THE DISTRIBUTION IN THE BLOOD AND LYMPH OF PNEUMOCOCCUS TYPE III INJECTED INTRAVENOUSLY IN RABBITS, AND THE EFFECT OF TREATMENT WITH SPECIFIC ANTISERUM ON THE INFECTION OF THE LYMPH

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For several years, two of us have been interested in the passage of visible particles through the walls of blood vessels and lymphatics (1). During 1935, experiments[1] were carried out which showed that when rabbits were injected intravenously with large doses of a virulent Type III Pneumococcus, the animals developed a bacteremia, and within an hour the organisms could be demonstrated by culture to be present in the thoracic duct lymph (2). These non-motile bacteria presumably passed through the walls of blood capillaries into the tissue fluid, and then through the walls of lymph capillaries in order to reach the lymph stream.

While the thoracic duct carries lymph from all parts of the body, the greatest volume of fluid comes from the abdominal region. Judging by the protein content of liver and intestinal lymph, it is generally held that the blood capillaries in this huge region are more permeable than those in other parts of the body such, for example, as the skin and subcutaneous tissues. As a result of improvements in technique it became possible to cannulate lymphatics and collect lymph continuously from vessels in the subcutaneous tissues of the neck and occasionally the foot. It therefore seemed significant in rabbits infected with pneumococci to collect lymph draining from such areas. Should this lymph after intravenous injection of pneumococci contain the organisms, it would indicate that in the presence of a bac-

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[1] In collaboration with members of the Department of Bacteriology.

teremia the tissue spaces and lymphatics generally are invaded by pneumococci.

In addition to obtaining data on the bacterial content of the lymph from these sources, we have extended our observations on the penetration of pneumococcus antibody into the lymph following its introduction by the intravenous route. In the previous paper (2) it was shown that after the administration of rabbit or horse antipneumococcus Type III sera to infected rabbits, antibodies often fail to reach the lymph in demonstrable amounts, and even when shown to be present in considerable quantity do not lead to its sterilization. The unfortunate implications of such a situation from the standpoint of serum therapy are obvious. Our earlier experiments, however, were not extended longer than 4 hours after the injection of antiserum. Because of this it could be reasonably objected that subsequently the antibody might exert a beneficial effect on the lymphatic infection. We have, therefore, advanced the period of our observations to an interval of 18 hours following antiserum treatment. Furthermore, we have sought to eliminate, by the use of organisms in the state of maximum encapsulation, any possibility of transfer from blood to lymph in the mobile phagocytic cells.

Technique

Strains of Pneumococci.—The same rabbit virulent strain of Pneumococcus Type III (strain SV) (3) as previously employed was used in nearly all of the present work. Into a few animals was inoculated another strain of Pneumococcus Type III (strain CH) (4) which, although highly virulent for mice by the intraperitoneal route of infection, failed to kill rabbits even when given in large amounts. 16 to 20 hour rabbit blood broth cultures of these strains were usually the source of the infecting dose, but in one instance the serum of a rabbit dying after infection with strain SV was used.

Antisera.—Although several different lots of antipneumococcus Type III horse sera were injected in various experiments, that serum possessing an agglutinin titre of 1:400 was utilized in all but one of those which are reported in detail in this communication.[2] In one case an antiserum[3] showing an agglutinin titre

[2] This antiserum was obtained through the courtesy of Dr. Annabelle W. Walter, of the Bureau of Laboratories of the Department of Health of the City of New York.

[3] This antiserum was obtained through the courtesy of the Massachusetts State Antitoxin and Vaccine Laboratory.

of 1:20 was used. An anti-horse rabbit serum exhibiting a titre of 1:20,000 (dilution of the antigen) against normal horse serum served to determine the quantity of horse serum present in blood and lymph, following the administration of the antipneumococcus Type III horse serum. For this purpose the ring test technique, using undiluted anti-horse rabbit serum and falling dilutions of blood serum and lymph, was adopted. Readings were taken after 2 hours at room temperature.

Blood and Lymph Cultures.—Measured amounts of blood and lymph specimens were plated undiluted and after suitable dilution in infusion broth, by the blood agar pour plate method.

Cannulation of the thoracic duct in the rabbit has been previously described (2) but slight modifications in technique have been introduced. The left external jugular vein is followed to its junction into the subclavian vein and ligated at this point. This minimizes the amount of dissection, since it is no longer found necessary to ligate the subclavian vein. All branches entering the jugular vein are carefully tied, and it is then ligated a second time about 2 cm. from its junction point into the subclavian vein. The blocked thoracic duct will then show up clearly. The venous pocket so formed is first cannulated but, if this results in bloody lymph, direct cannulation of the thoracic duct is employed, using a minimum of dissection. This, with care, is successful over 90 per cent of the time.

The cervical lymphatics are picked up easily, particularly on the left side. After the thoracic duct has been blocked by ligating the jugular vein, the cervical lymphatic which enters it will likewise become distended and can easily be recognized.

The leg lymphatics in the rabbit are extremely small and unusually delicate. In order to expose them at the ankle, 0.1 cc. of 1 per cent trypan blue must be injected subcutaneously near the toes. The lymphatics can then be picked up on either side of the saphenous vein. The binocular dissecting microscope is used both for separating the lymphatic from its connective tissue and for cannulation for which the smallest possible quartz cannula is used.

EXPERIMENTAL

To obtain information concerning the question of whether pneumococci emigrate from the blood vessels and pass into the lymphatics draining areas other than that of the abdominal region, the right cervical and right leg lymphatic ducts of a normal rabbit (weight, 1.7 kilos) were cannulated after nembutal anesthesia (1.5 cc. of 5 per cent nembutal intravenously). The deposit obtained by centrifugalization of 40 cc. of a 16 hour blood broth culture of Pneumococcus Type III (strain SV) was resuspended in 1 cc. of sterile broth and injected into the ear vein (4 hours after anesthesia). Samples of blood from the right jugular vein and specimens of lymph from the three sources noted above were taken from time to time and cultured. The experiment was continued for about 4 hours.

It will be seen from an inspection of the data presented in Table I that the organisms appeared promptly (within 15 to 20 minutes) in

TABLE I

Rabbit 1. Massive Intravenous Injection of Pneumococci. Recovery of Organisms in Thoracic Duct, Cervical, and Foot Lymph Almost Immediately Afterwards

Time	Organisms per cc. of				Remarks
	Blood	Thoracic duct lymph	Cervical lymph	Foot lymph	
p.m.					
1:10					Animal inoculated intravenously with organisms from 40 cc. of 16 hr. B.B. culture SV
1:12	5.3×10^7				
1:25		875			
1:30			2800		
1:45		1400			
1:50				3 in 1 small drop	
2:11		2300			
2:15			9600		
2:18	1.8×10^7				
2:38				1 in 2 small drops	
2:40		1500			
2:47			7200		
3:05				3 in 2 small drops	Cannula afterwards pulled out of leg lymphatic
3:10		1300			
3:19	2.2×10^7				
3:40			1450		
3:55					Cannula removed from cervical lymphatic
4:05					
4:15	8.5×10^7				
4:50					
4:55	1.8×10^8				Experiment terminated

the cervical as well as in the thoracic duct lymph. The numbers of cocci in each did not greatly differ, although on the whole those in the cervical lymph appear to be somewhat greater. In several of our

experiments definitely more organisms were cultured from neck lymph than from thoracic duct lymph. It is possible that the relatively large numbers of pneumococci in the former may be accounted for in part by the massage which is necessary in its collection. Adjacent lymph nodes as well as vessels are included in the field of massage. Even slight pressure on lymph nodes causes a rapid increase in the lymphocytes in efferent lymph. Thus it is not improbable that massage also increases the number of organisms in the lymph by forcing some of those in the nodes and tissue spaces of the neck into the lymph stream. Organisms were also found in foot lymph in the first specimen which was taken within 50 minutes after intravenous injection of culture. In the rabbit the amounts of foot lymph which can be secured at any one time are so small that accurate measurement of volume is impracticable, and therefore figures comparable with those for thoracic duct lymph and cervical lymph are not presented. We can say only that pneumococci may also quickly gain entrance to the lymphatics of the foot, although without much doubt in far smaller numbers. The results indicate that into these regions of the lymphatic bed, which are ordinarily considered less permeable to the entrance of proteins and other substances, the bacteria in question may penetrate with facility.

Since it had been found (5) that in cultures of Pneumococcus Type III (strain SV) of the age employed a certain percentage of the microorganisms appeared to be vulnerable to phagocytic attack and were thus removed from the circulating blood, we wished to eliminate this possible means of intracellular transfer from blood to lymph. It has been shown (5) that completely encapsulated organisms, such as occur in the blood of a rabbit dying of infection with this strain, remain in undiminished quantities in the blood, which indicates a complete resistance to ingestion by phagocytes. Following cannulation of cervical and thoracic lymph ducts, a normal rabbit was therefore injected intravenously with 13 cc. of the blood serum from a second rabbit suffering from a severe bacteremia. The bacterial content of blood and lymph specimens taken at intervals thereafter was determined. The results of this experiment are included in Table II. Here again there has been rapid passage of organisms across the

vascular lymphatic barriers in the case of both cervical and thoracic duct lymph, although the possibility of movement of cocci in phagocytes has been substantially ruled out. As further evidence for the failure of phagocytic carriage to account for these findings we may mention experiments not here reported in detail in which large numbers of Pneumococcus Type III (strain CH) were injected. These organisms had already largely lost their capsules during the course of

TABLE II

Rabbit 2. Intravenous Injection of Heavily Infected Serum from a Second Rabbit Moribund as a Result of Pneumococcus Infection. Phagocytosis as a Means of Transfer from Blood Thus Eliminated

Time	Organisms per cc. of			Remarks
	Blood	Thoracic duct lymph	Cervical lymph	
p.m.				
12:30				13.0 cc. of infected rabbit serum injected intravenously
12:32		0		
12:35	1×10^8			
12:45		20		
12:47			250	
12:55	8.2×10^7			
1:27		20,000		
1:32			6000	
2:35	3.4×10^8			
2:40				Thoracic duct lymph suddenly became bloody
2:45			9000	
3:30	3.7×10^8			
3:45			9000	
4:00				Experiment terminated

growth and in consequence the great majority were readily removed from the blood stream, presumably by phagocytic cells (5). In this condition these cocci did not appear at all in the cervical lymph, while in that from the thoracic duct, although most specimens were sterile, in a few an extremely small number of pneumococci were detected. These observations show that when massive phagocytosis of the organisms takes place the lymph is either not invaded at all or only to a minimal degree.

Having established the fact that the pneumococci migrate into the cervical and foot lymph as well as the thoracic duct lymph, and that this migration is not mediated through the agency of phagocytic ingestion, we next proceeded to reinvestigate the effect of antiserum on the organisms in these sites, partly because lymph derived from different regions was now available and partly to study the course of events during periods farther removed from the time of inauguration of serum treatment than those previously recorded. Details of two typical experiments on this point are given in Tables III and IV.

The animal which yielded the data summarized in Table III was inoculated with a small dose of pneumococci 23½ hours before the administration of 15 cc. of antipneumococcus Type III horse serum[3] (agglutinin titre 1:20). The organisms in the blood, which were present in moderate numbers when the serum was given, were temporarily reduced within a brief period to zero, although subsequently they reappeared. In this case the initial number of pneumococci in the thoracic duct lymph was comparatively small, and this may have undergone a transient reduction following antiserum but soon increased. Cervical lymph on the other hand contained relatively large quantities of cocci. These also underwent some diminution in the course of the experiment, although sterility was not obtained within the 7 hours after the injection of antiserum. Two samples of leg lymph proved sterile on culture.

The protein content of cervical and thoracic duct lymph was similar but the cell counts were uniformly higher in the cervical specimens. In both types of lymph the cells were almost entirely lymphocytes and the presence of pneumococci did not alter the differential counts. On the average the large mononuclears were found to comprise from 2 to 6 per cent of the total number of cells while polymorphonuclear leucocytes were absent.

These observations, then, indicate that within the 7 hours following antiserum administration the lymph fails to become free of pneumococci. It should be noted, however, that the antiserum contained a rather low titre of antibody as measured by its agglutinating capacity. This might account for its failure to lead to the elimination of the cocci in the lymph. In the experiments which follow, similar results were secured although an antiserum exhibiting an agglutinin titre far greater was used.

TABLE III

Rabbit 3. Intravenous Infection Followed by Antipneumococcus Type III Horse Serum after 23½ Hours

Time	Organisms per cc. of				General data and remarks
	Blood	Thoracic duct lymph	Cervical lymph	Foot lymph	
Mar. 17, 1936 *a.m.*					
11:00					Intravenous inoculation of 0.2 cc. of 16 hr. B.B. culture of SV. Rectal temperature 103.0°F.
Mar. 18, 1936 *a.m.*					
9:10					1.2 cc. of 5 per cent nembutal intravenously
10:05					Rectal temperature 105.7°F.
10:25	2400				
10:33					15 cc. antipneumococcus Type III horse serum intravenously (H536, N. Y.)
10:45	50				
11:50					Leucocytes in blood 2600 per c.mm.
p.m.					
12:30					Protein in thoracic duct lymph 3.55 per cent
12:37					Erythrocytes in thoracic duct lymph 500 per c.mm. Leucocytes in blood 3200 per c.mm. Rectal temperature 106°F.
12:40					Protein in cervical lymph 3.56 per cent
12:50					Leucocytes in thoracic duct lymph 37,200 per c.mm.
1:00					Leucocytes in cervical lymph 55,400 per c.mm.
1:15	0				Erythrocytes in cervical lymph 500 per c.mm.
1:20		7			
1:35			1200		
1:55					Protein in leg lymph 1.23 per cent
2:25					Leucocytes in thoracic duct lymph 34,400 per c.mm. Erythrocytes in thoracic duct lymph 500 per c.mm.
2:45					Leucocytes in blood 4600 per c.mm.

TABLE III—*Concluded*

Time	Organisms per cc. of				General data and remarks
	Blood	Thoracic duct lymph	Cervical lymph	Foot lymph	
p.m.					
2:55					Protein in cervical lymph 3.63 per cent
2:58					Protein in thoracic duct lymph 3.33 per cent. Leucocytes in cervical lymph 129,800 per c.mm. Erythrocytes in cervical lymph 3400 per c.mm.
3:05					Rectal temperature 106.1°F.
3:12				0	
3:25			2000		
3:35		0			
4:05	0				
4:20	120				Thoracic duct lymph 49,400 leucocytes per c.mm., and 1200 erythrocytes per c.mm.
4:22				0	
4:40		30			
4:47			900		
4:50					Thoracic duct lymph, leucocytes 63,400 per c.mm. and erythrocytes 1200 per c.mm.
4:55					Rectal temperature 108°F.
4:56	77				Leucocytes in blood 3400 per c.mm.
5:00					Cervical lymph, leucocytes 129,400 per c.mm. and erythrocytes 9600 per c.mm.
5:12			250		
5:16				0	
5:19		10			
5:25					Experiment terminated

The data assembled in Table IV were obtained in the case of a rabbit which received two doses of antiserum, at 33 hours and again at 50 hours, following intravenous inoculation of a small dose of strain SV culture. Examination of the results shows that the first dose of antiserum reduced the organisms present in the circulating blood, but 16 hours later, at the beginning of the observations following cannulation of the lymphatics, organisms were detected in the blood

TABLE IV

Rabbit 4. Intravenous Infection with Small Dose of Pneumococci, Followed 33 Hours Later by Intravenous Administration of Antipneumococcus Type III Horse Serum. Recovery of Organisms in Thoracic Duct and Cervical Lymph, Both before and after a Second Injection of Antiserum (17 Hours Subsequent to First Serum Treatment). Titration of Blood and Lymph for Presence of Antibodies and Also Horse Serum Vehicle

Time	Organisms per cc. of			Agglutinin titre *vs.* Type III Pneumococcus	Precipitinogen titre horse serum *vs.* anti-horse rabbit serum	General data and remarks
	Blood	Thoracic duct lymph	Cervical lymph			
May 4, 1936						
p.m.						
2:00	Animal inoculated intravenously with 0.05 cc. of 21 hr. blood broth culture of SV					Rectal temperature before inoculation 102.7°F. Leucocytes in blood before inoculation 6500 per c.mm.
5:00						Rectal temperature 103.5°F.
May 5, 1936						
p.m.						
3:15						Rectal temperature 105.8°F.
10:40	5300			0 (blood)	0 (blood)	
11:00						Rectal temperature 105.7°F. Intravenous injection of 7.5 cc. antipneumococcus horse serum (New York)
11:30	2000			1:50 (blood)	1:600 (blood)	
May 6, 1936						
a.m.						
9:15						1.6 cc. nembutal intravenously
9:45						Leucocytes in blood 4800 per c.mm.
p.m.						
12:30						Rectal temperature 102°F.
12:55						Thoracic duct lymph, 25,400 leucocytes per c.mm., 6200 erythrocytes per c.mm.

1:30						Thoracic duct lymph, protein 3.74 per cent
2:30						Leucocytes in blood, 4800 per c.mm.
2:40				0 (thoracic duct lymph)	1:200 (thoracic duct lymph)	
2:50	2500			0 (blood)	1:300 (blood)	
3:10		1.1×10^5				Thoracic duct lymph, 70,400 leucocytes per c.mm., 1200 erythrocytes per c.mm.
3:20			180 per small drop			
3:30				0 (thoracic duct lymph)	1:200 (thoracic duct lymph)	
3:40						Cervical lymph, 137,200 leucocytes per c.mm., no erythrocytes seen
3:45	2500			0 (blood)	1:300 (blood)	Rectal temperature 104.5°F.
3:55		1.9×10^5				
3:57			∞ colonies per drop			
4:00						Intravenous inoculation of 5 cc. of antipneumococcus horse serum (New York)
4:18				0 (thoracic duct lymph)	1:300 (thoracic duct lymph)	
4:25			∞ colonies per drop	0 (cervical) lymph)	1:600 (cervical lymph)	
4:30	100 or less			1:3 (blood)	1:600 (blood)	
4:55			6.7×10^6			
5:03		1.2×10^6				
5:05			∞ colonies per 0.05 cc.			Thoracic duct lymph, 52,800 leucocytes per c.mm., 1400 erythrocytes per c.mm.
5:07	14,500			0 (blood)	1:800 (blood)	
5:10				0 (thoracic duct lymph)	1:500 (thoracic duct lymph)	Blood, leucocytes 1200 per c.mm.
5:11						Cervical lymph, 81,800 leucocytes per c.mm., 400 erythrocytes per c.mm.

TABLE V

Rabbit 5. Intravenous Infection with Small Dose of Pneumococci, Followed after 24 Hours by Intraperitoneal Administration of Antipneumococcus Type III Horse Serum. Titration of Antibody in Blood and Lymph as well as Determination of the Presence of Organisms in These Fluids

Time	Organisms per cc. of				Agglutinin titre *vs.* Type III Pneumococcus	Precipitinogen titre horse serum *vs.* anti-horse rabbit serum	General data and remarks
	Blood	Thoracic duct lymph	Cervical lymph	Foot lymph			
May 18, 1936							
p.m.							
4:55	Animal inoculated intravenously with 0.1 cc. of 23 hour blood broth culture of SV						
May 19, 1936							
a.m.							
11:00	3.3×10^4						Rectal temperature 103.8°F.
p.m.							
4:55	9.7×10^4				0 (blood)	0 (blood)	Rectal temperature 105.6°F.
5:05							10 cc. antipneumococcus Type III horse serum (New York), intraperitoneally
6:05	180				1:3 (blood)	1:200 (blood)	Rectal temperature 104.6°F.
May 20, 1936							
p.m.							
12:20					1:1 (thoracic duct lymph)	1:300 (thoracic duct lymph)	
12:36		1720					
12:55							Blood, 10,600 leucocytes per c.mm.
1:00							Thoracic duct lymph, 50,800 leucocytes per c.mm., 1200 erythrocytes per c.mm.

1:10					1:1 (thoracic duct lymph)	1:300 (thoracic duct lymph)	
1:11	0						
3:00				0			
3:40							Blood, 7400 leucocytes per c.mm.
3:54				0			
4:10	530				1:6 (blood)	1:400 (blood)	
4:15		1.1×10^5					
4:20							Thoracic duct lymph, 67,400 leucocytes and 400 erythrocytes per c.mm.
4:45					1:1 (thoracic duct lymph)	1:300 (thoracic duct lymph)	
4:50							Cervical lymph, 139,000 leucocytes and 3600 erythrocytes per c.mm.
4:55				0			Blood, 8600 leucocytes per c.mm.
5:05			7060				Thoracic duct lymph, 32,400 leucocytes and 3400 erythrocytes per c.mm.
5:10	530						
5:25		1.9×10^5					Experiment terminated

in numbers practically equivalent to those observed shortly after antiserum was first introduced. A finding which calls for particular emphasis is the fact that a definitely greater number of cocci were present at this time in the thoracic duct lymph and probably also in the cervical lymph, than were cultivated from the blood. 17 hours after the first dose of antiserum a second injection of 5 cc. was given. This resulted in a reduction of approximately 95 per cent of the organisms in the blood. The larger numbers in the thoracic and cervical lymph showed no evidence of decrease but continued to rise. This may serve to explain the subsequent large increase in the bacterial count of the blood at the close of the experiment, which may well have resulted from the influx of organisms from other intact lymphatics, such as the remaining cervical duct which had not been cannulated.

Titrations to determine the presence of agglutinins in blood serum and lymph, indicate that the considerable amount of antibody in the serum immediately after the first injection of antiserum had completely disappeared by the time the operation on the following day was completed. A small amount of antibody in the blood serum was found in the sample taken just after the second antiserum injection, but this was speedily exhausted. No agglutinating antibody could be detected in any sample of lymph. That the horse serum vehicle had gained entrance to the lymphatic system in abundance was readily demonstrated by means of precipitin tests with anti-horse rabbit serum. The failure to discover antibody in the lymph and its rapid disappearance from the blood, can be attributed without much doubt to the large numbers of organisms present, as well as their soluble antigenic products which would speedily neutralize considerable quantities of agglutinin. In general the results clearly indicate that, even at intervals of many hours following antiserum treatment, the pneumococci may be found in large numbers in the lymph, where a second intravenous injection of antibody fails to reduce them.

The experiment recorded in Table V serves to confirm these findings. In addition it shows that, although demonstrable antibody may be observed in the lymph after 24 hours following intraperitoneal injection of antiserum in the case where the number of cocci

in the lymph is moderate, nevertheless antibody at least in this quantity is insufficient to effect a permanent reduction or to inhibit their multiplication.

DISCUSSION

In this paper are presented the results of experiments which supplement and extend those reported in 1935. They make it quite apparent that, during the course of the severe bacteremia following intravenous infection of the rabbit with virulent Pneumococcus Type III, the bacteria readily pass through the vascular walls, migrate through the tissue spaces, and enter lymphatic vessels. This emigration from the blood stream can take place not alone through the capillaries of the highly permeable abdominal region, since during this investigation it has been found that the organisms are present in both the lymph of the cervical duct and of the lymphatic vessels of the foot, as well as in that of the thoracic duct. Thus one may fairly conclude that hematogenous infection of the lymphatics may occur at almost any site.

Although, in our former experiments, we felt that it was unlikely that the conveyance of the pneumococci from blood to lymph was effected by phagocytic cells which had ingested the organisms but had failed to destroy them, there was nevertheless the possibility that entrance to the lymphatics was gained by this means. Through the use of completely encapsulated organisms (animal), it has been shown that these readily infect the lymph although entirely resistant to phagocytic attack, while pneumococci in the decapsulated state which are easily taken up by phagocytes enter the lymph in minimal numbers or not at all.

Further information concerning the effect of the intravenous administration of antiserum on the organisms in the lymphatics has been acquired which indicates that even after many hours and repeated doses the antiserum remains without decisive action in reducing the severity of the lymphatic infection. This failure may depend on a number of factors which can be surmised from the data recorded in this and the previous paper, as well as from the bacteriological, immunological, and physiological facts concerning the mode of resistance of the body against infection with this organism and

the passage of proteins across the vascular lymphatic barriers. Since it is well known that antibody alone is not sufficient to bring about the destruction of the pneumococcus, but that subsequent to sensitization with the immune substance ingestion by the phagocytic cell with attendant intracellular digestion must follow, it is obvious that unless such cells are present in adequate numbers in the lymphatic system, the antibody in concentrations however great will not of itself lead to the elimination of the cocci. In the course of our work, cytological studies on blood and lymph have shown that in the blood the leucocyte count tended to be low, with a large proportion of the cells represented by lymphocytes. In the thoracic duct lymph, the increasing content of pneumococci throughout various experiments seems to have caused no marked changes from the cell counts and relationships which have been noted in normal rabbits. In uninfected animals variations from 11,300 to 44,960 per c. mm. have been reported (6),[4] and differential counts have indicated that about 88 per cent are small lymphocytes. In one of our experiments on an animal with heavily infected thoracic duct lymph, four differential counts showed 92, 95, 88, and 95 per cent small lymphocytes. The remaining cells were large lymphocytes. No polymorphonuclear leucocytes were found and large monocytes only after prolonged search. Thus it would appear that the only cells available for phagocytosis are tissue cell phagocytes of the lymph nodes and it is probable that these are too few to cope with the heavy infection which exists under the conditions of our experiments. The absence, then, of an effective cellular defence in the lymph may well represent one factor in the failure of antiserum to function effectively. A second is suggested by the rapid fall in titre of antibody following injection of antiserum into the blood stream of the infected rabbit, which was observed in many cases. This points to the neutralization of a large proportion of antibody by the cocci and their soluble antigenic products. The same relative reduction would take place in the quantity of any antibody reaching the infected lymph. Evidently it is necessary to introduce very large amounts of antibody to overcome this effect even in the blood stream. It would appear to be even more difficult to attain sufficient concentration of im-

[4] Drinker and Field (6), page 122.

mune substances in the lymph because of the more or less fixed ratio which exists between the concentrations of blood protein and lymph protein, whether this be homologous or foreign. This ratio is about 2 to 1 for the homologous protein of blood and lymph in rabbits. Various workers (6)[5] have shown that the antibody content of lymph from various sources compared with that of the blood follows closely the ratios for the normal protein content of the two fluids. Presumably by increasing the vascular permeability following the administration of antiserum, antibody in larger quantity might be delivered to the lymphatic system. Available measures for increasing vascular permeability nevertheless are generally so radical that their use subsequent to antiserum treatment seems inadvisable.

SUMMARY

Experiments are described which show that in rabbits infected intravenously with virulent Type III pneumococci, these organisms are found not only in the thoracic duct lymph, as previously reported, but also in lymph from the cervical and leg lymphatics. The nonmotile bacteria must have crossed both vascular and lymphatic endothelium in reaching the lymph. Intracellular transportation by phagocytes is apparently not the means by which this is effected.

The intravenous and intraperitoneal injection of large amounts of homologous type-specific antibody fails even after many hours to terminate or permanently reduce the pneumococcal infection of the lymph.

The failure of antiserum to sterilize the lymph is discussed.

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[5] Drinker and Field (6), page 127.