

THE FATE OF MICROCOCCUS AUREUS INTRODUCED INTO
THE BLOOD STREAM OF DOGS.*

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In the work here reported an attempt has been made to determine as accurately as possible by histological means the manner in which the *Micrococcus aureus* after entering the blood stream is disposed of by the living tissues. The fate of the cocci has been followed from a minute or two after the injection until their general disappearance from the tissues. The rapidity with which living bacteria introduced into the blood stream may be removed from it has been known since the early days of bacteriology. Wyssokowitsch,¹ working with both pathogenic and non-pathogenic bacteria, demonstrated by cultural methods that when these were injected into the blood stream of the rabbit, they were quickly stored up in certain organs, particularly the spleen, liver, and bone marrow. In his histological investigation Wyssokowitsch experimented with the *Micrococcus tetragenus* and the typhoid bacillus. The cocci were found twenty-four hours after the injection, especially in the capillary vessels of the liver; later, however, some of them were found imbedded in the endothelial cells of this organ. In the spleen they were seen only after repeated injections of large numbers of the germs, and here they were incorporated in the large endothelial cells of the pulp. Only exceptionally were they met with in the interstitial cells of the kidney. The typhoid bacillus behaved similarly; the majority of the bacilli were found free in capillary vessels, but some of them were lying in endothelial cells, and also in the cells of the interstitial tissue.

Later Werigo² produced an anthrax bacillemia in rabbits and made a careful microscopic study of the organs, particularly of the liver and spleen, for bacteria. His results will

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be referred to later. Much more recently Filatow,³ Arima,⁴ Kyes,⁵ and Bull⁶ have added considerably to our knowledge of the subject. Unfortunately Filatow's original paper is not at hand. Working with a non-pathogenic organism, *B. subtilis*, he injected it into rabbits and found it in the spleen, liver, and also in the eye, which had been previously injured.

Arima⁴ worked with the *Micrococcus aureus* and with the colon and typhoid bacilli. Suspensions of the bacteria in saline solution were injected intravenously into rabbits and these were killed after varying intervals of time. Pieces of different organs were ground up and plate cultures made from each organ and from the various body fluids. After half an hour bacteria were seldom found in the blood. They were most abundant in the liver, next in the spleen and bone marrow. The kidney contained but few. From these experiments he was of the opinion that the destruction of the bacteria varies according to the extent of the attraction which the different organs have for them. It is greatest in those organs which attract them most and this destruction is carried out especially in the liver.

Kyes⁵ experimented on pigeons with the pneumococcus, in order to determine the distribution of the bacteria and their fate in the body of this refractory host. The results obtained were as follows: The pneumococci injected intravenously were rapidly withdrawn from the general blood stream and localized especially in the liver and the spleen and, in a much smaller number, in the lung and bone marrow. Also he found that the ultimate localization of the pneumococcus in the liver and the spleen was in the fixed phagocytes present in these organs, and the destruction of the bacteria took place within this type of cells.

Bull,⁶ studying the fate of typhoid bacilli injected intravenously into rabbits, found they were promptly agglutinated and quickly removed from the blood stream and then ingested by polymorphonuclear leucocytes in the liver, spleen, and possibly other organs. They were then destroyed within these cells. In a later study⁷ upon the fate

of pneumococci after intravenous injection into dogs, he found that their very rapid disappearance from the circulation was due to their agglutination and their accumulation in the lungs, liver, spleen, etc. After twenty-four to forty-eight hours they reappeared in the blood. He suggests that the incubation period of the infectious diseases is due to the fact that infecting agents must become adapted to the adverse conditions in the newly-infected host before they can multiply sufficiently to produce symptoms of the disease.

In our work we have with a few exceptions used dogs as the experiment animals, and the *Micrococcus aureus* as the infecting organism. Unless otherwise stated, the routine was as follows: eighteen to twenty-four-hour agar slant cultures of the *Micrococcus aureus* were grown, the growth from four slants suspended in ten cubic centimeters of sterile physiological salt solution was centrifugalized to remove any clumps of bacteria, and the suspension of bacteria then injected into the left ventricle of the dog's heart. At the end of the specified time the animal was killed by injecting a very large dose of strychnine directly into the heart. Strychnine injected in this way causes practically instantaneous death. The tissues were fixed in Zenker's fluid and in alcohol, imbedded in paraffin and sectioned in a uniform thickness of five microns. Mallory's eosin-methylene blue stain was used for histological purposes and the Gram-Weigert method, for the better demonstration of bacteria. Three different strains of the *Micrococcus aureus* were used, each of only a moderate degree of virulence. No attempt was made to increase the virulence by passage. In order to determine the relative number of bacteria present in the sections, at least twenty fields (Leitz one-twelfth oil immersion, Reichert No. 6 ocular) were carefully examined in each section, a count made of all bacteria seen in these, and the average number in each field calculated from the total thus obtained.

Our main object being to study the localization of bacteria in the different organs and tissues, we did not attempt to choose animals of equal size or of the same race, nor to

make the dose exactly proportional to the body weight. We can deduce from the following experiments only approximately the time of the complete disappearance of bacteria from the tissues once invaded. On the other hand, this method has enabled us to determine fairly accurately the relative number of bacteria in the different tissues at varying periods after the injection. It must be borne in mind in interpreting the results obtained that the resistance of different dogs against the infecting agent varies and that the relative size of the organs, particularly the spleen, is subject to individual differences.

In the following list of experiments only one protocol for each of the periods will be given. Certain duplicates were carried out, but as the results in these duplicates were consistent with those here recorded, they will be omitted for the sake of brevity.

No. 1 (Dog 43). — Medium size. Micrococcus strain A injected. The animal was killed immediately as soon as the injection was finished, and was autopsied at once. Pieces were first taken from the lung. This was completed one minute after the injection. The excision of pieces of the other organs and tissues was completed within three minutes or less after the injection.

Blood: Smears made from the blood in the right ventricle within three minutes after the injection showed 5–6 cocci in an entire smear, all of which had been ingested by polymorphonuclear leucocytes.

Lung: A large number of bacteria was found. More than one-half of them were free. The other cocci had been ingested almost exclusively by polymorphonuclear leucocytes. The average number of cocci per field was 70.

Spleen: Moderate numbers of bacteria were scattered in the tissue. More than one-third of them were free. Others had been ingested by polymorphonuclear leucocytes and splenic cells. Few bacteria were found in the walls of sheathed arteries. The average number of cocci per field was 10.

Liver: The average number of polymorphonuclear leucocytes per field was 1.3. Somewhat more bacteria were present than in the spleen. Nearly two-thirds of them were free. Most of the other cocci had been taken up by polymorphonuclear leucocytes. Few bacteria were in endothelial cells. The average number of cocci per field was 12.

Bone marrow: No bacteria seen. Kidney: A few bacteria were present, and these were within polymorphonuclear leucocytes. Wall of intestine: Very few cocci were found, and these were in the villi. Heart muscle: No bacteria seen. Skeletal muscle: No bacteria seen.

No. 2 (Dog 41). — Medium size. Micrococcus strain C injected. The animal was killed ten minutes after the injection.

Blood: Smears showed a moderate number of cocci, mostly within polymorphonuclear leucocytes. There were also very few free bacteria.

Lung: The tissue was not hyperemic, but polymorphonuclear leucocytes were much increased in number. There was no exudate in the alveoli. An abundance of microorganisms was found, and almost all of them had been ingested by polymorphonuclear leucocytes. Only a small number of endothelial cells contained bacteria. Free bacteria were scarcely met with. The average number of cocci per field was 69.

Spleen: The tissue showed no leucocytosis. Numerous cocci were found. Most of them had been ingested by polymorphonuclear leucocytes. Fewer were within splenic cells. Several were found in the walls of sheathed arteries. A few cocci were free. The average number of cocci per field was 20.

Liver: The tissue was not particularly hyperemic. There was no leucocytosis, the average number of polymorphonuclear leucocytes per field being 1.1. Numerous microorganisms were seen in the capillary vessels. More than one-half of them had been ingested by polymorphonuclear leucocytes, often a large number being in one cell. Many others were found in endothelial cells. There were also many free bacteria. The average number of cocci per field was 18.

Bone marrow: Very few bacteria were present, mostly outside of cells. The average number of them per field was .35. Mesenteric lymph gland: No bacteria seen. Kidney: Few bacteria were found and these were in the glomeruli. Wall of intestine: No bacteria seen. Heart muscle: No bacteria seen. Skeletal muscle: Very few bacteria (.05 cocci per field) were present.

No. 3 (Dog 38). — Small size. Micrococcus strain A injected. The dog was killed after twenty minutes.

Lung: Polymorphonuclear leucocytes were much increased, but there was no exudation into the alveoli. Many bacteria were found. Almost all of them had been ingested by polymorphonuclear leucocytes, exceptionally by endothelial leucocytes. The average number of cocci per field was 10.

Spleen: There was a moderate leucocytosis, but no noticeable hyperemia. Numerous cocci were found. Most of them had been ingested by splenic cells and polymorphonuclear leucocytes. A few others were free. The average number of cocci per field was 25.

Liver: Polymorphonuclear leucocytes were moderately increased, the average number in each field being 5. Many cocci were found and most of them had been ingested by polymorphonuclear leucocytes and endothelial cells. The average number of cocci per field was 15.

Bone marrow: Very few bacteria (.15 per field) were found. Mesenteric lymph gland: No bacteria seen. Kidney: The tissue showed beginning tubular nephritis. Very few cocci were found and these were in

the glomeruli. They had been ingested by polymorphonuclear leucocytes. Heart muscle: No bacteria seen.

No. 4 (Dog 28).— Small size. This was the first animal used in this series, and fewer bacteria were injected than in the other dogs. Twenty cubic centimeters of a 24-hour-old bouillon culture of *Micrococcus aureus* (Strain A) were injected into the left ventricle of the heart. The animal was killed thirty minutes after the injection.

Spleen: A slight increase of polymorphonuclear leucocytes was found. There was a moderate number of cocci, and some of them had been ingested by polymorphonuclear leucocytes. Other bacteria were partly free and partly had been engulfed by splenic cells. Frequently several bacteria were seen imbedded in the walls of sheathed arteries. The average number of cocci per field was 6.

Liver: Polymorphonuclear leucocytes were moderately increased, the average number of them in each field being 6. A small number of cocci was found and more than one-half of them were in polymorphonuclear leucocytes. Not infrequently endothelial cells contained some bacteria. Other bacteria were free. The average number of cocci per field was 4.5.

Bone marrow: Very few bacteria (.1 per field) were found. Mesenteric lymph gland: No bacteria seen. Kidney: The tissue showed a picture of tubular nephritis of moderate degree. No bacteria seen. Heart muscle: No bacteria seen.

No. 5 (Dog 29).— Small size. *Micrococcus* strain A injected. The animal was killed one hour after the injection.

Spleen: Polymorphonuclear leucocytes were moderately increased. Numerous bacteria were found in the tissue. Many had been ingested by polymorphonuclear leucocytes and by splenic cells, rarely by endothelial leucocytes. Besides these there were some free bacteria. The average number of cocci per field was 21.

Liver: Many polymorphonuclear leucocytes were found, their average number in each field being 10. The tissue was hyperemic. Numerous cocci were found in the capillary vessels, and most of them had been ingested by polymorphonuclear leucocytes, fewer by endothelial cells. There were also several free bacteria. The average number of cocci per field was 18.

Bone marrow: Very few bacteria (.45 per field) were present. Mesenteric lymph gland: No bacteria seen. Kidney: The tissue showed tubular nephritis with numerous epithelial casts. Few bacteria were found and these were especially in the glomeruli. They were usually within polymorphonuclear leucocytes. Heart muscle: Very few bacteria (.15 per field) were found.

No. 6 (Dog 30).— Small size. *Micrococcus* strain A injected. The dog was killed two hours after the injection.

Spleen: Polymorphonuclear leucocytes were distinctly increased in

number. Many bacteria present, and most of them had been ingested by polymorphonuclear leucocytes and splenic cells, less frequently by endothelial leucocytes. Several bacteria were found in the walls of sheathed arteries. Few were free. The average number of cocci per field was 18.

Liver: Numerous polymorphonuclear leucocytes were present in sinusoids, the average number per field being 26. A marked hyperemia was present. Many cocci were present and practically all of them had been ingested by polymorphonuclear leucocytes. There was only a small number of free bacteria. Endothelial cells which had ingested bacteria were scarcely met with. The average number of cocci per field was 13.

Kidney: The general picture was that of tubular nephritis with some casts. There was a hyperemia of moderate degree. A few bacteria were found and these were in the glomeruli.

Heart muscle: No bacteria seen.

No. 7 (Dog 50).—Large size. Micrococcus strain D injected intravenously under ether. The animal was killed with chloroform eight hours after the injection.

Blood: Smears showed an apparent leucocytosis of a slight degree. No bacteria were present.

Lung: A marked congestion and leucocytosis. No exudation except slight hemorrhage in a few alveoli. No bacteria seen.

Spleen: Not particularly hyperemic. Polymorphonuclear leucocytes much increased, especially round the peripheral zone of Malpighian corpuscles. Minute fibrin network was seen in many places in the pulp. Few bacteria (1.9 per field) were found in the tissue. One-third of them had been taken up by splenic cells, another third of them remained free, and the others were found within the walls of the sheathed arteries.

Liver: Marked congestion of the tissue. A very marked leucocytosis (21 leucocytes per field) was present. Few bacteria (2.3 per field) were found. Nearly two-thirds were free, others found in endothelial cells and polymorphonuclear leucocytes as well.

Bone marrow: Very few cocci (.15 per field) present. Mesenteric lymph gland: No bacteria seen. Kidney: A rather marked degeneration of the renal epithelium. Many epithelial and hyaline casts. Very few bacteria found, especially in glomeruli. None were found in the vessels of the medulla even after a prolonged search. Wall of intestine: No bacteria seen. Heart muscle: No bacteria seen. Skeletal muscle: No bacteria seen.

No. 8 (Dog 51).—Large size. Micrococcus strain D injected intravenously under ether anesthesia. The animal was killed with chloroform sixteen hours after the injection.

Blood: Leucocytosis of a moderate degree. Very few cocci were present, all extracellular.

Lung: Slight congestion. No exudation in alveoli. Leucocytes much increased. No bacteria seen.

Spleen: Slight congestion in places. Leucocytes moderately increased. Few bacteria (2.9 per field) were found. More than two-thirds of them free; the rest mostly in splenic cells.

Liver: A marked congestion of the tissue. Leucocytes much increased (11 per field). Few cocci (1.3 per field) were present. About two-thirds of them free; others in endothelial cells.

Bone marrow: No bacteria seen.

Mesenteric lymph gland: Polymorphonuclear leucocytes much increased. A marked hemophagocytosis in lymph sinuses. No bacteria seen.

Kidney: The tissue generally hyperemic. Capillary vessels engorged, especially in the medulla near the cortex. A marked degeneration of the renal epithelium. Several epithelial casts in the medulla. Within some of the capillary vessels of the medulla, dense masses of polymorphonuclear leucocytes. In several places in the medulla, accumulations of innumerable cocci within the lumina of capillary vessels. Occasionally these bacterial masses did not fill the lumen of the vessel, and had no evidence of clotting of blood around them. They lay against the endothelial cells of one side of the vessel.

Wall of intestine: No bacteria seen. Heart muscle: No bacteria seen.

No. 9 (Dog 42). — Medium size. Micrococcus strain C injected. The injection was made intravenously under ether and the animal killed with chloroform twenty-four hours later.

Blood: Smears showed a marked leucocytosis. No bacteria were found.

Lung: There was a marked hyperemia and a moderate increase of polymorphonuclear leucocytes. No exudate in the alveoli. No bacteria were present.

Spleen: The tissue was hyperemic. Polymorphonuclear leucocytes were much increased. In many places, especially in the peripheral parts of the Malpighian corpuscles, minute foci of fibrinous exudation were seen. The bacteria were present in small number. They were mostly lying free; some of them had been incorporated by splenic cells. Hardly any of the polymorphonuclear leucocytes contained cocci. The average number of cocci per field was 3.8.

Liver: The organ was hyperemic. Also there was a marked leucocytosis. Polymorphonuclear leucocytes averaged 16 per field. Few bacteria were found. Most of them were free. A small number of endothelial cells contained a few bacteria. The average number of cocci per field was 3.1.

Bone marrow: No bacteria seen. Mesenteric lymph gland: A very marked increase of polymorphonuclear leucocytes was found. No bacteria seen. Kidney: The picture was that of a tubular nephritis of severe form. The tissue was in general hyperemic. Polymorphonuclear leucocytes were increased, and in several places they had collected in large

numbers. Innumerable bacteria were found in areas in the medulla, while in the cortex only few were present. Many of them were ingested by polymorphonuclear leucocytes, but the majority were found free in the lumina of capillary vessels. These accumulations of cocci fairly resembled emboli, but a careful examination led to the conclusion that they were not emboli, but simply colonies growing in the lumina of the vessels. Heart muscle: No bacteria seen. Skeletal muscle: No bacteria seen.

No. 10 (Dog 49).—Medium size. Micrococcus strain D injected intravenously under ether anesthesia. The animal was killed with chloroform forty-eight hours after the injection.

Lung: Polymorphonuclear leucocytes much increased. Moderate congestion in places. Also slight hemorrhage and emigration of leucocytes in a few alveoli. No bacteria seen.

Spleen: A marked congestion of the tissue. Polymorphonuclear leucocytes much increased. Fibrinous exudation in some places. Very few cocci (.3 per field) were found both intra- and extracellular.

Liver: Hyperemic. Polymorphonuclear leucocytes were increased (7.6 per field). Few bacteria (1.1 per field) were found, about one-half lying within endothelial cells. Others in leucocytes or free.

Bone marrow: Very few bacteria (.4 per field) were present. Mostly intracellular. Mesenteric lymph gland: No bacteria seen. Kidney: An advanced degeneration of the renal epithelium. Abscesses of different sizes and hemorrhagic purulent infiltration in many spots. Numerous cocci were found both intra- and extracellular. Wall of intestine: No bacteria were seen. Heart muscle: Beginning abscess formation. Several cocci were found in the infiltrated foci, mostly intracellular. Skeletal muscle: No bacteria seen.

No. 11 (Dog 46).—Medium size. Micrococcus strain D injected intravenously, ether anesthesia. The animal killed with chloroform after seventy-two hours, in a very weak condition.

Blood: Smear showed a very marked leucocytosis. A few cocci were found, all extracellular.

Lung: The tissue was hyperemic. Polymorphonuclear leucocytes were markedly increased. No exudation into alveoli. No bacteria seen.

Spleen: The tissue was a little hyperemic. Polymorphonuclear leucocytes were present in considerable numbers, and they infiltrated the pulp. No bacteria seen.

Liver: The tissue was not particularly hyperemic, but contained many polymorphonuclear leucocytes. No bacteria seen.

Bone marrow: No bacteria seen.

Mesenteric lymph gland: A great many polymorphonuclear leucocytes were found in the tissue. No bacteria seen.

Kidney: Hemorrhagic purulent foci with or without fibrin nets were found in many places. The renal epithelium was degenerated to a marked

degree. Many bacteria were present in purulent foci. In this part of the medulla abundant bacterial growth in several places was found. Wall of intestine: No bacteria seen. Heart muscle: No bacteria seen. Skeletal muscle: No bacteria seen.

No. 12 (Rabbit 8). — One slant culture of *Micrococcus aureus* (Strain D) was suspended in 4 cubic centimeters of sterile physiological saline solution and injected intravenously. The animal was killed after ten minutes.

Blood: Very few bacteria were found in smears.

Lung: The tissue showed leucocytosis of a moderate degree. An abundance of bacteria (about 100 cocci per field) was found. Practically all bacteria were intracellular and almost all of them were within polymorphonuclear leucocytes, few in endothelial cells, and in epithelial cells of alveoli. The nuclei of many leucocytes were obscured by ingested cocci. Very few cocci were free.

Spleen: Seventeen cocci per field were found in the tissue. They had mostly been ingested by splenic cells, much less frequently by polymorphonuclear leucocytes. Few cocci were free.

Liver: More bacteria were present than in the spleen. The average number of them per field was 35. Most of them had been taken up by endothelial cells, less frequently by polymorphonuclear leucocytes. Several cocci were free.

Bone marrow: The average number of cocci per field was 9. More than one-half were intracellular. Kidney: Several cocci were found in glomeruli, mostly ingested by leucocytes. Cocci in smaller numbers were present in the interstitial tissue. Wall of intestine: No bacteria seen. Heart muscle: Very few bacteria (.1 per field) were found. Skeletal muscles: Very few bacteria (.05 per field) were found.

No. 13 (Rabbit 3). — Two slant cultures of *Micrococcus aureus* (Strain A) were suspended in 6 cubic centimeters of sterile saline solution and injected intravenously. The animal was killed after half an hour.

Lung: The tissue showed hyperemia of a slight degree. Polymorphonuclear leucocytes were much increased. There was no exudate in the alveoli. A large number of bacteria was found. Most of them had been ingested by polymorphonuclear leucocytes. Several of the endothelial and alveolar epithelial cells contained some cocci. Very few were lying free outside of cells. The average number of cocci per field was about 110.

Spleen: The tissue was a little hyperemic. Polymorphonuclear leucocytes were slightly increased. An abundance of microorganisms was present. Most of them had been ingested by leucocytes and splenic cells in about equal numbers. Decidedly fewer bacteria were free. The average number of cocci per field was about 70.

Liver: The tissue was not particularly hyperemic. There was no remarkable increase of polymorphonuclear leucocytes. An abundance of

bacteria was found. Most of them had been ingested by endothelial cells and polymorphonuclear leucocytes in about the same numbers. Besides these there were many free cocci. The average number of cocci per field was about 120.

Bone marrow: A moderate number of bacteria was present. More than one-half of them were free, the others were intracellular. The average number of cocci per field was 6. Kidney: A slight degeneration of renal epithelium was found. No casts seen. Few cocci were found in the glomeruli, and most of them had been ingested by polymorphonuclear leucocytes. Heart muscle: No bacteria seen.

No. 14 (Rabbit 4). — One slant culture of *Micrococcus aureus* (Strain D) was suspended in 4 cubic centimeters of sterile saline solution and injected intravenously. The animal was killed after one hour.

Blood: No bacteria were found in smears.

Lung: The tissue showed leucocytosis of a moderate degree. Many bacteria were found. Most of them had been ingested by polymorphonuclear leucocytes, somewhat fewer were found in the cytoplasm of endothelial and alveolar cells. The average number of cocci per field was 9.5.

Spleen: An abundance of bacteria was found in the tissue. Many of them had been ingested by leucocytes, but more had been taken up by the splenic cells. There were also many free cocci. The average number of cocci per field was 48.

Liver: The tissue was not particularly hyperemic. Polymorphonuclear leucocytes were a little increased. Many bacteria were found, and about one-half of them had been ingested by endothelial cells. A much smaller number was in the polymorphonuclear leucocytes. Besides these there were many free bacteria in capillary vessels. The average number of cocci per field was 13.

Bone marrow: The average number of cocci per field was 2.2. Kidney: There was almost no histological change, except for the presence of a few hyaline casts. Very few bacteria were found and these were especially in the glomeruli. Wall of intestine: Very few bacteria (.05 per field) were found. Heart muscle: Very few bacteria (.2 per field) were found. Skeletal muscle: Very few bacteria (.05 per field) were present.

TABLE SHOWING DISTRIBUTION OF COCCI IN DIFFERENT TISSUES.

Number.	Time.	Blood.	Lung.	Spleen.	Liver.	Bone Marrow.	Lymph Gland.	Kidney.	Intestine.	Heart Muscle.	Skeletal Muscle.
1. (Dog 43)	1-3 minutes.	Very few.	70	10	12	0	Few.†	Very few.	0	0
2. (Dog 41)	10 "	"	69	20	18	0.35	0	Few.	0	0	0.05
3. (Dog 35)	20 "	10	25	15	0.15	0	Very few.	0	
*4. (Dog 28)	30 "	6	4-5	0.1	0	0	0	
5. (Dog 29)	1 hour.	21	18	0.45	0	Few.	0.15	
6. (Dog 30)	2 hours.	18	13	"	0	
7. (Dog 50)	8 "	0	0	1.9	2.3	0.15	0	Very few.	0	0	0
8. (Dog 51)	16 "	Very few.	0	2.9	1.3	0	0	Masses.	0	0	0
9. (Dog 42)	24 "	0	0	3.8	3.1	0	0	"	0	0
10. (Dog 49)	48 "	0	0.3	1.1	0.4	0	"	0	+	0
11. (Dog 46)	72 "	Very few.	0	0	0	0	0	"	0	0	0
12. (Rabbit 8)	10 minutes.	"	100	17	35	9	Few.	0	0.1	0.05
13. (Rabbit 3)	30 "	110	70	120	6	"	0	
14. (Rabbit 4)	1 hour.	0	9.5	48	13	2.2	Very few.	0.05	0.2	0.05

* Fewer bacteria injected than in the other dogs.

† In this table "Very few" indicates less than one coccus per field; "Few," between one and five per field; "Masses," that they could not be counted. In the kidney, the distribution of cocci was so irregular that no closer average was practicable.

DISCUSSION.—The results of these experiments are in general in accord with those of other investigators who have studied the fate of bacteria after injection into the blood stream. There are, however, certain differences. These may be due in part to the species of animal which we used chiefly, the dog; or to the bacterium employed or to the large amounts of the culture which were injected; or finally, and certainly in part, to the fact that we examined tissues from animals killed almost immediately after the injection had been made as well as at later periods. In considering the distribution of the bacteria in the organs and tissues, particularly those in the lung, it is to be remembered that the injections were made into the left ventricle of the heart in the case of all dogs killed within two hours or less after the injections were given and that the bacteria thus had to pass through a set of capillary vessels before reaching the lung. This method of injection into the left side of the heart instead of intravenously was chosen in order to avoid the lodging of the bacteria in the capillaries of the lung before entering the general circulation. The animals in which the intravenous route was chosen were those to be kept for a considerable period and in those the injections were not made into the heart for fear of producing a purulent pericarditis.

The organs in which the bacteria were quickly lodged in large numbers were the lungs, spleen, and liver. On the other hand, they were not found at all, or were present in insignificant numbers, in the bone marrow, the lymph nodes, the intestinal wall, the skeletal muscles, and for the first few hours at least, in the kidneys and myocardium. Moreover, the rapidity with which they decreased in the organs in which they were detained in such large numbers, and more particularly in the lung, is striking. The spleen, liver, and lungs, acting either independently or perhaps by some combined method, are the organs chiefly active in the destruction of the *Micrococcus aureus* thus injected into dogs, and will be discussed separately.

Lung. — The number of bacteria in the lung showed remarkable differences according to the time which had elapsed between the injection and the death of the animal. When the lung was removed within one minute after the injection, a great number of cocci was present. The majority of these were free in the capillary vessels of the alveolar walls. Besides these there were many polymorphonuclear leucocytes and a few mononuclear cells which had already ingested some of the bacteria. Within ten minutes after the injection almost all of the bacteria had been taken up by the polymorphonuclear leucocytes, which by this time had increased decidedly in numbers over those present one minute after the injection. Here there were practically no free cocci in the sections of lung. After twenty minutes the total number of bacteria per field was distinctly less than they were one minute after the injection.

The results obtained by Werigo may well be recalled here. In studying the localization of bacteria in the lung in the early stages of an experimentally-produced bacteriemia, he killed two rabbits, one five minutes, one eight minutes after the injection of the anthrax bacillus. He found in both cases more bacilli in the lung than in the liver, and the great majority of them had already been ingested by leucocytes, which had accumulated in abundance in the capillaries — a result which is in strict accord with our findings in the dog killed ten minutes after the injection. From this he surmised that the ingestion of bacteria by leucocytes is likely to take place in the capillary vessels of the lung, although he did not demonstrate this assumed earlier stage, where the bacteria were extracellular. Our experiments, however, confirm his assumption by showing the large number of bacteria free in the capillaries of the lung in the animal killed one minute after the injection. It appears evident, therefore, that almost immediately following the injection of the micrococci into the blood stream of the dog, they accumulate, for some unknown reason, in enormous numbers in the capillaries of the lungs. They are there ingested by leucocytes, which collect there in rapidly-increasing numbers, and are then

either very quickly destroyed, or; as appears probable, are in considerable part promptly carried by these wandering cells to other parts of the body, principally to the liver and spleen. The exact period at which they completely disappear has not been determined, as the lung was not examined in some of our earlier experiments, including the thirty-minute, the one-hour, and the two-hour dog. However, in twenty minutes they showed a very marked decrease, and in the eight-hour dog none whatever were found. In the rabbit there also appears to be a similar prompt accumulation in the lungs, followed by a rapid disappearance. We did not here observe the early extracellular stage, as our first rabbit was killed ten minutes after the injection. At this time the lung still showed many more bacteria than either the spleen or liver, a condition which was reversed in the rabbit killed one hour after the injection. Werigo suggested that large microorganisms like the anthrax bacillus may be more readily arrested in the capillaries than smaller bacteria. But, as is apparent from our experiments, large size of bacteria is by no means a necessary factor for their temporary detention in the lung.

Spleen. — As shown by the protocols and the accompanying table, the bacteria not only accumulated rapidly in the spleen and liver following the injections, but remained there for a considerable period. Between one and ten minutes after the injections there was an increase in the number of cocci in these organs, followed by a nearly stationary period, or one with moderate decrease, to a number which remained low between eight and twenty-four hours, followed by a further decrease, to complete disappearance.

Wyssokowitsch, as already seen, found that the microorganisms were taken up by the endothelial cells of the splenic pulp. Kyes confirmed this finding in his experiments with pneumococci in pigeons, saying that the "Phagocytosis by fixed tissue cells is the basis of this accumulation, to which, in the spleen is added a supplementary factor in the filtering action of the modified vascular wall of an artery peculiar to that organ." He also states that wandering-cell

phagocytosis is not a considerable factor in the immunity of the pigeon to the pneumococcus. A somewhat different result was obtained by Werigo. He found that during the first period of anthrax infection in rabbits all bacilli found in the spleen were ingested either by leucocytes or pulp cells, although the rate of phagocytosis in both kinds of cells varied according to different stages of the infection. Immediately after injection, almost all bacteria were present in the splenic pulp, and a comparatively small number of them were engulfed by leucocytes. Then the number of bacteria in leucocytes gradually increased, and twenty minutes after the injection the majority of the bacteria were found inside the leucocytes. During the following stationary period, free bacteria were also present.

Our results are more nearly in accord with those of Werigo than with those of Kyes. The splenic and endothelial cells are undoubtedly an important factor for the elimination of the bacteria from the blood, but the wandering cells also play an important part here. In the earlier stages following infection, a considerable part of the bacteria present were in the polymorphonuclear cells, and the destruction of the cocci, judging by their microscopic appearances, took place in these cells as well as in the fixed cells. This marked intracellular bacteriolysis by both types of cells was very evident. But, in addition, it is noticeable that as long as cocci could be found at all in the spleen, a considerable part of them were extracellular. In fact the relative number of extracellular bacteria increased as the total number decreased, so that in the dog killed sixteen hours after the injection more than two-thirds of them were free, and later nearly all were free. These were found as single bacteria and did not give the impression of having multiplied here. This continued extracellular location of bacteria in the spleen and liver, apparently in good condition judging by their staining properties, appears to us to be a probable important factor in the later development of abscesses in less resistant tissues.

In all cases bacteria, frequently in considerable numbers, were seen imbedded in the walls of the sheathed arteries, an observation already made by Kyes in the spleen of pigeons.

In the spleens removed eight to forty-eight hours after injection, the number of bacteria was decidedly reduced, and polymorphonuclear leucocytes contained practically no organisms in their cytoplasm. The same thing was observed by Werigo in the spleen as well as in the liver. In the spleen of the seventy-two-hour dog no bacteria at all could be recognized.

Liver. — Since the liver is much more bulky than the spleen, the total number of bacteria detained in it is undoubtedly much larger than in the spleen, although the average number per microscopic field found in this organ is somewhat less than it is in the spleen. The localization of microorganisms in the liver has been variously described by different investigators. Wyssokowitsch found the *Micrococcus tetragenus*, as well as the typhoid bacillus which had been injected twenty-four hours previously, especially in the capillary vessels of the liver, closely attached to the walls, and only later were a part of the bacteria incorporated by endothelial cells. Werigo found many bacilli, considerably more than in the spleen, within seven and a half minutes after the injection of anthrax bacillus. Soon afterwards this number decreased very rapidly, and during nearly the entire course of the disease it remained very small. During the first period, most of the bacteria had been taken up by hepatic macrophages, and a much smaller number had been ingested by leucocytes. But, during the stationary period, the number of bacteria ingested by leucocytes gradually increased till it became equal to or sometimes surpassed the number ingested by macrophages. Free bacteria were not met with till the commencement of the third period. According to Kyes, practically all pneumococci found in the liver of pigeons were engulfed by the hemophages.

In all cases in our experiments numerous bacteria were found in the liver in the early stages. Later they decreased very much in number, this organ corresponding closely with the spleen in this respect. Furthermore, the localization of bacteria in the tissue is worthy of note. At first most of them were found inside of cells, viz.: endothelial cells and

polymorphonuclear leucocytes, besides a few transitional leucocytes. But in addition there were many free bacteria. The phagocytosis by both kinds of cells was about equal, though at times that of the wandering cells predominated. With the lapse of time the relative number of bacteria within polymorphonuclear leucocytes increased more and more, while those within endothelial cells correspondingly decreased. At the end of two hours from the beginning almost all bacteria had been ingested by polymorphonuclear leucocytes. In later stages the relative number of free bacteria had gradually increased as in the spleen, while the number of bacteria seen in each field had been remarkably reduced. This extracellular location of the cocci here, as well as in the spleen, even forty-eight hours after the injection, is to be emphasized.

Kidney. — In the early stages following the injection of the bacteria, only very small numbers were found in the kidney and these were almost invariably in the glomeruli and were usually within polymorphonuclear leucocytes. They were found in this small number in nearly every animal with no evident increase even up to eight hours after the injection. A careful search of sections of the kidneys of the dog killed eight hours after the injection has not shown any cocci in the medullary portion. It was in the kidneys of the next dog, killed sixteen hours after the injection, that the masses of bacteria inside the vessels of the lower part of the medulla were first evident. The picture here was the common one of beginning abscess formation. The micrococci were found chiefly in capillary vessels and at times did not even fill the lumen of the vessel, and this when serial sections were studied to exclude the possibility that these might be parts of larger masses. These very small bacterial masses not only did not appear to be parts of an embolus but, at the earliest stages at which we have found them, they had not caused any clotting of blood about them. It is our conclusion that they lodge here against the endothelial cells, either singly or in small numbers, and grow as colonies inside the vessels, and the resulting solid masses of bacteria,

completely filling the vessels, are what have always been described as emboli. Our study of this phase of the subject is not yet completed and will be reported later.

Reviewing the results obtained in the different organs, it is evident that the great majority of the micrococci introduced into the blood stream are quickly taken up by the endothelial cells of the liver and spleen, or by polymorphonuclear leucocytes in these organs or in the lung. The latter cells are apt to change their location in the body, and the number of bacteria present in any organ during the early stage of infection depends in considerable part upon the number of these wandering cells which have accumulated there. For instance, they were present in the lung in large numbers very shortly after the bacterial injection, while the liver at this time showed no increase in leucocytes. The latter organ next showed a rapid increase of polymorphonuclear leucocytes, containing numerous bacteria. A similar process, somewhat less pronounced, was also noticeable in the spleen. It seems justifiable to assume that the gradual accumulation of leucocytes loaded with bacteria in the liver and spleen is in part at least due to their transportation from the lung, where they were at first in such great numbers. These leucocytes carrying bacteria were then detained almost exclusively in the liver and spleen, where the bacteria gradually underwent disintegration. The real reason for their detention here is of course beyond our recognition in a study of this nature, and for the present must be explained by the chemotactic attraction between the liver or spleen tissue on one side, and leucocytes loaded with bacteria, and therefore presumably altered in their minute chemical constitution, on the other.

A factor which makes difficult the recognition of a part of the bacteria deposited in organs and tissues is their rapid destruction within the cells. It has been our common experience to find that bacteria ingested by endothelial cells or leucocytes show varying stages of bacteriolysis, and cultures made of the tissues containing such bacteria often prove to be sterile. In other words, in such cases microscopical findings do not coincide with the results of culture. For this

reason the microscopical examination, though not enabling us to recognize all of the bacteria present, is preferable to the cultural method in order to determine the relative numbers of bacteria distributed in the different organs and tissues of the body.

If we exclude the lung, where the accumulation of bacteria seems to be quite temporary, from our consideration, then principally two organs, viz., the spleen and liver of the tissues studied, are those concerned in the destruction of the *Micrococcus aureus*. However, in spite of the large numbers of bacteria deposited in these organs, neither the spleen nor liver showed any histological evidence of pronounced injury from their presence. These facts may tend to show what a strong resistance to the *Micrococcus aureus* these organs possess among the different tissues of the host.

The failure to find bacteria in the vessels of the medulla of the kidney, even eight hours after injection, with their presence in such large numbers later, leads to the conclusion that they did not locate here directly following the injection, but were temporarily held in some other part of the body. One is inclined to infer that the extracellular cocci seen both in the spleen and liver, and continuing as long as any bacteria were evident in either of these organs, may explain the source of this kidney infection. Many of the extracellular cocci in each of those organs stained well and did not have the appearance of bacteria undergoing lysis. It seems a plausible explanation, though not proven, that the free cocci after remaining in the spleen and liver until a certain degree of immunity had been acquired by them against opsonins, agglutinins, and the bacteriolytic substances of the blood and of the organ in which they were lodged, were later carried in the blood stream to the kidney where, for some unknown reason, the conditions preventing their growth are less marked than in most other parts of the body. Such a supposition would explain our failure to find cocci in the vessels of the medulla of the kidney in the earlier stages of the infection and their later appearance there. Also it appears to us that they probably are not deposited in these vessels as emboli, but as already indicated, lodge singly or

in small numbers against the lining endothelium and form colonies within the vessels. In this connection the findings in the blood smears are of value. No systematic study of the blood at all periods following the infection was attempted, but smears were studied from the blood of animals killed very quickly after the injections and from those that lived eight hours or more. In the smears made from animals during the early stages of infection the bacteria were very largely or entirely within the leucocytes. In the eight-hour dog no cocci were seen in the blood smear. In the sixteen-hour dog the few cocci found were extracellular, and this was the case also in the smears made from the only other animal killed later which showed any present. This extracellular location of the cocci may be due to lack of opsonin at this period, but it appears more probable that the explanation given above is the correct one, that these cocci had already acquired a certain degree of immunity against opsonins and bactericidal substances; that the time during which they were in the spleen or liver represents their adaptation period at the end of which they were able to maintain themselves free in the blood and develop in the kidney.

These results are in entire accord with those obtained by Bull, already referred to, in his work with the pneumococcus in dogs. The adaptation period of the *Micrococcus aureus* as obtained by us is comparatively short, between eight and sixteen hours.

Bull,⁸ in studying the fate of bacteria injected into the blood stream, concluded that, in the case of animals immune to any particular bacterium, there is almost immediate agglutination of that bacterium. We have looked for evidence of such agglutination of the *Micrococcus aureus in vivo* in dogs, because if present it might explain the very rapid removal of the cocci from the blood, and if it occurred, particularly in the pulmonary vessels, it would offer a plausible explanation of their being detained temporarily there in such large numbers. We have not found such agglutination among the extracellular cocci. Those found within the polymorphonuclear leucocytes were often present in such large numbers

in a single cell as to indicate that they had been agglutinated before being ingested.

CONCLUSIONS.

1. The *Micrococcus aureus* injected into the left ventricle of the heart in dogs is almost immediately stored up in relatively great numbers in the lung capillaries, being at first chiefly extracellular, and is there rapidly ingested by polymorphonuclear leucocytes.

2. Shortly after injection, the cocci are stored up in the liver and spleen, their presence here in considerable numbers being coincident with their decrease in the lung. In the liver and spleen they are found largely in wandering cells and in fixed cells, in both of which they rather quickly undergo bacteriolysis. Extracellular cocci are also present in each of these organs, and remain extracellular as long as any cocci can be demonstrated here. The bacteria completely disappear from these organs within forty-eight to seventy-two hours.

3. The cocci do not primarily lodge in the kidney in any considerable numbers, but are secondarily deposited and grow there after a period of adaptation elsewhere. Their location here is probably not embolic.

4. The cocci are found only in small numbers, or not at all, in the blood, bone marrow, lymph nodes, intestinal wall, and skeletal muscle.

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