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THE RELATION OF THE SPLEEN TO THE FIXATION OF ANTIGENS AND THE PRODUCTION OF IMMUNE BODIES

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BY

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THE RELATION OF THE SPLEEN TO THE FIXATION OF ANTIGENS AND THE PRODUCTION OF IMMUNE BODIES.*

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I. Introductory.

As soon as it was discovered that the normal physiological activity of an animal was dependent on the immune bodies contained in its body fluids which protect it against invasion by disease-bearing organisms, investigators sought to explain the mechanism of antibody production. As a result we have to-day the well-known theories of immunity: the side chain theory of Ehrlich and the phagocytic theory of Metchnikoff. It will be recalled that Ehrlich formulated a definite method according to which antibodies are formed in the living body without specifying any organ or tissue as particularly engaged in their production, whereas Metchnikoff ascribes their production to the direct or indirect activity of the phagocytes (macrophages and microphages).

Of all the tissues of the body the blood possesses the greatest number of polymorphonuclear leucocytes (microphages). And yet Hektoen and Carlson [1] have by transfusion experiments advanced direct proof that the blood takes no direct part in the fixation of antigen (goat's or rat's corpuscles) nor in the production of immune bodies for these orpuscles. What tissue or organ fixes antigen from three to forty-ight hours after intravenous injection?

* A preliminary report of this work was made before the American Physiological Society at the New Haven meeting in December, 1910; and a brief abstract of the work published in the " Proceedings " of that meeting.

II. Literature.

Concerned as it is with the production of the blood, the hemopoietic system (spleen, lymph glands, bone marrow) has been suspected of also elaborating the various antibodies found in the blood. The experimental evidence which has been obtained by some investigators in support of this supposition has not been confirmed by others.

1. **Splenectomy.** — Bardach [2] found splenectomized animals much more susceptible to the fatal influence of pathogenic organisms or their endotoxins than normal animals. Von Kurlow [3] reported that normal animals were no more resistant towards pathogenic bacteria than asplenic animals. Blumreich and Jacoby,[4] on the other hand, found that splenectomized animals were *more* resistant to pathogenic bacteria than the normal control animals.

According to Kraus and Schiffmann [5] splenectomy had no influence on the production of precipitins (horse serum) or typhoid agglutinins. In a number of cases Deutsch [6] found that splenectomy arrested the development of typhoid agglutinins. Jakuschewitch [7] reported that the serum of splenectomized animals had a greater hemolytic action for foreign corpuscles after immunization than the serum of normal animals which were similarly immunized. Szokalski [8] did not find that splenectomy exercised a retarding influence on the formation of specific hemolysins. In four splenectomized dogs injected with rat's corpuscles Hektoen [9] reported "a lower but otherwise typical antibody curve than is usually the case under otherwise comparable conditions" (Harvey lecture, 1909–1910).

2. **The antibody content of the spleen** was studied at various periods after immunization and compared with the antibody content of the serum and other organs of the same animal. A. Wassermann,[10] Pfeiffer and Marx,[11] van Emden,[12] Jatta,[13] M. Wassermann,[14] and Cantacuzène[15] discovered specific antibodies in the spleen, bone marrow, and lymph glands some days before they appeared in the blood of the animal and concluded that these tissues elaborated them. Rath,[16] Fodor and Rigler,[17] and Deutsch [6] denied that extracts of spleen or other organs possessed a higher titre (typhoid agglutinins) than the serum.

3. **Injury of the hemopoietic organs** by various methods was practised by some in a study of this problem. Benjamin and Sluka [18] report

that exposure of rabbits to the X-rays, which are known to have a deleterious influence on the blood-forming organs, greatly checked or entirely prevented the formation of precipitins.[19] Brezina injected into animals cytotoxic sera for spleen, bone marrow, and lymph glands, and found that such animals did not develop antibodies for *b. coli* in as great a concentration as his control animals.

4. Acute hemorrhage, which *stimulates* the blood-forming organs to marked activity, has been known for some time to cause a considerable increase in the formation of antibodies.[20] This fact has more recently been confirmed by Dreyer and Walker [21] and Hektoen and Carlson.[1]

5. On histological grounds we have the observations of Cantacuzène [22] and Freymuth.[23] The former finds an enormous overproduction of mononuclear leucocytes in the lymph glands, spleen, and bone marrow after the injection of horse serum; the latter reported evidence of increased cellular activity in the bone marrow and spleen on the second and third day after immunization with *b. typhosus*.

III. Methods.

Assuming with Ehrlich that the organs which fix antigen are also intimately concerned with the production of the specific immune bodies for these antigens and knowing that the antigens (goat and rat corpuscles) are removed from the blood from three to forty-eight hours after intravenous injection, successful transplantation of the spleen from one dog immunized twenty-four hours previously with an intravenous injection of antigen into a normal dog ought to be followed by the appearance of specific antibodies in the blood of the latter *providing* the spleen played any part in the fixation of antigen and production of the immune substances. On account of the technical difficulties involved this direct method of attacking the problem was abandoned, though the operation has been successfully performed by Dr. Carrel.[24]

Operative. — The problem was, therefore, attacked indirectly by the use of the following methods:

1. *Intraperitoneal injection of "immune" spleen.*[6] The spleens of dogs immunized by a single injection of antigen were removed asep-

tically and ground up by a sterile meat grinder. The pulpy mass was suspended in warm physiological salt solution and quickly introduced into the peritoneal cavity of a normal dog. Dogs into whose peritoneal cavities we introduced spleen pulp from normal animals served as controls. The dogs were bled at various intervals for about three weeks. The sera were preserved and tested under the same conditions and on the same suspension of antigen at the end of that period.

The following considerations form the basis for this procedure. If the splenic cells fix antigen, either or both of two things may happen when the "immune" spleen is introduced into the peritoneal cavity of a normal dog. The splenic cells may escape death and give rise to the specific antibodies in the second dog; or the antigen may be split off on the death of the splenic cells and reaching the circulation may again attach itself to suitable receptor and thus stimulate the production of specific antibodies.

2. *Splenectomy.* — If the spleen takes a significant part in the production of antibodies, removal of that organ ought to markedly influence the *rate* and the *extent* of their production. In other words, an animal possessed of a spleen ought to produce the specific antibodies more quickly and in greater ultimate concentration. In all previous work, with exception of the few experiments reported by Hektoen,[9] these two points did not receive the consideration which they deserve in studying this problem.

Dogs were used in all experiments. Following splenectomy various time intervals were allowed for recovery from the effects of the operation before actively immunizing them with a single intravenous injection of goat's or rat's corpuscles; for it was thought that the longer the time allowed for recovery the greater the possibility of other organs vicariously taking up the function of the removed spleen. For each splenectomized animal we provided on the same day a control animal having the same age, weight, and size, and in order to make conditions as comparable as possible performed a laparotomy at which occasion the spleen was temporarily removed from the abdominal cavity and replaced. Under these experimental conditions the differences which were subsequently noted could be unqualifiedly attributed to the fact that one animal was bereft of a spleen; since both had been subjected to anesthesia and surgical manipulations and had synchronously recovered from the debilitating effects of an operation under identical conditions.

Collection, Preservation, and Testing of the Sera. — After immunization the animals were bled daily for the first nine days in order to study exactly the progress of the immunity. In order to get as complete an immunity curve as possible they were also bled on the twelfth, sixteenth, and twenty-first day. The various blood samples were procured by inserting a sterile hypodermic needle into one of the large superficial leg veins. Since we kept the sera three weeks before testing them on the same suspension of antigen, it was necessary to prevent marked deterioration by storing them in a cold chamber at approximately 0° C.

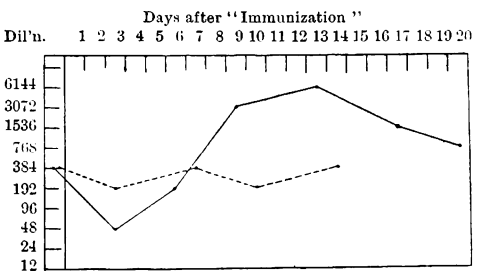

FIGURE 1. — Hemolysins for goat's corpuscles. An emulsified "immune" spleen was injected intraperitoneally into Dog A; a normal spleen was similarly introduced into Dog B. ———— = Dog A. ..... = Dog B.

On the day of testing the goat immune sera were heated to 49° C. for thirty minutes to prevent lysis of the goat's corpuscles which would interfere with the agglutinin and opsonin determinations. To determine the extent of lysis the same heated serum was recomplemented by the addition of the proper amount of fresh guinea pig's serum.[1]

IV. RESULTS.

1. **Intraperitoneal injection of "immune" spleen.** — Figs. 1 and 2 show at a glance the results obtained by this method of experimentation.

Fig. 1. Specific hemolysins for goat's corpuscles. Dog A received intraperitoneally the emulsified spleen of a dog immunized twenty-four hours previously with an intravenous injection of 1 c.c. of a 10 per cent suspension of goat's corpuscles per kilo body weight. Dog B received intraperitoneally an emulsion of spleen of a normal animal.

Fig. 2. Specific hemagglutinins for rat's corpuscles. In this experiment Dog A received the emulsified "immune" spleen. Dog B received intraperitoneally ground up bone marrow from the same dog which furnished the "immune" spleen.

In a preliminary report of this work [25] we published a similar result as regards the formation of specific agglutinins for goat's corpuscles.

In two instances we used a bacterial antigen (*b. typhosus*). The experiments were performed in the following manner. Three days previous to splenectomy Dog A received a large subcutaneous injection of *b. typhosus* killed by heating for half an hour at 60° C. On the fourth day after the injection the spleen was removed aseptically, emulsified, and injected into the peritoneal cavity of Dog B. Dog C received an intraperitoneal injection of 20 c.c. defibrinated blood of Dog A. The two dogs were bled at regular intervals. In one experiment we obtained the following result:

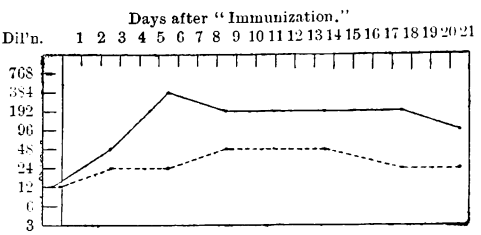

FIGURE 2. — Agglutinins for rat's corpuscles. An emulsified "immune" spleen injected intraperitoneally into Dog A; "immune" bone marrow similarly introduced into Dog B. ———— = Dog A. ..... = Dog B.

	Days after "Immunization."					
	1	3	6	9	12	21
Dog B	1/20	1/40	1/100	1/100	1/100	1/50
Dog C	1/20	1/20	1/20	1/20	1/20	1/20

A second experiment similarly performed yielded negative results.

The results of these experiments demonstrate clearly that the spleen fixes antigen. The mechanism of subsequent antibody production whether by growth of living splenic cells or a second fixation of antigen liberated by the dead or dying splenic cells remains an open question.

The same method has been employed with "immune" lymph glands, bone marrow, liver, and heart muscle. The results were practically negative. In one experiment the introduction of "immune" lymph glands into the peritoneal cavity of a dog was followed by a slight increase in the lysins for goat corpuscles. The result of an intraperitoneal introduction of "immune" bone marrow is recorded in Fig. 2. The rise noted in this experiment is only suggestive of possible results which might follow the injection of a greater amount of "immune" bone marrow than we could procure from one dog.

2. **Splenectomy.** — As above stated, we resorted to the method of splenectomy to (*a*) study daily the rate of antibody formation and (*b*) to get comparative figures on the ultimate extent of immunization in the asplenic and normal animals. In addition, we thought that the longer the time interval allowed for recovery from the splenectomy before immunization the less would be the difference between the control and splenectomized animals in these respects; and that immunization effected immediately after removal of the spleen would be followed by a slower elaboration of the immune bodies than if time were given the organism to adjust itself to the new conditions by either a hyperplasia or an increased activity on the part of the remaining tissue which is to an extent responsible for the production of the antibodies.

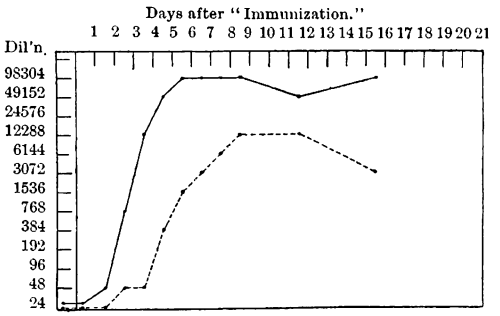

FIGURE 3. — Curves showing difference in rate of formation of lysins from goat's corpuscle and the concentration reached between a splenectomized and a control dog. ..... = Dog 7, splenectomized. ———— = Dog 8, control.

It would be both useless and tedious to discuss in detail the results obtained from nineteen splenectomized and control dogs which were immunized immediately and on the fifth, eleventh, fourteenth, sixteenth, twenty-first day, and three-quarters of a year after splenectomy and laparotomy. Our own summary which follows will show at a glance what results this method yielded with regard to the particular points under investigation. On the other hand, we thought it advisable to submit the following tables of the individual experiments together with a curve which shows graphically the general nature of our results.

As in the previous charts the figures represent the highest dilution of the serum in which the specific antibodies could be detected. *E. g.*, 6 represents a dilution of 1/6; 1536, 1/1536; 98304, 1/98304.

TABLE I.

CONCENTRATION OF THE SPECIFIC HEMOLYSINS IN THE SERA OF 8 ASPLENIC AND 7 CON

INTERVALS AFTER

Series.	Dogs.	Operation.	Days between operation and immunization.	Norm.	1	2	3
I	1	Splenectomy	0	...	...	...	1536
	2	Laparotomy	0	...	...	...	1536
	3	Splenectomy	0	...	...	...	384
	4	Laparotomy	0	...	...	...	384
	5	Splenectomy	0	...	...	...	...
II	7	Splenectomy	5	24	24	24	48
	8	Laparotomy	5	24	24	48	768
III	9	Splenectomy	11	192	192	192	192
	10	Laparotomy	11	48	48	48	384
IV	11	Splenectomy	14	...	...	96	96
	12	Laparotomy	14	...	...	48	...
V	13	Splenectomy	16	...	...	384	768
	14	Laparotomy	16	...	...	192	192
VII	19	Splenectomy	275	...	...	...	6
	20	Laparotomy	275	...	...	...	6

TABLE I.

TROL DOGS (SUBJECTED TO LAPARATOMY) IMMUNIZED WITH GOAT'S BLOOD AT VARIOUS THE OPERATION.

Days after immunization.								
4	5	6	7	8	9	12	16	21
1536	1536	3072	3072	3072	1536	6144	6144	49152
3072	6144	6144	24576	49152	49152	3072	6144	6144
384	192	192	192	1536	1536	3072	1536	3072
1536	24576	12288	98304	12288	24576	12288	1536	1536
12	24	768	768	1536	....	....	....	....
48	384	1536	3072	6144	12288	12288	3072	....
12288	49152	98304	98304	98304	98304	49152	98304	....
192	192	384	384	768	1536	3072	768	....
6144	98304	98304	98304	98304	98304	98304	24576	....
48	48	96	96	....	384	768	768	384
24	192	768	768	....	768	384	....	....
384	1536	6144	49152	....	6144	12288	12288	12288
768	49152	98304	49152	98304	4 152	49152	12288	6144
48	384	768	3072	1536	....	3072	1536	....
384	3072	3072	3 72	6144	....	3072	1536	....

TABLE II.

CONCENTRATION OF HEMAGGLUTININS IN THE SERA OF 10 SPLENECTOMIZED AND 9 CON
SPLENECTOMY OR

Series.	Dogs.	Operation.	Days between operation and immunization.	Norm.	1	2	3
I	1	Splenectomy	0	..	..	..	6
	2	Laparotomy	0	..	..	..	6
	3	Splenectomy	0	.	..	..	6
	4	Laparotomy	0	..	..	..	6
	5	Splenectomy	0	..	..	..	..
II	7	Splenectomy	5	6	6	6	6
	8	Laparotomy	5	6	..	6	24
III	9	Splenectomy	11	3	3	3	3
	10	Laparotomy	11	3	6	6	24
IV	11	Splenectomy	14	..	..	12	6
	12	Laparotomy	14	..	..	6	6
V	13	Splenectomy	16	..	..	12	6
	14	Laparotomy	16	..	..	6	12
VI	15	Splenectomy	21	..	..	24	24
	16	Laparotomy	21	..	..	24	24
	17	Splenectomy	21	..	..	24	24
	18	Laparotomy	21	..	..	24	48
VII	19	Splenectomy	275		..	..	6
	20	Laparotomy	275		..	..	24

TABLE II.

TROL DOGS IMMUNIZED WITH GOAT'S OR RAT'S BLOOD AT VARIOUS INTERVALS AFTER SIMPLE LAPAROTOMY.

Days after immunization.								
4	5	6	7	8	9	12	6	21
0	0	0	6	6	6	6	6	12
12	48	96	384	192	192	192	96	96
6	12	48	48	48	96	48	24	24
24	48	96	192	192	384	384	192	96
6	6	12	24	48	..	..	..	..
12	12	24	48	48	48	48	48	..
96	38	768	1536	1536	1536	768	768	..
6	6	12	12	96	48	48	24	..
96	1536	1536	3072	3072	3072	1536	768	..
0	0	6	6	..	24	48	48	24
12	24	48	48	..	48	48	..	..
12	6	24	48	..	96	96	48	24
12	48	192	96	..	192	96	96	48
12	..	24	48	..	384	384	192	..
24	96	192	384	..	1536	1536	768	192
48	96	96	192	..	192	384	192	192
384	384	384	384	..	768	1536	384	384
6	12	12	12	48	..	96	24	..
24	48	96	96	96	..	48	48	..

TABLE III.

CONCENTRATION OF HEMOPSONINS IN THE SERA OF 10 ASPLENIC AND 9 CONTROL SPLENECTOMY OR

Series.	Dogs.	Operation.	Days between operation and immunization.	Norm.	1	2	3
I	1	Splenectomy	0	..	..	..	24
	2	Laparotomy	0	..	..	..	12
	3	Splenectomy	0	..	..	..	6
	4	Laparotomy	0	..	..	..	24
	5	Splenectomy	0	..	..	..	..
II	7	Splenectomy	5	3	3	3	3
	8	Laparotomy	5	6	12	12	48
III	9	Splenectomy	11	6	6	6	12
	10	Laparotomy	11	12	12	24	96
IV	11	Splenectomy	14	.	..	0	0
	12	Laparotomy	14	..	..	0	0
V	13	Splenectomy	16	..	..	0	0
	14	Laparotomy	16	..	.	3	3
VI	15	Splenectomy	21	..	..	12	12
	16	Laparotomy	21	..	..	6	24
	17	Splenectomy	21	..	..	12	12
	18	Laparotomy	21	..	..	12	12
VII	19	Splenectomy	275	..	..	..	6
	20	Laparotomy	275	..	..	..	6

TABLE III

DOGS IMMUNIZED WITH GOAT'S OR RAT'S BLOOD AT VARIOUS INTERVALS AFTER LAPAROTOMY.

Days after immunization.								
4	5	6	7	8	9	12	16	21
24	24	24	48	48	24	6	48	48
12	24	24	48	48	48	24	24	48
12	12	12	24	48	24	24	24	24
24	48	48	96	192	192	96	192	..
6	6	6	24	24	..	..	..	..
3	6	12	24	48	48	48	48	..
48	48	..	192	384	384	96	192	..
12	12	12	12	24	24	48	96	..
96	384	384	768	384	192	192	192	..
0	0	0	3	..	6	6	6	6
0	0	3	6	..	6	6	..	..
6	6	12	24	..	24	48	24	24
(	12	24	24	..	24	48	48	24
12	24	..	48	..	96	96	48	..
48	192	192	768	..	768	768	384	192
12	24	24	48	..	192	192	96	96
24	24	48	96	..	192	384	192	192
6	12	24	24	24	..	48	24	..
12	24	48	48	96	..	48	48	..

Summary of results obtained by splenectomy. — In every instance the control animals developed the specific antibodies more rapidly and in higher concentration than did the corresponding splenectomized animals. We have plotted composite antibody curves of all the asplenic and control animals and for ready comparison have transferred them to the same chart so that the marked difference can be seen at a glance.

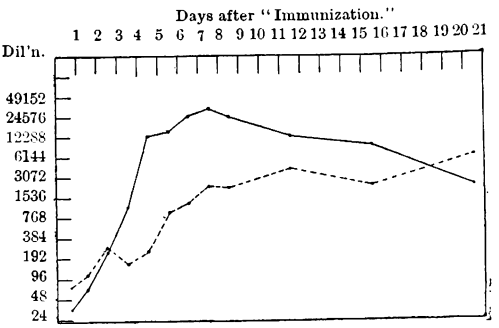

FIGURE 4. — Composite hemolysin curve of 7 control and 8 splenectomized animals, showing marked difference in the rate of formation and in concentration reached. ———— = 7 control dogs. ..... = 8 splenectomized dogs.

Fig. 4 shows the composite curves of the hemolysins. We call especial attention to the following two points:

1. The ultimate concentration of the hemolysins in the sera of the control animals (seven dogs) is much higher than in the sera of the splenectomized animals (eight dogs). If the tables are consulted, it will be found that on twenty-seven days the serum of the control animals laked goat's corpuscles in a dilution of 1/24,576 or over. On fourteen of the twenty-seven days the serum of these animals was found to possess lytic action in a dilution of 1/98,304 and on eight days was found active in a dilution of 1/49,152. Comparing these results with the splenectomized animals, we find that on twelve days only was the serum active in a dilution of 1/6144 or over. From the figure we see that the serum of the asplenic animals possessed only one-fourth the titre of the controls at the height of immunity (1/24,576 versus 1/6144).

2. The *rapidity* with which the control animals produced the hemolysins is very striking when it is compared with the rate of formation by the splenectomized animals which normally laked in a higher dilution. In one instance the rise is distinctly abrupt; in the case of the splenectomized dogs the rise to the point of maximum concentration is more drawn out.

Fig. 5 shows the same general results as regards the hemagglutinins. The more abrupt ascent of the curve of the control animals

shows how much more rapidly the dogs possessed of a spleen formed the specific agglutinins. On the fifth day the serum of the control animals caused agglutination in a dilution of 1/96. Four days later the serum of the splenectomized dogs was found active in a dilution of 1/48. On that day and on the preceding day the serum of the control animals had eight times that value.

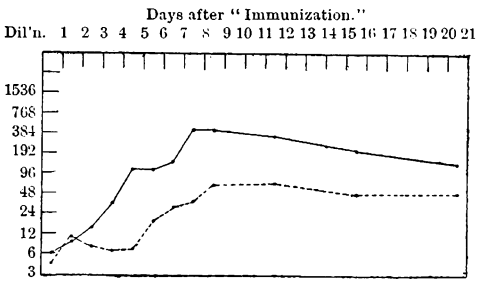

FIGURE 5. — Composite hemagglutinin curve of 9 control and 10 asplenic dogs, showing marked difference in the rate of formation of antibodies and in the ultimate concentration reached. ———— = 9 control dogs. . . . . = 10 asplenic dogs.

The composite curves of the hemopsonins show the same relationship between the controls and the splenectomized animals. A glance at Fig. 6 shows how slowly the asplenic animals formed opsonins. On the eighth day of immunity the serum of the control dogs was at least four times as active as the serum of the splenectomized animals.

In many of the individual experiments the height of the immunization was reached by the normal dogs several days earlier than by the asplenic dogs (see Fig. 3. Also hemagglutinins of Dogs 9 and 10, and the opsonins of Dogs 15 and 16).

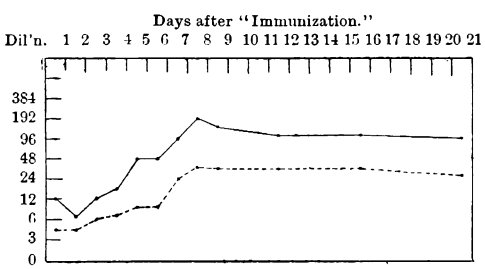

FIGURE 6. — Composite hemopsonin curve of 9 control and 10 asplenic dogs, showing differences in rate of formation of antibodies and in ultimate concentration reached. ———— = 9 control dogs. . . . . = 10 asplenic dogs.

These results, which show conclusively that normal animals produce specific hemolysins, hemagglutinins, and hemopsonins more rapidly and in greater concentration than the corresponding splenectomized animals, admit of but one interpretation, namely, that the spleen takes a very active part in the elaboration of these particular immune bodies. The possibility that normally in active immunity the spleen stimulates the antibody-

producing organs to activity by means of some internal secretion or hormone does not merit serious consideration, since we have shown that the spleen fixes antigen.

We are not, however, justified in attributing to the spleen the same function when we consider bacterial antigens and their specific antibodies. If the spleen destroys erythrocytes in the normal intact animal, we could have reasoned *a priori* that it would also destroy those foreign corpuscles which the experimenter chose to inject into the blood stream. But, being foreign to the organism, the spleen would react towards this foreign corpuscle (corpuscular antigen) by the overproduction of specific destructive agents — the antibodies in question. The evidence presented in this paper supports this hypothesis. We have, however, no well-founded reasons for supposing that the spleen reacts in like manner towards bacterial antigen. In one experiment we could demonstrate that the spleen fixed bacterial antigen (*b. typhosus*). In another instance, however, we obtained negative results.[11]

It will be recalled that the animals were immunized at various periods after splenectomy and laparotomy to determine whether in the absence of the spleen other tissues or organs would sooner or later take up the function of that organ. We have no evidence on this point which is conclusive. Our methods revealed only *gross* differences in the rate of antibody formation and extent of immunization. Such a compensatory hyperactivity on the part of other organs possibly exists. In one instance (Table VII) we found that a complete compensation had not been in effect three-quarters of a year after splenectomy. We are, nevertheless, inclined to believe from a study of our results that other organs or tissues eventually take up the function of the removed spleen. A great number of experiments with only a slight difference in strength of each successive dilution of the serum will settle this point.

V. Conclusions.

1. **The spleen fixes antigen.** — When an optimum dose of antigen (goat's or rat's blood) is injected intravenously into a dog, the antigen is partly fixed by the spleen; for if the spleen of the dog is removed, emulsified, and introduced into the peritoneal cavity of a normal dog,

the specific immune bodies appear in the serum of the latter. The introduction of normal spleen into the peritoneal cavity is not followed by an increase of the antibodies in the serum of the recipient. The introduction of "immune" heart muscle, liver, bone marrow, and lymph glands did not give positive results.

2. **The spleen is concerned, directly or indirectly, in the immune bodies.** — Asplenic dogs no not produce hemolysins, hemagglutinins, or hemopsonins (*a*) as rapidly nor (*b*) in as high a concentration as the corresponding control dogs.

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