

STUDIES UPON EXPERIMENTAL PNEUMONIA IN RABBITS.

IX. THE PART OF THE LEUCOCYTE IN THE IMMUNITY REACTION.*

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It has been shown that pneumonia is much more fatal in aplastic than in normal rabbits and that the latter are less resistant to the disease than animals in which a leucocytosis has been artificially produced (1). Recently Gay (2) has reported a hyperleucocytosis following the intravenous injection of antigen in immunized animals, thus indicating the importance of the leucocyte in the immunity reaction as well as in the initial defense. It was therefore determined to study the part of the leucocyte in the immunity reaction.

In the following paper the effect of the intravenous injection of pneumococci in normal, aplastic, and in actively and passively immunized normal and aplastic rabbits will be presented.

METHOD.

It is possible to follow the extent of the bacteriemia in pneumonia by determining the number of organisms in relation to the number of red blood cells. This method is not satisfactory when the organisms are present in small numbers; then they can not be counted accurately or are entirely overlooked. Therefore the following method of making blood cultures was devised. The animals' ears were shaved and the skin was sterilized with 95 per cent. alcohol. One of the larger veins was then punctured with a sterile needle, and a loop of blood was immediately smeared over the surface of a rabbit blood agar slant. This method allows of rapidly repeated cultures and does not interfere with the condition of the animal.

All the cultures in the experiments to be reported were made in the manner described. In all the same amount of blood was taken. The tubes were incubated for forty-eight hours before observations were recorded.

* Received for publication, January 23, 1915.

THE FATE OF INTRAVENOUSLY INJECTED PNEUMOCOCCI AND ITS RELATION TO THE LEUCOCYTIC REACTION IN NORMAL ANIMALS.

Fourteen normal rabbits were given an overwhelming dose of pneumococci intravenously. Blood cultures and smears were made at short intervals until the animals died. In several a number of leucocyte counts were made. The results were similar in all cases (table I).

TABLE I.

Date (1914).	Time.	Procedure.	No. of pneumococci in blood smears.	No. of colonies in blood cultures.	White blood count per c.mm.	Result.
June 3	9 A.M.	11,700
	10 A.M.	8 c.c. pneumococci intravenously
	10.10 A.M.	Too few to count	Innumerable
	11 A.M.	Too few to count	Innumerable
	3 P.M.	400,000 per c. mm.	Innumerable
	5 P.M.	800,000 per c. mm.	Innumerable
	7 P.M.	1,335,000 per c. mm.	Innumerable	2,200
June 4	9 P.M.	3,800,000 per c. mm.	3,900
	1 A.M.	4,400
	10 A.M.	Death.

It may be seen from table I that following an intravenous injection of a lethal dose of pneumococci in a normal rabbit the organisms increase steadily in number until the animal dies. Further, there is a marked and persistent fall in the number of leucocytes in the peripheral circulation.

THE FATE OF INTRAVENOUSLY INJECTED PNEUMOCOCCI AND ITS RELATION TO THE LEUCOCYTIC REACTION IN APLASTIC ANIMALS.

Experiments with several rabbits in which the leucocytes had been reduced to below 1,000 per cubic millimeter by the daily injection of benzol showed that these animals are more susceptible than normal rabbits to the intravenous injection of pneumococci. No special experiments were undertaken to determine the leucocytic reaction, as the result of such a procedure seemed sufficiently evident from previous experiments in which pneumococci had been injected intratracheally in aplastic animals. Here a bacteriemia developed while the number of leucocytes remained uninfluenced.

THE FATE OF INTRAVENOUSLY INJECTED PNEUMOCOCCI AND ITS
RELATION TO THE LEUCOCYTIC REACTION IN PASSIVELY
IMMUNIZED RABBITS.

Three rabbits were passively immunized and then injected intravenously with an overwhelming dose of homologous pneumococci. Blood cultures were taken at short intervals over a period of twenty-seven to seventy-two hours. The results in all were practically identical (table II).

TABLE II.

Date (1914).	Time.	Procedure.	No. of colonies in blood cultures.	Result.
June 8	9 A.M.	20 c.c. immune serum
	10 A.M.	10 c.c. virulent pneumococci intravenously
	10.05 A.M.	Very many
	10.30 A.M.	None
	11 A.M.	None
	2 P.M.	None
June 9	8.30 P.M.	None
	1 P.M.	None	Recovery.

Table II shows that, following the intravenous injection of a lethal dose of pneumococci in a passively immunized rabbit, the organisms are present in large numbers for the first five minutes, but are no longer demonstrable in the circulation thirty minutes after the injection.¹

¹In the other two rabbits in this series the time of disappearance of the organisms was one hour and three hours, respectively. All three animals recovered completely.

THE FATE OF INTRAVENOUSLY INJECTED PNEUMOCOCCI AND ITS
RELATION TO THE LEUCOCYTIC REACTION IN ACTIVELY
IMMUNIZED RABBITS.

In this experiment fifteen rabbits were used. The animals received a number of gradually increasing doses of pneumococci until they possessed a decided immunity. They were then injected intravenously with a lethal dose of pneumococci of the same strain. Blood cultures were taken and the leucocytes counted at short intervals after the injection. The chart of animal 2 (table III) is characteristic of this group.

TABLE III.

Date (1914).	Time.	Procedure.	No. of colonies in blood culture.	White blood count per c.mm.	Result.
Apr. 17 to May 23		Gradually increasing doses of pneumococci intravenously from 0.5 c.c. to 9.0 c.c.			
June 3	9.15 A.M.			10,800	
	10.15 A.M.	8 c.c. of pneumococci in- travenously			
	10.20 A.M.		Innumerable		
	10.30 A.M.		Very many		
	10.45 A.M.		Many		
	11.15 A.M.		50		
	12.15 P.M.		12		
	2.15 P.M.		None		
	4.15 P.M.		None		
	7.15 P.M.		None	23,300	
	10.15 P.M.		None	37,300	
June 4	1.15 A.M.		None	27,900	
	10.15 A.M.		None		Recovery.

Table III shows that in actively immunized rabbits the injection of a lethal dose of pneumococci is followed by a rapid disappearance of the organisms from the circulation, and that there is a decided leucocytosis reaching its maximum about twelve to fifteen hours after injection. This leucocytosis occurs many hours after the disappearance of the organisms from the circulation.

THE FATE OF INTRAVENOUSLY INJECTED PNEUMOCOCCI AND ITS
RELATION TO THE LEUCOCYTIC REACTION IN APLASTIC
PASSIVELY IMMUNIZED RABBITS.

Eight rabbits were given daily doses of benzol subcutaneously until the number of their leucocytes was below 1,000 per c.mm. At this stage they were passively immunized and then injected with a lethal dose of the homologous pneumococci. Blood cultures were taken at short intervals, and in one animal leucocyte counts were made before and after the injection of the organisms. All these animals reacted identically (table IV).

TABLE IV.

Date (1914).	Time.	Procedure.	No. of colonies in blood cul- tures.	White blood count per c.mm.	Result.
May 27 to June 2		Daily subcutaneous in- jections of benzol			
June 3	8.30 A.M.			340	
	8.45 A.M.	10 c.c. immune serum intravenously			
	9.30 A.M.	10 c.c. immune serum intravenously			
	9.45 A.M.	8 c.c. pneumococci intra- venously			
	9.50 A.M.		Many		
	10.15 A.M.		36		
	10.45 A.M.		6		
	11.45 A.M.		1		
	1.45 P.M.		1		
	3.15 P.M.		6	160	
	7.45 P.M.		None	80	
June 4	9.45 A.M.		Very many		
June 5	3.45 A.M.		Innumerable		Death.

Table IV shows that after the intravenous injection of a lethal dose of pneumococci in passively immunized aplastic rabbits there is a rapid disappearance of the organisms from the circulation; within two to four hours they have practically disappeared. The disappearance of the organisms from the blood is, however, only temporary. The bacteriemia recurs and there is a steady increase in the number of organisms until the animal dies. In addition, it may be noted that the injection of the pneumococci in these animals is not followed at any time by an increase in the number of leucocytes in the peripheral circulation.

THE FATE OF INTRAVENOUSLY INJECTED PNEUMOCOCCI AND ITS
RELATION TO THE LEUCOCYTIC REACTION IN ACTIVELY
IMMUNIZED APLASTIC RABBITS.

Three animals were actively immunized in the same manner as those previously mentioned. When they possessed a decided immunity they were given daily subcutaneous injections of benzol until their leucocytes were reduced to below 1,000 per c.mm. At this stage they were each injected intravenously with a lethal dose of pneumococci of the same strain used before, and blood cultures were taken at short intervals thereafter. In these animals leucocyte counts were also made several times after the injection of the organisms.

The reaction in all cases was the same and is illustrated by table V.

TABLE V.

Date (1914).	Time.	Procedure.	No. of colonies in blood cul- tures.	White blood count per c.mm.	Result.
Apr. 17 to May 23		Gradually increasing doses of pneumococci injected intravenously up to 9 c.c.			
May 25 to June 2		Daily subcutaneous in- jections of benzol			
June 3	8.50 A.M.	8 c.c. pneumococci intra- venously			
	9.00 A.M.		Innumerable	280	
	9.20 A.M.		Innumerable		
	9.50 A.M.		36		
	10.20 A.M.		3		
	11.20 A.M.		1		
	1.20 P.M.		None		
	7.20 P.M.		None	380	
	9.20 P.M.		None	560	
June 4	12.20 A.M.		None	500	
	9.20 A.M.		None	380.	
June 5	3.20 A.M.		None	580	Recovery.

Table V shows that, following the intravenous injection of a lethal dose of pneumococci in actively immunized aplastic animals, there is a permanent disappearance of the organisms from the circulation, and the rabbit recovers.

In these actively immunized aplastic animals there is no increase in the number of leucocytes at any time after the injection of the pneumococci.

SUMMARY AND DISCUSSION.

Following the intravenous injection of an overwhelming dose of pneumococci in normal animals there is a rapidly increasing bacteriemia which reaches its maximum with the death of the animal.

Immunized animals, whether the immunity is active or passive, whether the animals have their leucocytes, or whether these have been destroyed by benzol, react differently. They have in common the ability to cause the organisms to decrease rapidly in number, and, as far as could be determined by the methods used, to disappear absolutely in a very short time from the circulation. This may be called the immediate reaction.

In actively and passively immunized normal rabbits the disappearance of the organisms from the blood is followed by their destruction in the body and the ultimate recovery of the animal. This same result occurs also in actively immunized rabbits deprived of their polymorphonuclear leucocytes by benzol. On the contrary, in passively immunized rabbits, deprived of their leucocytes in the same way, the immediate disappearance of the organisms from the circulation is followed, after a lapse of from six to twenty-four hours, by a recurrent, gradually increasing bacteriemia and the death of the animal.

It is evident that the immediate reaction is no index of the ultimate result.

This finding, that the result of the intravenous injection of an overwhelming dose of pneumococci in immunized animals may be divided into two stages, immediate and ultimate, has been utilized in the interpretation of the experiments that have been reported above.

1. *Normal Rabbits.*—In the normal rabbit the injection of a lethal dose of pneumococci is followed by the same immediate and ultimate response. The animal develops a rapidly progressing bacteriemia and dies.

2. *Passively Immunized Normal Rabbits.*—A diametrically opposed finding is obtained when the animals are passively immunized. Then following the intravenous injection of pneumococci the organisms rapidly disappear from the circulating blood and the animal recovers. The only apparent difference between these two groups of experiments is the introduction of a relatively small quantity of immune serum. Tentatively, therefore, it may be concluded that the immune serum is responsible both for the immediate and ultimate reaction in this case.

3. *Passively Immunized Aplastic Rabbits.*—A normal rabbit that has been previously benzolized, and in this way deprived of its myeloid elements, can not be successfully immunized. Here the immune serum injected even in excessive quantities is followed by the immediate reaction, but the ultimate result is entirely different,—the animal dies.

It may be concluded, therefore, that the serum is the potent factor in bringing about the immediate disappearance of the organisms from the circulation.

Furthermore, it is evident that the white blood cells of the myeloid tissue are necessary in order that passively immunized animals may recover following the introduction of a lethal dose of pneumococci. So far it seems that two elements are essential in the immunity process; *i. e.*, immune bodies and white blood cells.

Corroborative evidence of the importance of the white blood cell in this reaction is offered by the hyperleucocytosis which follows the injection of antigen in actively immunized animals (Gay). This hyperleucocytosis occurs in actively immunized rabbits injected with pneumococci, after the organisms have disappeared from the circulation. It occurs at about the same time that the septicemia recurs in passively immunized aplastic rabbits.

4. *Actively Immunized Aplastic Rabbits.*—The experiments with actively immunized aplastic rabbits complicate the conception of the part of the white blood cells in the immunity process. When actively immunized, benzolized animals are injected with a lethal dose of pneumococci, the immediate reaction occurs just as in the passively immunized aplastic animal. Available antibodies are present and cause the disappearance of the organisms from the circulation. The ultimate reaction differs from that in the passively immunized aplastic animal. The latter develops a recurrent bacteriemia and dies. The actively immunized aplastic animal recovers.

It would seem that the white blood cell is no longer necessary in an animal that has been actively immunized, but that it must be present for the passive protection of the animal.

The ultimate reaction in immunized rabbits seems to be dependent upon some action of the white blood cells. In the passively immunized animal this may occur at the time of, or following, the im-

mediate reaction; and if it can not occur, owing to the absence of the white blood cells, the animal subsequently dies. In the actively immunized aplastic rabbit this action has apparently occurred at a previous time and is sufficiently developed to protect the animal even though the leucocytes have been destroyed.

It may be assumed that the function of the white blood cell is exercised not directly, but perhaps indirectly, by some influence exerted on other body cells. This interrelation or interaction between the white blood cells and other cells in the body constitutes a third factor essential to the ultimate protection of the animal.

Additional evidence of this action of the white blood cell is furnished by further experiments with actively immunized aplastic rabbits. Several immunized rabbits were benzolized and then injected with a lethal dose of pneumococci. The results were the same as those indicated above. The organisms disappeared rapidly from the circulation and the animals recovered.

An interval of five days was allowed to elapse and the animals were injected again with the same amount of pneumococci. This time the reaction was different. The organisms disappeared rapidly from the circulation, but there was a recurrent bacteriemia and the animals died. In these animals the favorable ultimate result after the first inoculation of a lethal dose of pneumococci probably depended upon the presence of the immune bodies and the third factor mentioned above. After the subsequent inoculation of pneumococci the immediate reaction was followed by the recurrence of the bacteriemia and the death of the animals. It may, therefore, be inferred that the third factor present at the time of the first injection had been destroyed, and, owing to the absence of white blood cells at the time of the subsequent inoculation, it was not formed again.

It may be concluded that there are at least three elements necessary in the immunization process: (1) immune bodies, (2) white blood cells, and (3) a third factor which is dependent for its existence upon the presence of white blood cells at the time of inoculation of the pneumococci. Furthermore, this third factor may be removed if the animal is inoculated at a time when it is aplastic.

CONCLUSIONS.

1. The result of the intravenous injection of pneumococci in immunized rabbits may be divided into two stages,—immediate and ultimate.

2. The immediate reaction is not decisive of the ultimate result.

3. The immunity process seems to be dependent upon at least three factors: immune bodies, white blood cells, and a third factor which is dependent for its existence upon the presence of the white blood cells at the time of the inoculation of the pneumococci.

This third factor may be removed by rendering an immunized normal rabbit aplastic and then injecting it with a minimal lethal dose of pneumococci.

The result of the injection of this antigen into immune rabbits varies according to the presence of the three factors mentioned above. The immune bodies cause an immediate disappearance of the organisms from the circulation. The third factor causes the permanent absence of the organisms from the circulation and the recovery of the animal. The white blood cells seem to be essential for the production of this third factor.

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