the same tubes as the hemolysins. The method employed to determine whether or not agglutination had occurred, was inspection of the rim of sedimented corpuscles. When the corpuscles, after sedimentation by standing in the ice-box, show a perfectly smooth, knife-edged border, no agglutination has occurred. If the border is slightly or decidedly roughened, agglutination has occurred At first this method was carefully supplemented by microscopic examination, but it was soon found so accurate that in the later experiments we depended entirely upon the observation of the rim of the corpuscles, and dispensed with the use of the microscope.

A. Normal animals.—The concentration of lysins and hemagglutinins in the body fluids of normal animals varies within rather narrow limits. This variation is great enough, however, to make it necessary that the comparison be made between the body fluids of the same animal. The following experiment shows the behavior of the body fluids of the normal dog.

Table I shows that the concentration of hemolysins is greater in the serum than in the other body fluids; thoracic lymph is next, at least in the case of rabbit corpuscles; and neck lymph is third.

This difference in the hemolytic power of serum or other body fluid against the corpuscles of different species of animals has been explained by Ehrlich and his coworkers on the basis of a multiplicity of amboceptors and complements, some of which are specific, some

TABLE 1.

COMPARATIVE HEMOLYTIC AND AGGLUTINATING POWER OF THE BODY FLUIDS OF A NORMAL DOG ON RABBIT AND RAT CORPUSCLES.

DILUTION	SERUM				NECK LYMPH				THORACIC LYMPH			
	Rabbit		Rat		Rabbit		Rat		Rabbit		Rat	
	Lysis	Aggl.	Lysis	Aggl.	Lysis	Aggl.	Lysis	Aggl.	Lysis	Aggl.	Lysis	Aggl.
1:6	100	−	10	+	3	+	0	0	40	+	0	+
1:12	40	+	0	0	0	+	0	0	0	+	0	+
1:24	0	+	0	0	0	0	0	0	0	+	0	+
1:48	0	0	0	0	0	0	0	0	0	+	sp	−
1:96	0	0	sp*	−	0	0	sp	−	0	0	0	0
1:384	0	0	0	0	0	0	0	0	0	0	0	0

DILUTION	PERICARDIAL FLUID				CEREBROSPINAL FLUID				AQUEOUS HUMOR			
	Rabbit		Rat		Rabbit		Rat		Rabbit		Rat	
	Lysis	Aggl.	Lysis	Aggl.	Lysis	Aggl.	Lysis	Aggl.	Lysis	Aggl.	Lysis	Aggl.
1:6	0	+	0	0	0	0	0	0	0	0	sp	−
1:12	0	+	0	0	0	0	sp	−	0	0	sp	−
1:24	0	0	sp	−	0	0	0	0	0	0	0	0
1:48	0	0	0	0	0	0	0	0	0	0	sp	−
1:96	0	0	0	0	0	0	sp	−	0	0	0	0
1:384	0	0	0	0	0	0	0	0	0	0	0	0

* It will be noted that in this table several tubes are marked "sp." By that symbol is meant hemolysis not due to the ordinary hemolysins. The appearance of the partially laked corpuscles is entirely different from that in the ordinary hemolytic test. The hemoglobin can be seen diffusing from the sedimented corpuscles, while the supernatant fluid remains perfectly clear. The hemoglobin has the peculiar reddish purple tint of reduced hemoglobin, instead of the clear red of oxyhemoglobin. Furthermore, laking may appear anywhere in the series, frequently, where no hemolysis is to be expected, and is met more often in fluids like the cerebrospinal, or aqueous humor, which are normally not hemolytic, than in the other fluids. Rat corpuscles seem more susceptible to this form of hemolysis than rabbit corpuscles. Complement seems to inhibit this form of hemolysis.

non-specific. In most cases the thoracic lymph of normal dogs is hemolytic for rat corpuscles, altho in the experiment cited above, such was not the case.

As may be seen from the table above, the concentration of agglutinins may be higher in the thoracic lymph than in the serum. Such, however, is not the usual finding. In 10 experiments on normal dogs we found in seven the concentration of agglutinins highest in the serum; in two it was highest in the thoracic lymph; and in one

the concentration was the same in both. The fact that the concentration of agglutinins may be greater in the thoracic lymph than in the serum, renders it hard to see how these antibodies can come from the blood by pure filtration, for in that case, we should expect the hemolysins to run a parallel course—a thing which they do not do—or else we must assume that the agglutinins pass through membranes more readily than the hemolysins. It would be necessary, also, on the basis of filtration, to assume sudden great changes in the concentration of the agglutinins in the blood, for on no other basis could we explain the fact that the concentration of agglutinins would be so much lower in the serum by the time the lymph reached the upper end of the thoracic duct, than it was at the time the lymph was formed. Of course other explanations are possible: there may be an active secretion of the agglutinins into the lymph from the blood, or the agglutinins, after being formed in the area drained by the thoracic duct, are thrown into the lymph, reaching the blood by that route. Much more investigation must be made before any conclusion can be reached on this point.

The pericardial fluid when collected under the best conditions never shows hemolysins for rabbit corpuscles. Agglutinins may or may not be present. In four of our ten supposedly normal dogs hemolysis was noted, in only one case amounting to more than 10 per cent. Of these four animals, two were in poor condition, emaciated, and generally run down, and both these dogs yielded excessive amounts of pericardial fluid; in the other two cases, the pericardial was found to contain a few erythrocytes. Agglutinins were found in all four of these cases and in three others, making a total of seven in ten. From these experiments we are inclined to believe that hemolysins are not found in the pericardial fluid of normal dogs. The fact that some animals showed hemolysins in the pericardial fluid we would explain as a pericardial transudate in two cases, and to admixture with blood in two cases. We did not test whether it was amboceptor, or complement, or both which was absent from the fluid, altho we have evidence on this point in immune animals. Agglutinins for rabbit corpuscles may or may not be present in the pericardial fluid of normal dogs.

As will be seen from Table 1 the cerebrospinal fluid and aqueous