THE EFFECT OF SPLENECTOMY UPON THE PRODUCTION OF ANTIBODIES *

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Contradictions displayed by the experimental results concerning the part of the spleen in the production of specific antibodies disallow generalizations in this regard. On the one hand, findings are recorded which indicate that antigens are localized in the spleen;[1] that antibodies are detected in the spleen earlier than in the blood;[2] that later in the course of their production the concentration of antibodies in the spleen is greater than in the blood;[3] and finally that the removal of spleen reduces the power of an animal to produce antibodies.[4] On the other hand, an equal number of investigations are advanced to show that the spleen does not display a concentration of antibody greater than that of the blood;[5] and that the removal of the spleen does not decrease the degree of antibody production.[6]

These contradictions in experimental results are doubtless in large measure due to differences in the materials and methods employed by the various investigators, but the confusion which exists demands more systematic quantitative experimentation and above all an analysis of the changes produced, in terms of cell activity.

It is the purpose of this paper to present results which I have obtained in modifying antibody production in rabbits by the removal of the spleen. The findings relate to the production of specific hemolysins for sheep erythrocytes in healthy full-grown rabbits, and fall under two headings: I. Changes in the Degree of Antibody Production, and II. Changes in the Site of Antibody Production.

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I. Changes in the Degree of Antibody Production

Reference has already been made to the fact that certain observers believe splenectomy to decrease antibody production, while others contend that no such change results.

I have found that with the same materials, either of these results may be produced. The determining factor is the amount of antigen employed. When a small antigen dose is used, the antibody is produced less rapidly and to a less degree in splenectomized rabbits than in non-splenectomized animals. On the other hand, when larger doses of antigen are employed the production of antibody is of the same degree in splenectomized and non-splenectomized rabbits.

The following table (Table I) shows the difference in the antibody content of the serum of splenectomized and non-splenectomized rabbits five days following two intravenous injections of 2 c.c. of a 50% suspension of washed erythrocytes. The titrations were made with inactivated serum, 1. c.c. of 5% erythrocytes and twice the minimal activating dose of pooled guinea pig serum.

Table I. — Comparative Antibody Titer, Splenectomized and Non-Splenectomized Rabbits

Number of Rabbit	Two injections 2 c.c. 50% sheep erythrocytes 10 hrs. apart. Serum drawn 5 days later		
	Lapse between Laparotomy and Injection	Hemolytic Doses in 1 c.c. Serum	Average Titer
Splenectomized			
S94	21 days	100	42
S97	17 "	10	
S98	15 "	20	
S100	14 "	40	
Non-splenectomized			
N12	21 days	200	250
N13	21 "	200	
N14	14 "	400	
N15	6 hrs.	200	

In the above table it may be observed that with the relatively small amount of antigen employed, the amount of antibody present in the blood on the fifth day, was six times greater in the non-splenectomized rabbits than in those from which the spleen had been removed.

This observation shows that in the initial response the antibody content of the serum is much greater in the rabbits with spleens. Further, the following experiment (Table II) with continued observations over a period of forty-eight days, shows that the great difference between the splenectomized and non-splenectomized rabbits is not confined to the early stages of antibody production but holds throughout the process.

TABLE II. — COMPARATIVE ANTIBODY TITER, SPLENECTOMIZED AND NON-SPLENECTOMIZED RABBITS

Number of Rabbit	One Injection .5 c.c. 50% Sheep Erythrocytes								
	Lapse between Splenectomy and Injection	Hemolytic Doses in 1 c.c. Serum drawn on given days following injections							
		2	4	6	10	15	20	25	48
Splenectomized									
S86	28 "	0	0	40	100	40	20	20	10
S101	21 "	0	0	0	40	13	10	4	2
S102	10 "	0	0	10	28	10	10	10	2
Non-splenectomized									
N16	.....	0	1	666	1000	400	285	133	66
N17	.....	2	20	1000	2860	1000	400	400	133
N18	.....	28	40	133	400	200	133	133	40
Average Titer ...	Splenectomized	0	0	17	56	21	13	11	5
	Non-splenectomized	10	20	600	1400	530	240	220	80

In the above table it is seen that, judged by the average content of the serum, the animals with spleens produced throughout the period of observation approximately twenty fold the amount of antibody displayed by the animals without spleens.

In contrast to the above results are those obtained when large amounts of antigen are employed. The following experiment (Table III) shows that when a total of twenty cubic

TABLE III. — COMPARATIVE ANTIBODY TITER, SPLENECTOMIZED AND NON-SPLENECTOMIZED RABBITS

Number of Rabbit	Four Injections 5 c.c. 50% sheep erythrocytes 12 hours apart. Serum drawn 5 days after last injection		
	Lapse between Splenectomy and last Injection	Hemolytic doses in 1 c.c. Serum	Average Titer
Splenectomized			
S85	14 days	1000	730 (Splenectomized)
S86	14 "	100	
S87	9 "	400	
S88	9 "	1000	
S90	6 "	1000	
S91	4 "	1000	
S92	4 "	1500	
S93	1 day	40	
Non-splenectomized			
N9	....	400	700 (Non-splenectomized)
N10	....	400	
N11	....	1000	
N23	....	1000	
N24	....	400	
N25	....	1000	
N26	....	1000	

centimeters of the same antigen used in the previous experiments is employed, the average antibody content of the serum on the fifth day following the last injection is approximately the same in the splenectomized and the non-splenectomized rabbits.

The following experiment (Table IV) in which also a relatively large amount of antigen was used and in which the splenectomy was performed months prior to injection, shows that the equality in serum antibody content in the two groups of animals obtains throughout the course of active antibody production.

From the series of experiments given below, the fact is apparent that rabbits from which the spleen has been removed display much less antibody in their serum in response to the injection of a small amount of antigen than do rabbits from which the spleen has not been removed, — but that, when a

TABLE IV. — COMPARATIVE ANTIBODY TITER, SPLENECTOMIZED AND NON-SPLENECTOMIZED RABBITS

Number of Rabbit	Four Injections of 2 c.c. 50% sheep erythrocytes in 8 days				
	Laspe between Splenectomy and Injection	Hemolytic Doses in 1 c.c. Serum drawn on given Week following last Injection			
		1	2	3	5
Splenectomized					
S10	330 days	1000	2000	1000	200
S14	328 "	400	400	....	...
S33	270 "	2000	1000	400	200
S60	240 "	2000	2000	1000	400
S76	233 "	200	400	200	100
S80	228 "	400	200	200	100
Non-splenectomized					
N1	.....	1000	1000	400	200
N2	.....	2000	1000	1000	400
N3	.....	1000	2000	1000	400
N4	.....	400	400	200	200
N5	.....	400	400	200	200
N6	.....	1000	1000	400	100
N7	.....	2000	2000	1000	200
N8	.....	400	400	200	100
Average Titer	Splenectomized	1000	1000	560	200
	Non-splenectomized	1025	1025	550	225

large amount of antigen is employed, the display of antibody in the serum of splenectomized rabbits is equal to that of the non-splenectomized animals.

The significance of this finding will be discussed in connection with the observations reported in the following section.

II. CHANGES IN THE SITE OF ANTIBODY PRODUCTION

The fact that cellular antigens are very rapidly removed from the blood stream by fixed-tissue phagocytes was demonstrated by Kyes [7] in his studies upon the fate of pneumococci in birds. He found that where there is great activity on the part of the fixed-tissue phagocytes, there is a correspondingly great antibody production. It is the view of Kyes, that the specific antibodies are for the most part products of the par-

ticular fixed-tissue phagocytes which ingest the antigens and are substances elaborated primarily for the intracellular digestion of the phagocytosed protein antigen.

Cary,[8] working in the same laboratory, studied the fate of foreign erythrocytes injected into rabbits and demonstrated that here too the antigenic cells are rapidly removed from the blood stream by fixed-tissue phagocytes in the spleen and liver. In a second investigation, Cary[9] showed, by extraction methods, that just those organs which are rich in active fixed-tissue phagocytes are correspondingly rich in specific antibody content. He supported Kyes' view that fixed-tissue phagocytes are active producers of antibodies.

In a previous publication,[10] I have confirmed Cary's findings concerning the destruction of foreign erythrocytes by macrophages in the spleen and liver, and have shown further that a similar destruction occurs also in the bone marrow. In fact the destruction in the latter tissue is more extensive than in the liver.

Concerning the physiological phagocytic destruction of the rabbit's own erythrocytes which normally is limited to the spleen, I have shown that upon removal of the spleen, this function is assumed by fixed macrophages in the bone marrow and in the liver, and I have further shown that in the absence of the spleen the activity of these fixed-tissue phagocytes is much greater in the destruction of *foreign* erythrocytes than in the normal animal — especially in the bone marrow.

If now, the active fixed-tissue phagocytes ingest foreign erythrocytes and produce antibodies to them and if upon splenectomy, the number of such active phagocytes is increased in the liver and bone marrow, it might well be expected that the amount of antibody demonstrable in these two locations following the injection of foreign erythrocytes would be greater in splenectomized than in non-splenectomized rabbits.

That such is actually the case I have been able to demonstrate and offer this further evidence in support of Kyes' view that antibodies are produced by fixed-tissue phagocytes.

In the experiments leading to this conclusion, I injected sheep erythrocytes into splenectomized and non-splenectomized rabbits and by extraction methods determined the relative antibody content in the various tissues under consideration. The method of extraction was essentially the same as that used by Cary. A weighed amount of tissue was placed in a heavy metal tube and rapidly frozen by immersing the tube in liquid air. After thawing, the tissue was finely ground in a mortar with sterile sea sand, and to it were added fourteen parts of 50 per cent glycerine in salt solution. The resulting emulsion was transferred to a sterile bottle and to it was added a small amount of chloroform and sufficient tuluol to form a substantial covering film. The bottle was tightly corked and incubated at 37°C. for six days, being shaken several times daily. At the close of the extraction, the total emulsion was centrifugalized and the aqueous solution pipetted off. This solution was again thoroughly centrifugalized until perfectly clear; was heated at 56°C. for thirty minutes and finally titrated for its specific hemolytic action upon sheep erythrocytes in the presence of twice the minimal activating dose of pooled guinea pig serum. As a routine the fresh serum was titrated for hemolytic value, as was also an extraction of the serum carried out in parallel with that of the cellular tissues. It was found that this treatment did not appreciably deplete the antibodies of the serum.

The following experiment in which the described method was employed, shows the amount of antibody extracted from tissues and serum of splenectomized rabbits compared with the amount obtained from the same source in non-splenectomized animals.

Six rabbits (3 splenectomized) were injected with 12 c.c. of 50% sheep erythrocytes, 3 c.c. being given intravenously every twelve hours for two days. Nine days following the last injection, the animals were killed by exsanguination from the carotid artery. Weighed amounts of tissue and serum were subjected to extraction and a hemolysis titration made of the fresh serum. Six days later the several extractions, diluted one-half, were titrated for specific hemolysin content. Because

of the dilution occasioned by addition of the extraction fluid, the hemolysin could only be determined when one gram of the tissue yielded at least thirty hemolytic doses and a yield below this amount was therefore neglected in tabulating the comparisons. The decimals given in the table following, designate the gram weight of tissue or serum which yielded one complete lytic dose of the specific hemolysin. The hemolysin was determined to be specific for sheep erythrocytes and operated only when activated by complement. Control extractions of the corresponding tissues of normal rabbits yielded no hemolytic substances.

TABLE V. — COMPARATIVE ANTIBODY CONTENT OF TISSUES OF SPLENECTOMIZED AND NON-SPLENECTOMIZED RABBITS

Number of Rabbit	Four Injections of 3 c.c. 50% Sheep Erythrocytes in 48 hours. Tissues taken 5 days following last Injection			
	Amount of each Tissue which yielded one hemolytic Dose			
	Serum	Spleen	Bone Marrow	Liver
Non-splenectomized				
19	.001 gm.	.0033 gm.	....	....
20	.0005 gm.	.0005 gm.	....	....
21	.003 gm.	.01 gm.	....	....
Splenectomized				
S103 (13 days)	.0005 gm.	....	.001 gm.	.01 gm.
S104 (9 days)	.008 gm.	....	.03 gm.	....
S105 (9 days)	.003 gm.	....	.01 gm.	....

Consideration of the above table shows that of the tissues under discussion, the spleen and serum only, in the case of the unoperated animals, contained a demonstrable amount of antibody: the bone marrow and liver did not. In the case of the splenectomized animals, on the other hand, the bone marrow was uniformly rich in antibody content and the liver in one instance (S 103) showed a concentration equal to that found in the spleen of one of the unoperated animals (21).

In these findings can be seen proof that splenectomy, which results in the shift of the phagocytic destruction of foreign erythrocytes to the fixed-tissue phagocytes of bone marrow and liver, occasions a corresponding shift in antibody formation

to those locations. This I interpret to mean that the cells which ingest and destroy foreign erythrocytes, as antigen, are the producers of the specific antibody.

It has already been stated that when a relatively small amount of foreign erythrocytes is injected into rabbits as antigen, the resulting antibody content of the serum may be considerable in the animals with spleens but is very slight in splenectomized animals. In such instances, it might be expected from the previous results, that the animals with spleens would display a considerable concentration of antibody in that organ but that the splenectomized animals would show little or no antibody concentration in the bone marrow and the liver as sites of antibody production. Such is the case. Eight rabbits (four splenectomized) were twice injected in ten hours with 2 c.c. of 50% sheep erythrocytes and their tissues removed for extraction five days later. The results of extraction expressed as in the preceding table, gave the following values (Table VI).

TABLE VI. — COMPARATIVE ANTIBODY CONTENT OF TISSUES OF SPLENECTOMIZED AND NON-SPLENECTOMIZED RABBITS RECEIVING SMALL ANTIGEN DOSE

Number of Rabbit	Two Injections of 2 c.c. 50% Sheep Erythrocytes in 10 hours. Tissues taken 5 days later			
	Amount of each tissue yielding one hemolytic Dose			
	Serum	Spleen	Bone Marrow	Liver
Non-splenectomized				
12	.005 gm.	.005 gm.	....	....
13	.005 gm.	.005 gm.	....	....
14	.0025 gm.	.0025 gm.	....	....
15	.005 gm.	.008 gm.	....	....
Splenectomized				
S94 (21 days)	.01 gm.	....	.033 gm.	....
S97 (17 days)	.1 gm.	....	....	....
S98 (15 days)	.05 gm.	....	....	....
S100 (14 days)	.025 gm.	....	....	....

This table shows that with the relatively small antigen injection, the rabbits with spleens displayed a relatively high serum titer and a correspondingly great amount of antibody in

the spleen, whereas the splenectomized animals, which had a low serum titer, had a scarcely demonstrable amount of antibody in the bone marrow and liver, the site of antibody production with larger doses of antigen.

In the previous section of this paper it was pointed out that with small doses of antigen the resulting antibody content of the serum is distinctly less in animals deprived of the spleen; but that when a relatively large amount of antigen is injected, the serum antibody content is the same in splenectomized and non-splenectomized animals. The explanation of these occurrences is not clear, but it is not unlikely that when the amount of antigen used is so small that a great proportion of it is taken up in the spleen, the antibody there produced is more extensively turned into the blood stream than it is in the splenectomized animals where the same amount of antigen is distributed to a greater number of phagocytic cells in the total bone marrow, liver and possibly other tissues, and where the conditions of blood flow are less favorable for the transfer of the intracellular antibody to the plasma. According to this view when a large amount of antigen is employed, the spleen ceases to be a determining quantitative factor in that the great bulk of the antibody is produced elsewhere under similar conditions, so that no distinct contrast occurs in the resulting total serum content of antibody in animals with and without spleens. For the present this explanation must be regarded definitely as conjecture. Such an explanation does however coincide with the facts now at hand and it must be borne in mind that the concentration of antibody in the blood is at best only indirect evidence as to the total amount of antibody elaborated by, and contained within, the essential producing cells.

In this connection I wish to record a series of experiments in which rabbits were given a "preparatory" injection of antigen, allowed a long rest period with almost complete disappearance of the antibody from the blood, and then injected with a single dose of antigen. The animals were killed three days following the final injection and their tissues extracted. In this series eight rabbits were used, four being splenectomized.

Three animals of each group received a single small "preparatory" injection and the remaining one in each group was given eight times that dose. After the rest period, all eight animals received the same final dose of antigen.

In both groups the animal which received the large preparatory dose displayed a much greater content of antibody in the tissues in response to the final antigen injection than did the three animals which received the small preparatory dose.

It would seem from these results that when a great number of fixed-tissue phagocytes are once engaged by a large amount of antigen, the animal, either with or without the spleen, is for a long period capable of a greater antibody response to a "fresh" antigen injection than normally or than when prepared by a small dose of antigen. In tabulated form the results are given below.

In Table VII it appears that the antibody display following a single uniform injection of antigen after "preparatory" injection and a long rest period, was much greater in rabbits which were "prepared" by large doses of antigen (N_2 and S 76) than in those receiving the smaller initial dose. Furthermore this display was most evident at the locus of maximum fixed-tissue phagocytosis, namely in the spleen of the unoperated animal (N_2) and in the bone marrow of the splenectomized rabbit (S76). In the case of this last animal it is of interest to note the very great concentration of antibody in the bone marrow in comparison with that of the serum.

Throughout these experiments evidence is gained that fixed-tissue phagocytes participate in the production of specific antibodies and that when by any procedure, the extent or site of their activity is modified, there results a corresponding change in the extent or site of antibody production.

The two major conclusions which I wish to make are:

I. That the removal of the spleen from rabbits profoundly modifies the production of specific hemolysins, both as to the site and as to the concentration in the blood stream. When, however, the amount of antigen employed is large, the resulting concentration of the antibody in the serum is equal in splenectomized and non-splenectomized rabbits.

TABLE VII. — COMPARATIVE ANTIBODY CONTENT OF TISSUES OF RABBITS "PREPARED" WITH LARGE AND WITH SMALL INJECTIONS OF ANTIGEN

Number of Rabbit	Preparatory Antigen* Injection	Rest Period	Serum Titer before final Injection	Final Antigen* Injection	Amount of Each Tissue which yielded one hemolytic Dose, 3 days after final injection				
					Fresh Serum	Extracted Serum	Spleen	Bone Marrow	Liver
Non-splenectomized									
N2	4 inj. of 2 c.c. in 7 days	21 weeks	.008 gm.	5 c.c.	.00025 gm.	.00025 gm.	.00005 gm.	.01 gm.	.033 gm.
N16	.5 c.c.	10 "	.015 gm.	5 c.c.	.0008 gm.	.0008 gm.	.0008 gm.	...	...
N17	.5 c.c.	10 "	.01 gm.	5 c.c.	.0008 gm.	.0008 gm.	.00025 gm.	...	...
N18	.5 c.c.	10 "	.025 gm.	5 c.c.	.001 gm.	.001 gm.	.0008 gm.	...	...
Splenectomized									
S76	4 inj. of 2 c.c. in 7 days	21 weeks	.05 gm.	5 c.c.	.005 gm.	.005 gm.		.0016 gm.	.025 gm.
S86	.5 c.c.	10 "	.1 gm.	5 c.c.	.01 gm.	.01 gm.		.015 gm.	...
S101	.5 c.c.	10 "	.5 gm.	5 c.c.	.0025 gm.	.0025 gm.		.015 gm.	.025 gm.
S102	.5 c.c.	10 "	.5 gm.	5 c.c.	.05 gm.	?		.033 gm.	...

II. That — when by splenectomy the macrophage activity of the bone marrow and liver is increased, there is a corresponding increase in the antibody-producing power in these tissues.

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