CONDITIONS INFLUENCING THE DISAPPEARANCE OF LIVING BACTERIA FROM THE BLOOD STREAM

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PLATES 5 AND 6

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It is generally agreed that bacteria introduced intravenously into animals are quickly removed from the blood stream and localized or "fixed" in the phagocytic cells, especially those of the reticulo-endothelial system. The primary removal apparently follows more or less closely the conditions governing the disposal of particulate material under similar circumstances. The rate of disappearance and the ultimate fate depend upon the number and type of microorganisms injected, their pathogenicity for the animal, the functional state of the phagocytes and, indirectly, of the body as a whole.

Various investigators have determined some of the conditions, *e.g.*, hunger, food ingestion, disease, principal sites of localization, which affect or modify the removal of bacteria from the blood stream in man and normal laboratory animals (1). But, aside from the few preliminary experiments of Hopkins and Parker (2) and Parker and Franke (3), practically no work has been reported concerning the effects of specific active immunization upon the removal of bacteria from the circulating blood. We wish to report the results of studies of this sort, emphasizing particularly certain morphological aspects of the problem.

Materials and Methods

Adult rabbits, usually males, were immunized by the intravenous injection of a 24 hour growth on agar slants of microorganisms suspended in 0.85 per cent solution of sodium chloride and heated at 60°C. for 1 hour. One series of animals was immunized against *Staphylococcus albus* and another series against *Bact. paratyphosum* B. As a rule, a total of from 4 to 6 cc. of the suspension was given in daily injections of 1 cc. each. After a period of time varying usually from 1

to 3 weeks, these rabbits and normal ones of approximately the same size were simultaneously given intravenous injections of equal quantities of a 24 hour broth culture of the homologous living bacteria in order to determine the comparative rates of disappearance from the blood stream. Broth cultures were used so that the bacteria might be in a good state of dispersion with as few clumps as possible.

At intervals of 5, 15, 30, 45, 60 minutes, etc., blood was taken with aseptic precautions from the heart of the normal and the immune animal and plated in duplicate amounts in infusion agar, pH 7.3 to 7.5., the plates incubated at 37°C. and colony counts made at the end of 48 hours. Dilutions were required only in the sample of blood withdrawn 5 minutes after the intravenous injection. Such a method is subject to the usual error of interpretation that each colony does not necessarily represent one bacterium; therefore the results are recorded as the number of colonies per cubic centimeter of blood.

Smears and sections of the various organs and tissues were also made at different intervals in order to observe morphological changes as well as variations in concentration of the bacteria in the tissues of the normal and immunized animals For sections, the tissues were fixed in Zenker-formol solution, embedded in celloidin, sectioned at 10 microns and stained with hematoxylin-eosin-azur 11 and by the Claudius modification of the Gram stain (4).

We used a constant dosage of bacteria rather than a definite quantity per kilo of body weight because of the probability that much of the variation in weight may be due to unequal quantities of food in the gastrointestinal tract, different amounts of adipose tissue and differences in size of bone. For example, we found that in eight pairs of rabbits in which the initial differences in weight varied from 10 to 30 gm., the corrected weights after removal of the gastrointestinal tract ranged from 40 to 225 gm. There is necessarily an element of error no matter which procedure is followed but we believe that the injection of a constant dosage insures a more comparable method. In most of our experiments we have also determined the weights after the removal of the gastrointestinal tracts and have thus been able to analyze the results more satisfactorily.

Numerous experiments on relatively large numbers of animals are advisable in order to rule out individual variations in reactivity of "normal" animals. It is doubtful whether rabbits that have been infected with coccidia or have had snuffles or infected wounds are really normal in so far as the reactivity of macrophages is concerned. Furthermore, differences in nervous states, especially of the autonomic nervous system, as emphasized particularly by Müller (5) and Petersen (6), necessitate recognition of the importance of uniformity of handling, feeding and routine care so that reactivity may be as constant as is experimentally possible. In later stages of our work much less variation occurred, owing to the use of rabbits reared under exceptionally favorable conditions, and known to be absolutely free from "snuffles" and practically free from coccidial infection.

The Passage of Staphylococci through the Lungs of Normal and Immune Rabbits

Inasmuch as opinions vary concerning the relative importance of the lungs in the removal of bacteria from the blood stream, it is desirable to know just how and in what numbers microorganisms pass through this area. To determine this, equal amounts of a suspension of *Staphylococcus albus*, usually from 1 to 3 cc. of a 24 hour broth culture, were injected simultaneously into the marginal ear veins of normal and of actively immunized rabbits. At exactly 2 minutes, 4 minutes, 5 minutes and at later intervals up to 1 hour, the lungs were fixed in Zenker-formol solution, sectioned and stained.

Fig. 1 illustrates the findings in a normal rabbit 2 minutes after intravenous injection. This is a camera lucida drawing of two pulmonary veins and shows the staphylococci evenly dispersed in the lumina with occasional ones engulfed by leucocytes. The bacteria are compact and well stained, show no evidence of any agglutinative tendency and are apparently passing rapidly and extracellularly through the lungs on their way to the general systemic circulation. In the immunized animal at this period there was no increased number of bacteria within the pulmonary tissues, and although polymorphonuclear leucocytes were present in greater numbers than normal, practically none contained staphylococci. In neither rabbit were mononuclear cells particularly active in phagocytosis of the microorganisms. Sections taken from the lungs of normal and immune rabbits 4 and 5 minutes and later after injection showed practically no extracellular microorganisms and only a few in polymorphonuclear leucocytes and macrophages.

It is evident from these experiments that the countless numbers of staphylococci in the venous blood stream pass almost instantaneously through the blood vessels of the lungs of both normal and immune rabbits. A comparatively small number are caught within the capillaries; this may be due to mechanical obstruction or chance collisions with leucocytes. The bacteria show no evidences of agglutination or swelling such as will be seen in the livers and spleens of these same rabbits. We conclude that under the conditions of our experiments the lungs play an insignificant part in "fixing" such antigen as staphylococci.

These results agree with those of Werigo (7) in his studies of anthrax in the rabbit, in which he found that within 8 minutes after intravenous injection numerous bacilli had been engulfed by polymorphonuclear leucocytes within the lungs, whereas the pulmonary macrophages played little if any part in the reaction. Within 3 hours, however, very few bacilli were seen in the lungs. Werigo believed that the microorganisms were momentarily caught mechanically, were phagocytosed by polymorphonuclear leucocytes and were then transported to the liver and spleen. On the other hand, Hopkins and Parker (2) observed that streptococci injected intravenously into cats were removed from the blood stream within from 10 to 30 minutes and were found most abundantly in the lungs, more being within macrophages than polymorphonuclear leucocytes. They observed no evidence of agglutination of the bacteria within the pulmonary tissues, neither in films of the blood, crushed preparations nor in sections. Their experiments with the injection of streptococci into rabbits, however, agree with Werigo in that the microorganisms tended to localize in the liver and spleen rather than in the lungs. Arima (1) also found very few live bacteria in the lungs of rabbits 5 minutes after the intravenous injection of *Staphylococcus aureus*, *B. coli* and *B. typhosus*. He assumed that they were either killed by the bactericidal action of the blood or were transported to other organs.

The Disappearance of Living Bacteria from the Blood Stream of Normal and Actively Immunized Rabbits

The relative rates of removal of living staphylococci and paratyphoid bacilli from the blood streams of normal and of actively immunized rabbits were determined by the methods described above. Thirty-two pairs of animals were tested. The characteristic response is shown in Chart 1, there being a uniformly accelerated rate of removal of the bacteria from the blood stream of the immune animal. There seems to be a distinct acceleration in rate of removal of the bacteria in the normal rabbit after from 15 to 30 minutes, but in most instances there are, comparatively, many more bacteria per cubic centimeter of blood in the normal animals, even as late as 75 minutes after injection. The time of complete disappearance was not determined, but most workers agree that practically all bacteria, regardless

of virulence, are primarily removed from the blood stream within a few hours.

The actual data from sixty of these rabbits are given in Tables I and II. Seventeen of the animals were immunized against *Staphylococcus albus* and compared with seventeen normal rabbits as to the comparative rates of removal of living staphylococci of the same strain, while thirteen others were immunized against *Bact. paratyphosum* B and similarly compared with thirteen normals. With the exceptions

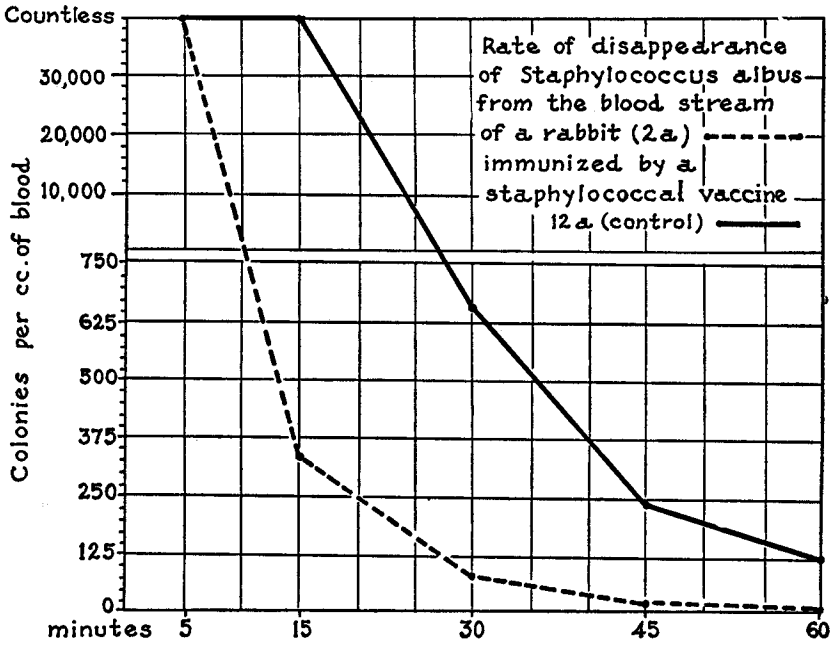

CHART 1

of Rabbits 9 and C28, and 46 and C66, in Table I, all of the immune animals have a lower colony count per cubic centimeter of blood than have their corresponding controls. These exceptions may be due to experimental error or to abnormal reactivity of the supposedly normal animals.

An analysis of the comparative weights of the animals after the removal of the gastrointestinal tracts (Table I) shows that eight of the immune rabbits weighed at least 20 gm. more than their controls while

TABLE I

The Disappearance of Staphylococci from the Blood Stream as Influenced by Previous Intravenous Immunization with a Staphylococcus Vaccine

Rabbit	Weight* 1 2	Time after termination of immunization	Time after intravenous injection, of cardiac puncture	Colonies per cc. of blood
	gm.	*days*	*min.*	
50	2,245–1,980	24	5	12,000
C70	2,290–1,875	—	5	Countless
5	2,240–1,700	15	16	590
C24	2,310–1,805	—	15	2,395
11	2,210–1,710	20	20	175
C30	2,165–1,690	—	17	1,400
12	2,250–1,780	20	19	20
C31	2,110–1,625	—	17	295
40	2,120–1,680	11	16	885
C60	2,150–1,715	—	15	990
41	1,985–1,590	11	15	117
C61	1,940–1,535	—	15	203
9	2,205–1,815	18	31	50
C28	2,195–1,730	—	30	28
10	2,090–1,615	18	33	27
C29	2,020–1,695	—	32	655
42	2,535–2,010	15	32	30
C62	2,545–2,110	—	30	83
43	1,800–1,355	14	30	41
C63	1,780–1,345	—	30	75
7	2,005–1,650	16	35	100
C26	1,980–1,475	—	35	182
8	2,400–1,765	16	47	31
C27	2,345–1,600	—	45	43
1	1,900–1,520	12	63	11
C20	1,930–1,325	—	62	120

* 1 = original weight. 2 = weight after removal of gastrointestinal tract.

TABLE I—*Concluded*

Rabbit	Weight* 1	2	Time after termination of immunization	Time after intravenous injection, of cardiac puncture	Colonies per cc. of blood
	gm.		*days*	*min.*	
2	2,225–	1,715	13	64	16
C21	2,220–	1,710	—	62	29
46	2,315–	1,870	18	62	49
C66	2,340–	1,740	—	60	10
3	2,150–	1,720	14	123	2
C22	2,195–	1,810	—	122	45
				hrs.	
47	2,225–	1,835	23	24	0
C67	2,195–	1,860	—	24	0

five normal animals weighed more than their immune partners in the experiment. Nevertheless, the trend in all pairs, except as above noted, was toward the more rapid elimination of the bacteria from the blood streams of the immune animals. Therefore the variations in weight have apparently not affected the comparison of bacterial elimination following the use of a constant dosage of bacteria for each pair of animals tested.

These experiments were done at time periods varying from 6 to 46 days after the termination of immunization but no significant differences were evident within this range. Presumably the optimal period should be from 7 to 21 days, assuming that the disappearance rates are dependent upon the union of sensitized macrophages with well opsonized bacteria.

The question arises as to whether this accelerated rate of removal may not be the result of non-specific stimulation resulting from immunization rather than from specific causes. We have evidence, to be reported in a later paper, that non-specific stimulation may, under certain conditions, accelerate the removal mechanism, but in general, we are convinced that specific active immunization more effectively facilitates the removal of the bacteria injected. Thus, Chart 2 illustrates the type of curve obtained when animals immunized against staphylococci were tested as to their ability to eliminate *Streptococcus*

TABLE II

The Disappearance of Bact. paratyphosum B from the Blood Stream as Influenced by Previous Intravenous Immunization with A. Bact. paratyphosum Vaccine

Rabbit	Weight* 1 2	Time after termination of immunization	Time after intravenous injection, of cardiac puncture	Colonies per cc. of blood
	gm.	*days*	*min.*	
47	1,735–1,250	7	15	86
C58	1,655–1,280	—	15	141
48	2,235–1,730	7	15	52
C59	1,955–1,390	—	15	525
49*a*	2,005–1,970	9	33	535
C59*a*	2,100–1,965	—	33	3,370
45	1,315– 910	6	65	9
C56	1,370– 975	—	65	45
46	1,570–1,070	6	65	2
C57	1,550–1,180	—	65	9
1	2,060	8	65	27
C14	2,050	—	65	87
5	1,865–1,415	15	65	16
C18	1,695–1,340	—	65	41
30	2,055–1,610	10	92	5
C50	2,070–1,570	—	92	205
41	2,275–2,040	10	125	5
C51*a*	2,105–1,940	—	125	56
42	2,365–1,990	12	123	3
C52	2,250–1,705	—	123	14
9	2,345–1,895	46	135	1
C21	2,330–1,840	—	125	15
2	1,825–1,430	11	260	6
C15	1,800–1,285	—	260	10
3	1,810–1,310	11	325	10
C16	1,845–1,305	—	325	330

* 1 = original weight. 2 = weight after removal of gastrointestinal tract.

hemolyticus from the blood stream. As is seen, the curves for the immune and normal animals are practically identical; in fact, in the two animals charted, the immune reacted less effectively than the normal and died on the following day.

It is evident, then, that active immunization changes the reactivity of rabbits in the elimination from the blood stream of certain living bacteria. Further analysis shows that the bacteria are removed and

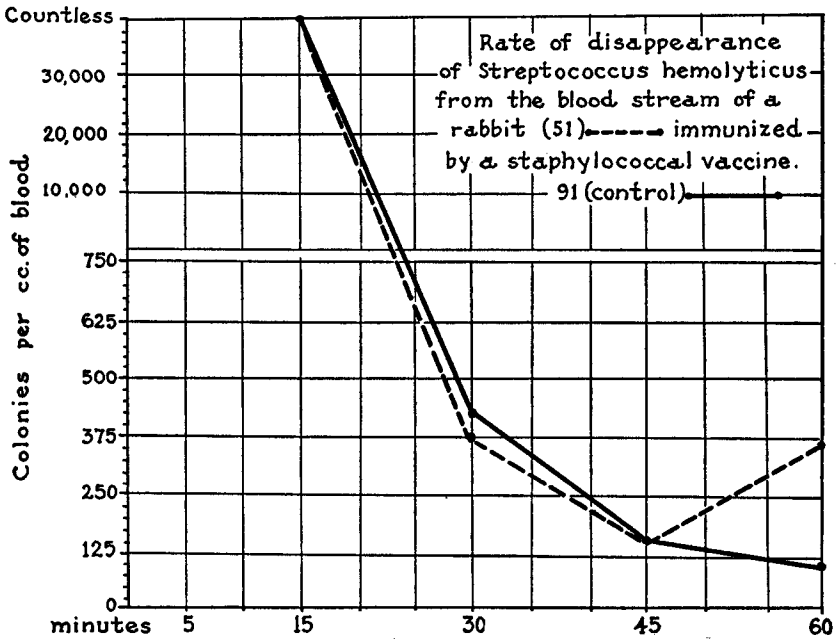

CHART 2

"fixed" in various organs and tissues, particularly in the liver and spleen, where they are killed and digested.

The Mechanism of Removal of Bacteria from the Blood Stream by the Liver and Spleen

The microscopical examination of sections from the livers and spleens of these rabbits revealed an enormous concentration of the bacteria in the immune animals with unmistakable evidence of an increased degree of disintegration of the microorganisms themselves.

It was apparent that some immunity mechanism was in action and that in all probability the more rapid removal of the bacteria from the blood stream in the immune animals was caused by the mechanism which was facilitating their fixation. The most striking feature, however, was the extraordinary speed of the process in the immune animals, astonishing changes occurring within 2 minutes after the intravenous injection of the staphylococci. Fig. 2 illustrates this effect in the spleen. It is a composite drawing, made by the aid of a camera lucida, of four oil immersion fields from smears of splenic pulp fixed by methyl alcohol and stained by Giemsa stain. These smears were made from the spleens of two rabbits, a normal and one previously immunized against the staphylococcus, exactly 2 minutes after the simultaneous injection into the marginal ear veins of each rabbit of equal quantities of the same suspension of staphylococci. In the smear from the normal rabbit there are relatively few bacteria, these are individually distributed, seem to be extracellular and are only slightly swollen. In the immune animal, however, there are many staphylococci which occasionally occur in clumps or masses with the individual microorganisms swollen and more faintly stained than are those in the normal rabbit. Evidently antibacterial substances have almost instantaneously affected the bacteria so that they have become swollen, seem to have an increased cohesiveness or decreased viscosity and stain relatively poorly. The bacteria appear to be extracellular, but splenic cells may have been ruptured in making the smears. At any rate, their concentration in the immune spleens and the extraordinarily rapid changes in their morphology are striking.

The spleens from these animals were fixed immediately in warm Zenker-formol solution, sectioned, and stained by the Claudius-Gram method. Figs. 4 and 5 are photomicrographs from them. In the normal spleen, as in the smears, there are relatively few staphylococci per oil immersion field, but, although the bacteria are distinctly stained, they are already somewhat swollen and are, for the most part, within macrophages. In the immune spleen, however, there are vast numbers of staphylococci per oil immersion field, they are indistinctly stained, are markedly swollen and are almost entirely within macrophages.

Similar changes occurred in the livers of animals examined 5 minutes

after injection of equal quantities of a suspension of staphylococci. In the normal animal an occasional staphylococcus was seen in Kupffer cells, whereas, in the immune animal, many large, swollen cocci were adherent to the walls of the sinusoids as well as within swollen Kupffer cells (Fig. 3). Many of the extracellular cocci seemed flattened as though larger portions of their surfaces were in contact with the lining membrane of the sinusoid.

At later stages sections from the spleens and liver have consistently shown a similar picture of greatly increased concentration and more marked phagocytosis of the bacteria in the immune organs. Furthermore, the ingested bacteria lose their staining properties and disappear much more rapidly in the spleens and livers of the immune animals, in spite of their greater concentration in such organs.

DISCUSSION

These experiments demonstrate the extreme rapidity with which certain reactions may occur within the immune body and suggest an important correlation between antigen and sensitized macrophages *in vivo*. The fact that the accelerated rate of removal of bacteria from the blood stream is dependent upon specific immunization indicates that neither the mere increase in macrophages nor their stimulation by non-specific agents is solely responsible for the clearance of bacteria from the blood. Furthermore, the marked changes in the size and degree of staining of the bacteria in the immune livers and spleens and their greater concentration there, suggest that the immune bodies probably act most effectively in the organs in which macrophages are most numerous. On the other hand, the presence of immune bodies in the blood stream does not determine bacterial localization throughout the tissues generally since the bacteria do not tend to become localized in the lungs, muscles, etc., and when seen there, they do not show the pronounced morphological changes observed in the immune livers and spleens.

We interpret the general mechanism as follows: Staphylococci injected intravenously into normal rabbits circulate throughout the blood stream in large numbers, probably making many passages through the organs and tissues of the body. In passing through the spleen and liver, especially, conditions more favorable for phagocy-

tosis may obtain, particularly those dependent upon slow blood flow, availability of macrophages and leucocytes, mechanical conditions favoring filtration, etc. Chance contacts between phagocytic cells and the relatively unchanged staphylococci induce a certain degree of phagocytosis, as seen in the polymorphonuclear leucocytes in the lungs and in the macrophages and leucocytes of the liver and spleen. Eventually this mechanism removes the bacteria from the circulating blood. Their further fate doubtless depends upon the virulence of the microorganisms and the digestive capacities, or in other words the functional state of the phagocytes, both macrophages and polymorphonuclear leucocytes. As Werigo showed in experimental anthrax infection, if these cells become inadequate, the bacteria again multiply and generalize.

In the immune animals, however, this normal mechanism disposed of the dead bacterial bodies during the preliminary period of immunization, at which time many macrophages in the liver and spleen removed and digested the bacterial particles. When at a later period, large numbers of living staphylococci run the gauntlet of these macrophages, there is an almost instantaneous swelling of the microorganisms, an increasing obstruction to their free passage through the immune liver and spleen and a tendency to clumping of the bacteria, with a resulting retention of such affected microorganisms in these organs.

The presence of swollen, clumped, poorly stained bacterial bodies suggests a marked alteration in their physical state, such as a decreased viscosity or increased cohesiveness, and the significance of the morphological changes may be explained by the experiments of Mudd and his associates (8) on the effects of immune bodies upon antigen. At any rate, it is apparent that the bacteria have been more effectively "fixed" and destroyed than under normal conditions.

The observations of Manwaring and Coe (9) merit renewed attention with respect to the above findings. These workers observed that pneumococci suspended in sterile Ringer's solution were removed rapidly in the livers of actively immunized rabbits when the organs were perfused *in situ* through the portal vein, whereas comparatively few bacteria were removed in normal livers under similar conditions. They reported that "smears and histological preparations made from

the perfused liver now show numerous pneumococci adherent to the capillary endothelium. Few if any agglutinated masses are seen, and little or no endothelial phagocytosis." After demonstrating that immune serum added to the perfusing fluid caused a similar effect whereas normal serum did not, they suggested the name "endothelial opsonin" for the serum component responsible for the retention of the pneumococci. The significant fact, however, is their demonstration of the bacteriotropic action leading to an increased degree of cohesion of the microorganisms to the lining membrane of the hepatic sinusoids, whereby the antigen is fixed and later engulfed and destroyed by the hepatic macrophages.

Hopkins and Parker (2) apparently observed a somewhat similar adherence of streptococci to the walls of capillaries of the lungs in cats injected intravenously with streptococci and in which the lungs were perfused 30 minutes later with salt solution and Helly's fluid. They stated that the streptococci were not washed out, were not within leucocytes but were, for the most part, either in large mononuclear cells or in an eosin-staining matrix which they thought might represent a section through endothelial protoplasm or some substance from the blood deposited around them. More recently Rich (10) has suggested a similar mechanism to explain the fixation of pneumococci introduced into the skins of rabbits previously injected with immune serum. He concluded that "local fixation appears to be effected primarily by a prompt and specific agglutination of the bacteria which impedes their free movement through the tissues" and which "appears to involve a phenomenon of adsorption to the tissues as well."

Bull (11, 12) has emphasized the importance of agglutination *in vivo* in the mechanism of removal of bacteria from the circulating blood, his opinion being that the microorganisms are agglutinated in the blood stream and removed by the polymorphonuclear leucocytes, especially in the liver and spleen. As the clumps of bacteria observed in his experiments were in smears from the blood and tissues, it is possible that they were formed, in part at least, in the process of making the smears. Zinsser (13) and Rheingold (1) have also presented evidence supporting the conception of agglutination in the circulating blood as a mechanism of defense. The latter observer described aggregates of *B. prodigiosus* in the capillaries and smaller vessels in the

livers of dogs injected intravenously with this microorganism, although he found no evidences of such a process in blood smears from the peripheral blood.

In our experiments we have at no time obtained any clear-cut evidence of such a phenomenon in the liver, spleen or lungs of normal or immune animals, although there may be a slight suggestion of such in Fig. 2. The small clusters of staphylococci may at least be regarded as evidence of an increased cohesiveness of the bacteria, but here again the clumps may have been formed in making the smears. No such indication of agglutination was apparent in the sections of the spleens and livers of these animals. As noted above, Manwaring and Coe observed no marked agglutination in the perfused livers, either normal or immune, nor did Hopkins and Parker in the tissues of cats and rabbits injected with streptococci.

Nevertheless, the absence of observable agglutination *in vivo* under the above conditions does not prove that such a process may not be of considerable importance as a means of localizing bacteria. The time factor may be the determining element. For instance, if immune tissues are so changed that bacteria are practically instantaneously arrested and then engulfed by phagocytes, obviously no agglutination could occur, as no opportunities for collisions between bacteria would be present. There is evidence, however, that when staphylococci remain extracellularly in immune tissues for a sufficient length of time, agglutination *in vivo* actually occurs, presumably because of the bacteriotropic effect of immune bodies in increasing the cohesiveness of the bacteria (14).

In whatever manner the bacteria may be fixed in the tissues, whether by actual adherence to the surfaces of normal cells or tissues, or by an agglutinating or agglomerating action whereby their dispersion through the tissues may be impeded or minimized, to that extent their generalization is limited and the dangers of disseminated infection lessened.

The relative importance of macrophages and polymorphonuclear leucocytes in the mechanism as a whole is of considerable interest. In such a complex system, however, it is questionable whether too much emphasis should be placed upon the comparative significance of two groups of mesenchymal cells whose functions are so similar

and apparently complemental. In the passage of staphylococci through the lungs many are unquestionably engulfed by polymorphonuclear leucocytes, although for the most part they pass quickly through and become generalized. In the liver and spleen, however, the bacteria are rapidly fixed by macrophages, although here, too, some are phagocytosed by polymorphonuclear leucocytes, which in turn are later engulfed by macrophages. We have repeatedly observed macrophages in the spleen containing many cocci side by side with polymorphonuclear leucocytes containing none. Apparently the primary reaction is mainly between the cocci, immune bodies and the cytoplasmic surfaces of the macrophages, accompanied or quickly followed by the accumulation of polymorphonuclear leucocytes attracted or retained there by chemotactic or electrotropic influences. Both types of cells then engulf the bacteria and destroy them provided that the virulence and numbers of the latter are not too great.

CONCLUSIONS

1. The simultaneous intravenous injection into normal and actively immunized rabbits of equal quantities of living staphylococci or paratyphoid bacilli is followed by a distinctly accelerated rate of removal of the bacteria from the blood streams of the immune animals.

2. This altered reactivity is due essentially to specific active immunization.

3. The bacteria pass rapidly through the capillary bed of the lungs, extracellularly and dispersed for the most part, and become generalized through the blood stream.

4. The bacteria are quickly removed from the circulating blood in the immune animals and less rapidly in the normal ones, by various organs, particularly the liver and spleen, where they accumulate in enormous numbers, become adherent to the lining membrane of the sinusoids of the liver and apparently to the macrophages of the spleen and are phagocytosed by the macrophages and leucocytes in these organs.

5. Associated with this effect are morphological changes in the bacteria as shown by swelling, loss of staining power and evidences of increased cohesiveness and decreased viscosity, these changes being apparent as early as 2 minutes after their intravenous injection.

6. Inasmuch as these changes are not seen to a marked degree within the lungs or other organs, they are probably the result of a local antigen-antibody reaction of a bacteriotropic type in the two organs generally considered to be most actively concerned with the production of immune bodies.

7. By means of this accelerated bacteriotropic effect in the actively immunized animals, phagocytosis is facilitated and intracellular digestion of the bacteria is enhanced.

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EXPLANATION OF PLATES

PLATE 5

FIG. 1. Drawing made with the camera lucida (×820) of two pulmonary veins from a normal rabbit which had been injected intravenously in the marginal ear vein 2 minutes previously with a heavy suspension of staphylococci. The micro-

organisms are mostly extracellular, are dispersed, of normal size and are well stained. An occasional polymorphonuclear leucocyte has engulfed staphylococci.

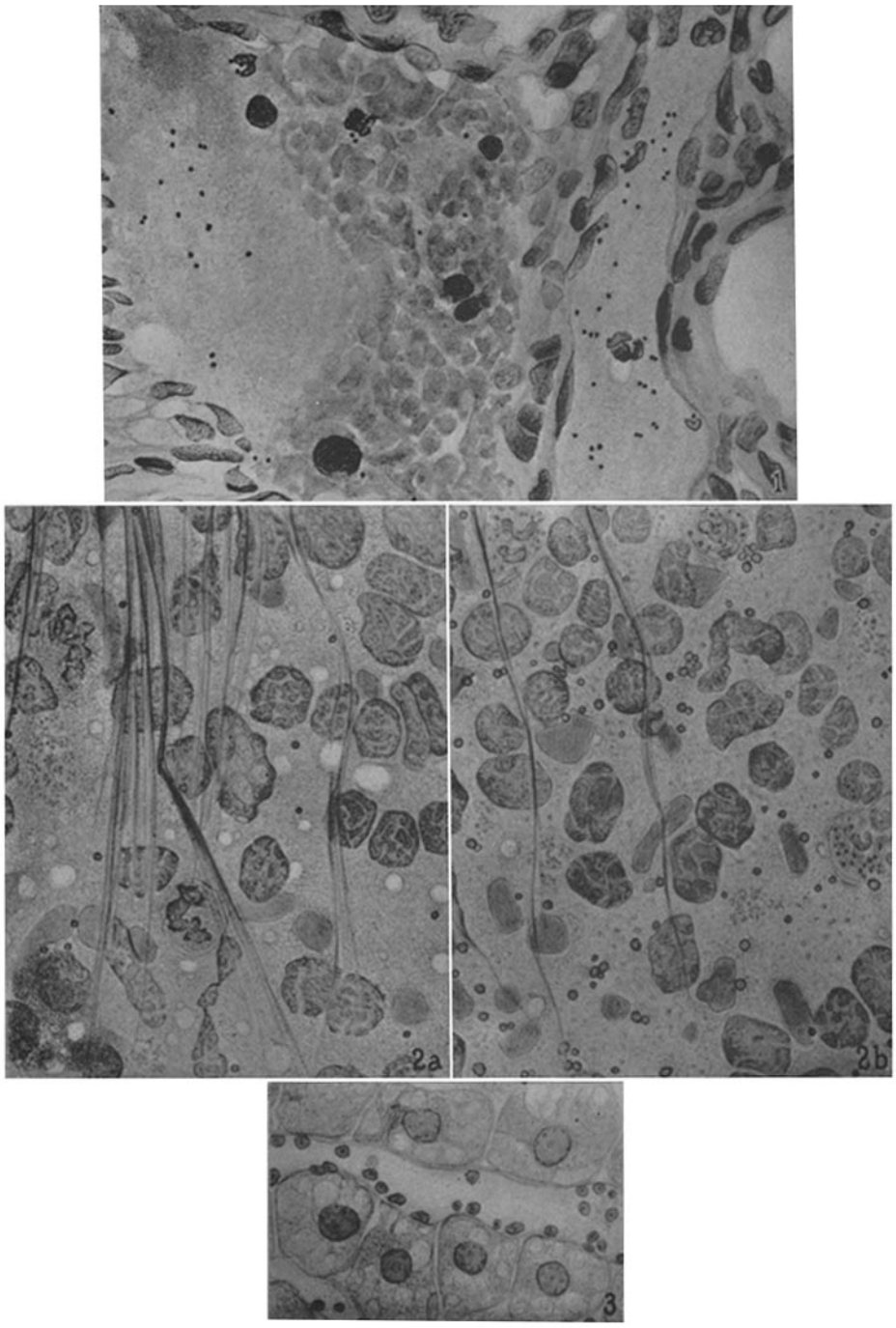
FIG. 2. Drawing made with the camera lucida of smears of splenic pulp from two rabbits injected simultaneously intravenously with equal quantities of the same suspension of staphylococci (×820). The smears were made 2 minutes after the injection, were fixed immediately by methyl-alcohol and stained by the Giemsa stain. Each picture is a composite of four oil immersion fields made under identical optical conditions. In the smear of the normal rabbit's spleen (*a*) there are comparatively few staphylococci, which are dispersed, are of practically normal size and are well stained. In the smear from the immune animal (*b*) there are many staphylococci, which are swollen, poorly stained and in places are in small clusters.

FIG. 3. Drawing made with the camera lucida (×820) of an hepatic sinusoid from an actively immunized rabbit 5 minutes after the intravenous injection of a heavy suspension of living staphylococci. The liver was perfused with sterile solution of sodium chloride followed by warm Zenker-formol solution. Note the adherence of the swollen microorganisms to the wall of the sinusoid although the erythrocytes have been washed out by the perfusing fluids.

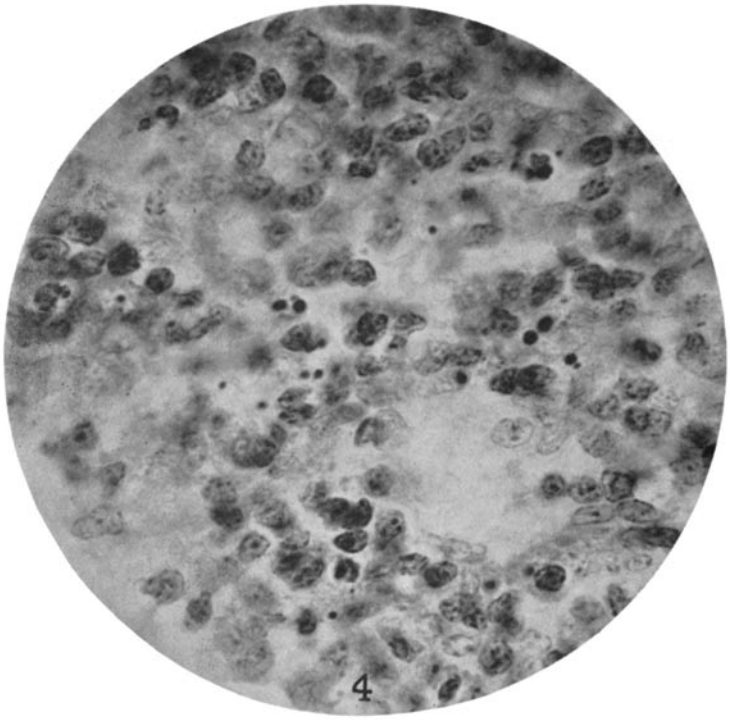
PLATE 6

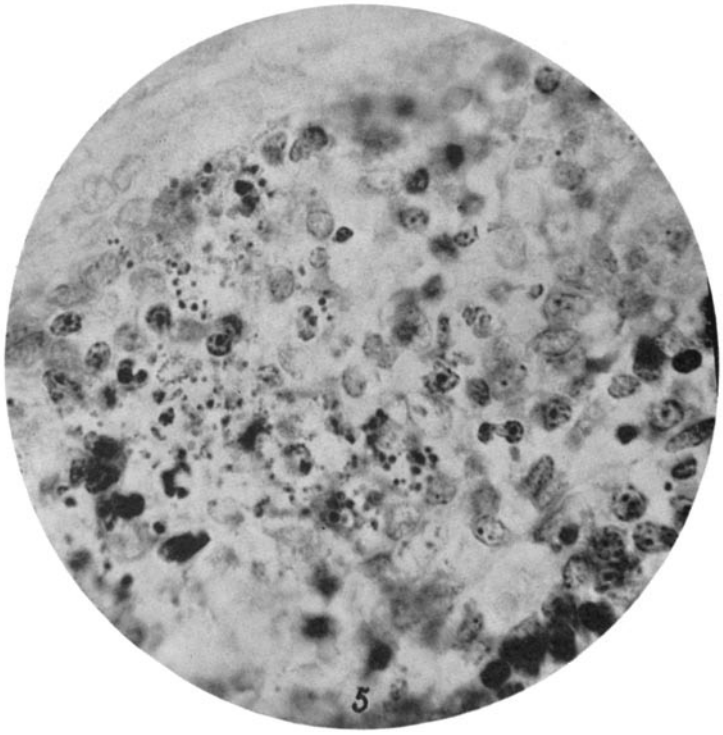
FIG. 4. Photomicrograph (×970) of a section of spleen from the normal rabbit described in Fig. 2. The spleen was fixed 2 minutes after the intravenous injection of the staphylococci and the section stained by the Claudius-Gram stain. Note the dispersed but well stained staphylococci, many of which, however, are swollen and either adherent to or within macrophages. Only a few polymorphonuclear leucocytes are present.

FIG. 5. Photomicrograph (×970) of a section of spleen of the staphylococcus-immune rabbit described in Fig. 2. The spleen was fixed 2 minutes after the intravenous injection of the staphylococci and the section stained by the Claudius-Gram stain. Note the marked concentration of staphylococci, the swollen as well as fading forms and the accumulation of polymorphonuclear leucocytes.



(Cannon *et al.*: Disappearance of bacteria from blood stream)





(Cannon *et al.*: Disappearance of bacteria from blood stream)