THE EMIGRATION OF PNEUMOCOCCI TYPE III FROM THE BLOOD INTO THE THORACIC DUCT LYMPH OF RABBITS, AND THE SURVIVAL OF THESE ORGANISMS IN THE LYMPH FOLLOWING INTRAVENOUS INJECTION OF SPECIFIC ANTISERUM

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Various workers have shown that large colloidal particles such as hemoglobin, and other soluble proteins, as well as certain dyes, pass readily from the blood stream to the lymphatics. It has also been repeatedly demonstrated that various insoluble materials including bacteria are absorbed into lymphatics from local sites, such as the cutaneous and subcutaneous tissues, peritoneum, pleura, etc. To the best of our knowledge little or no data exist concerning the penetration into the lymph of bacteria introduced into the blood stream. The first part of this paper reports the results of experiments designed to obtain information on this point. Having determined the fact that pneumococci do emigrate from the blood stream to the lymphatics, we were led to investigate the effect of specific antisera, intravenously injected, on the bacteria present in the lymph. This seemed pertinent inasmuch as there is a lack of information dealing with the effect of antibody on organisms after their arrival in the lymphatics.

A number of studies are, however, available upon the relative amounts of antibodies in blood and in lymph in non-infected animals. These have been reviewed in sufficient detail by one of us (1). They indicate that the antibody content of lymph is invariably lower than that of blood, and that the antibody concentration is relatively proportional to the protein content of the fluids examined. The fact that McMaster and Hudack (2) have recently shown that for some days

after intradermal injection of an antigen, agglutinins induced by it can be found in higher concentration in extracts of regional lymph nodes than in the blood, does not negative the generalization that lymph is poorer in antibodies than blood since such extracts are derived from gland cells, lymph, and traces of blood.   In a few of the infected animals studied by us we have made examinations for the presence of antibodies and their serum vehicle.

Materials and Methods

The microorganism employed has been a Type III pneumococcus, Strain SV, which was used by Tillett (3) in his experiments on the infectivity of this organism for rabbits.   The virulence for rabbits is such that an intradermal injection of 0.001 cc. of a 16 hour blood broth culture will kill the animal.   Tillett showed that the organism produces a bacteremia leading to death in 1 to 5 days depending upon the dosage.

16 and 20 hour rabbit blood infusion broth cultures were used in the experiments which follow, rabbits being inoculated with large amounts intravenously for immediate observation of the transfer of organisms from blood to lymph, and with small amounts for the study of conditions on the following day.   Measured samples of blood from the right jugular vein and of thoracic duct lymph were removed at intervals.   Serial dilutions of these were plated by pouring horse blood agar plates.

In a number of experiments the effects of antisera in sterilizing the blood and in failing to sterilize the lymph were observed.   Antisera of two sorts were used, Pneumococcus Type III rabbit antiserum prepared in the laboratory of Bacteriology, and Pneumococcus Type III horse antiserum prepared by the Massachusetts Antitoxin and Vaccine Laboratory.

The experimental rabbits were anesthetized by intravenous injections of nembutal in physiological saline, a fraction of the initial dose being repeated in order to maintain complete anesthesia throughout the experiment.   In order to expose the thoracic duct, the left external jugular vein is followed to its junction with the subclavian vein.   This is just above the clavicle which need not be removed, though a somewhat better exposure is secured if it is taken out.   After tying all entering branches, the subclavian and jugular veins are ligated 2 cm. from the point of junction.   Last of all, the subclavian vein is tied just central to the entrance of the thoracic duct which enters on the inner side of the junction of the veins.   The duct is extremely delicate, and close dissection of it is apt to be disastrous.   The venous pocket which has been formed is now opened and a cannula tied in it.   When this is first done the lymph is usually bloody, the source of the blood being small veins which are often impossible to locate.   In this latter case, a cannula is passed into the venous pocket and through it into the opening of the thoracic duct so as to be out of the region of entering veins.   It is absolutely essential that the thoracic duct lymph have no direct contamination by blood.

Such lymph in the rabbit, as in other animals, almost invariably contains red cells which are normal constituents of liver and intestinal lymph arising from the highly permeable capillaries in these regions and not from direct communication between the thoracic duct and small veins.

Emigration of Pneumococci from Blood Stream to the Lymphatics

The results obtained in rabbits following the intravenous injection of pneumococci and subsequent cultivation of blood and lymph may be classified under three headings on the basis of the number of bacteria introduced and the time elapsing after injection.

(a) *Large Dose of Pneumococci; Immediate Examination; Organisms Enter the Lymph within a Brief Period.*—The following protocol and Fig. 1 illustrate the sequence of events following the intravenous inoculation of the organisms derived from 20 cc. of blood broth culture. They persist in the blood stream in large numbers and enter the lymph within an hour.

Mar. 6, 1935.—Rabbit, weight 2.6 kilos. Nembutal anesthesia. *12:50 p.m.,* Preparation finished. The thoracic duct lymph appears free from blood. Rectal temperature 101.8°F. *1:15,* White cells in lymph 27,500 per c.mm.; red cells in lymph 700 per c.mm.

1:20, 20 cc. of 16 hour blood broth culture of SV concentrated to 0.8 cc. and injected in ear vein.

1:22, Blood culture 1, 57,000,000 pneumococci per cc. *1:26,* Lymph culture 1, 0 pneumococci per cc. *1:28,* White cells in lymph 26,900 per c.mm.; red cells in lymph 600 per c.mm. *1:45,* Rectal temperature 102.4°F.

1:52, Blood culture 2, 13,000,000 pneumococci per cc. *1:55,* Lymph culture 2, contaminated, 10 (?) pneumococci per cc. *1:57,* White cells in lymph 32,200 per c.mm.; red cells in lymph 400 per c.mm. *2:00,* Rectal temperature 102.3°F.

2:23, Blood culture 3, 7,000,000 pneumococci per cc. *2:24,* Lymph culture 3, 40 pneumococci per cc. White cells in lymph 39,300 per c.mm.; red cells in lymph 300 per c.mm. *2:29,* Rectal temperature 102.4°F.

3:40, Blood culture 4, 32,000,000 pneumococci per cc. Lymph culture 4, 2,700 pneumococci per cc. White cells in lymph 38,300 per c.mm.; red cells in lymph 300 per c.mm. *3:46,* Rectal temperature 102.6°F.

4:23, Blood culture 5, 90,000,000 pneumococci per cc. *4:27,* Lymph culture 5, 7,200 pneumococci per cc. White cells in lymph 36,000 per c.mm.; red cells in lymph 200 per c.mm. *4:34,* Rectal temperature 102.8°F.

4:58, Blood culture 6, 280,000,000 pneumococci per cc. *5:02,* Lymph culture 6, 24,000 pneumococci per cc. White cells in lymph 29,200 per c.mm.; red cells in lymph too few for count. *5:21,* Rectal temperature 103.1°F. *5:33,* Rectal temperature 103.0°F.

5:35, Blood culture 7, 230,000,000 pneumococci per cc. Lymph culture 7, 29,000 pneumococci per cc. White cells in lymph 31,200 per c.mm.; red cells in lymph 200 per c.mm. Experiment terminated.

The number of pneumococci per cubic centimeter of blood and lymph is shown in Fig. 1. The organisms in the lymph increased steadily but never became so numerous as in the blood.

(*b*) *Smaller Dose of Pneumococci; Immediate Examination; Organisms Markedly Reduced in Blood, Do Not Enter Lymph within 4½ Hours.* —When 1/20 of the dose of pneumococci used in the previous experiment was introduced, the bacterial count in the blood fell within 2 hours to 1 per cent of its original value. No organisms appeared in the lymph during the time of observation.

Feb. 1, 1935. Rabbit, weight 2.3 kilos. Nembutal anesthesia. *11:25 a.m.*, Thoracic duct isolated. Rectal temperature 99°F.

12:17 p.m., Injected 1.0 cc. 16 hour blood broth culture SV intravenously.

12:19, Blood culture 1, 153,000 pneumococci per cc.   *12:23*, Lymph culture 1, sterile (0.25 cc.).

12:47, Blood culture 2, 28,500 pneumococci per cc.   *12:49*, Lymph culture 2, sterile (0.25 cc.).   *12:52*, Rectal temperature 99°F.

1:07, Blood culture 3, 15,800 pneumococci per cc.   *1:10*, Lymph culture 3, sterile (0.25 cc.).

1:30, Blood culture 4, 5,000 pneumococci per cc.   *1:34*, Lymph culture 4, sterile (0.25 cc.). Rectal temperature 99°F.

2:02, Blood culture 5, 1,670 pneumococci per cc.   *2:07*, Lymph culture 5, sterile (0.25 cc.).

3:10, Blood culture 6, 1,410 pneumococci per cc.   *3:14*, Lymph culture 6, sterile (0.25 cc.). Rectal temperature 98.6°F.

3:45, Blood culture 7, 2,250 pneumococci per cc.   *3:48*, Lymph culture 7, sterile (0.25 cc.).

4:20, Blood culture 8, 1,570 pneumococci per cc.   *4:24*, Lymph culture 8, sterile (0.25 cc.). Rectal temperature 99.8°F.

4:51, Blood culture 9, 7,300 pneumococci per cc.   *4:55*, Lymph culture 9, sterile (0.25 cc.).   *5:15*, Rectal temperature 100°F. Experiment terminated.

(*c*) *Small Dose of Pneumococci; Examination after 20 Hours; Organisms Increasing in the Blood Stream and Lymph.*—Intravenous injection of 0.5 cc. of a 16 hour blood broth culture is followed after 20 hours by a bacteremia and the invariable presence of organisms in the lymph. This condition is apparent from the experiment recorded below, summarized in the form of a graph in Fig. 2, and from the

experiments which follow in which the effect of antisera on the pneumococci in the lymph was studied.

Mar. 26, 1935. Rabbit, weight 2.2 kilos. *5:00 p.m.*, Injected with 0.5 cc. of 16 hour blood broth culture of Strain SV. Rectal temperature 102.6°F.

Mar. 27. *9:00 a.m.*, Rectal temperature 104°F. Nembutal anesthesia. *9:50*, Rectal temperature 100.8°F. *12:00 m.*, Thoracic duct cannulated. Lymph clear.

1:00 p.m., Blood culture 1, 23,000,000 pneumococci per cc. *1:05*, Lymph culture 1, 3,300 pneumococci per cc. *1:06*, Rectal temperature 99.2°F.

2:20, Lymph culture 2, 6,800 pneumococci per cc. *2:24*, Blood culture 2, 43,000,000 pneumococci per cc.

3:20, Blood culture 3, 195,000,000 pneumococci per cc. *3:24*, Lymph culture 3, 25,000 pneumococci per cc. *3:26*, Rectal temperature 100.8°F. *3:27*, Experiment terminated.

Throughout the experiment counts of the numbers of red blood corpuscles in the lymph were made on every sample; none were found.

The three preceding experiments indicate that Pneumococcus Type III virulent for rabbits passes from the blood stream into the lymphatic system where it increases. The rapidity with which this migration is accomplished appears to be related to the numbers of pneumococci injected, since only in the case of the intravenous administration of a large quantity of culture are the organisms found in the lymph within 4 hours. With smaller doses lymphatic infection is not observed within this interval. This fact, however, does not necessarily imply that organisms do not reach the lymph during the period immediately subsequent to their introduction into the blood; for if the number migrating into the lymph bears a more or less constant relation to the number in the circulating blood, then with smaller doses those entering the lymph would be too few to be detected when samples of 0.25 cc. are plated. However this may be, it is certain that at some time after the inoculation of small doses, pneumococci do enter the lymph, for they are found there regularly at 22 hours, where they multiply and may even become more numerous than in the blood.

Effect of Specific Antiserum Injected Intravenously on Pneumococci Present in the Lymph

Since it was established in the foregoing experiments that 20 hours after injection organisms were regularly demonstrable in the thoracic
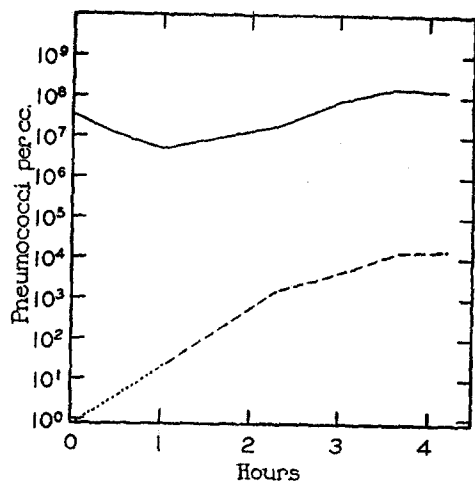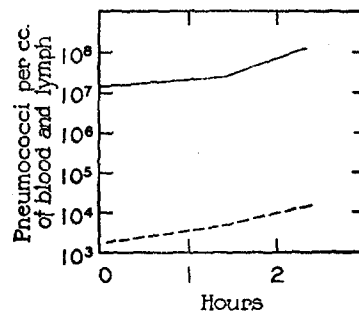
FIG. 1

FIG. 2

FIG. 1. ——, number of organisms in blood; - - -, number of organisms in lymph; ...., probable appearance of organisms in lymph.

FIG. 2. ——, number of organisms in blood; - - -, number of organisms in lymph.
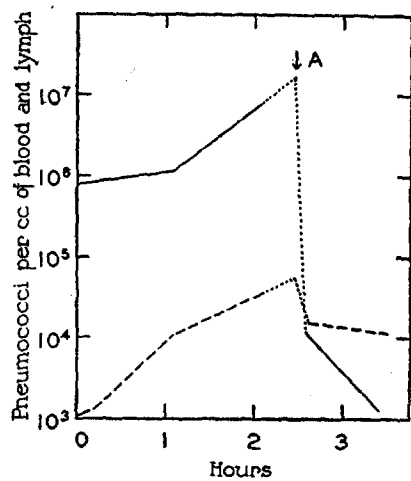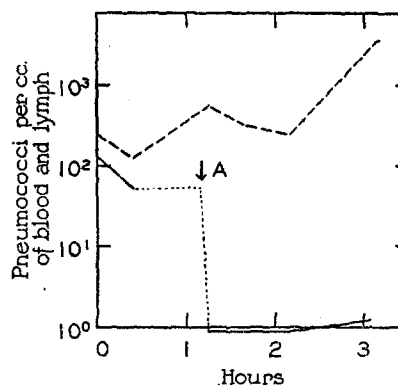

FIG. 3

FIG. 4

FIG. 3. ——, number of organisms in blood; - - -, number of organisms in lymph; ...., in blood and lymph curves, probable numbers of pneumococci before and at the time of antiserum injection. At A, 8.0 cc. of Antipneumococcus Type III rabbit serum were given intravenously.

FIG. 4. ——, number of organisms of blood; - - -, number of organisms in lymph. At A, 10.0 cc. of Antipneumococcus Type III horse serum were given intravenously.

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duct lymph, it became of interest in view of the well known sterilizing effect of antiserum on pneumococci in the blood to observe the action of this agent on the organisms present in the lymph. With this in view we have studied the result of intravenous treatment of animals thus prepared, employing Antipneumococcus Type III rabbit and horse sera.

(a) *Effect of Antipneumococcus Type III Rabbit Serum on the Organisms in the Blood and Lymph 23 Hours after Infection.*—

Apr. 29, 1935. Rabbit, 2.3 kilos. *3:30 p.m.,* 0.05 cc. of 1:2 dilution of a 20 hour blood broth culture intravenously. Rectal temperature 103.5°F.

Apr. 30. *9:00 a.m.,* Rectal temperature 106.0°F. Nembutal anesthesia. *9:30,* Rectal temperature 104.8°F. *10:40,* Rectal temperature 103°F. *2:20 p.m.,* Preparation finished. White cells in lymph 23,000 per c.mm.; red cells in lymph too few to count.

2:25, Blood culture 1, 900,000 pneumococci per cc. Lymph culture 1, 1,300 pneumococci per cc. *2:30,* Rectal temperature 103.2°F.

2:37, Lymph culture 2, 2,000 pneumococci per cc. *3:30,* Blood culture 2, 1,500,000 pneumococci per cc. Lymph culture 3, 12,000 pneumococci per cc. *3:32,* White cells in lymph 27,000 per c.mm.; red cells in lymph too few to count. Stained film of blood shows encapsulated organisms. *3:34,* Rectal temperature 102.6°F.

4:29, Blood culture 3, 9,000,000 pneumococci per cc. *4:31,* Lymph culture 4, 60,000 pneumococci per cc. White cells in lymph 12,400 per c.mm.; red cells in lymph 200 per c.mm.

4:53, 8.0 cc. Antipneumococcus Type III rabbit serum injected into an ear vein.

5:00, Blood culture 4, 15,000 pneumococci per cc. *5:02,* Lymph culture 5, 27,000 pneumococci per cc. White cells in lymph 18,700 per c.mm.; red cells in lymph 300 per c.mm. *5:20,* Rectal temperature 102°F.

5:50, Blood culture 5, 2,000 pneumococci per cc. *5:51,* Rectal temperature 102.8°F. *5:54,* Lymph culture 6, 15,000 pneumococci per cc. White cells in lymph 14,900 per c.mm. *5:59,* Experiment terminated.

The number of pneumococci in blood and lymph is shown in Fig. 3. Here the sterilizing effect of the antiserum upon the blood and the comparative absence of effect upon the lymph are strikingly shown. The organisms in the blood are reduced approximately 4,500 times after administration of antiserum. In contrast only a fourfold reduction occurs in the number of pneumococci in the lymph. It is also interesting to observe that at this stage of the infection the numbers of bacteria both in the blood and in the lymph were rapidly increasing.

(b) *Effect of Antipneumococcus Type III Horse Serum on the Organisms in the Blood and Lymph 22 Hours after Infection.*—

May 15, 1935. Rabbit, weight 2 kilos. *4:30 p.m.,* Inoculation intravenously with 0.1 cc. of 1:2 dilution of 20 hour blood broth culture of SV. Temperature at time of inoculation 103.4°F.

May 16. *9:00 a.m.,* Rectal temperature 102.4°F. *2:20 p.m.,* Preparation finished. Blood culture 1, 185 pneumococci per cc. Lymph cultures 1 and 2, average 450 pneumococci per cc. White cells in lymph 35,000 per c.mm.; red cells in lymph 500 per c.mm. *2:30,* Rectal temperature 100.6°F.

2:45, Blood culture 2, 75 pneumococci per cc. Lymph culture 3, 180 pneumococci per cc. White cells in lymph 40,000 per c.mm.; red cells in lymph 500 per c.mm.

3:30, 10 cc. Antipneumococcus Type III horse serum injected intravenously.

3:35, Blood culture 3, 0 pneumococci per cc. Lymph culture 4, 770 pneumococci per cc. *3:37,* Specimen of lymph taken for antibody titration.

3:40, Blood culture 4, 0 pneumococci per cc. White cells in lymph 67,000 per c.mm.; red cells in lymph 500 per c.mm.

3:58, Blood culture 5, 1 (?) pneumococcus per cc. Lymph culture 5, 560 pneumococci per cc. White cells in lymph 43,000 per c.mm. Red cells in lymph 400 per c.mm.

4:30, Blood culture 6, 0 pneumococci per cc. Lymph culture 6, 450 pneumococci per cc. White cells in lymph 36,600 per c.mm.; red cells in lymph 300 per c.mm. *5:05,* Lymph sample 7 (not cultured).

5:30, Blood culture 7, 2 pneumococci per cc. Lymph culture 8, 6,000 pneumococci per cc. White cells in lymph 38,000 per c.mm.; red cells in lymph 1,500 per c.mm. Experiment terminated.

The course of the experiment is shown in Fig. 4. It is of interest that at the beginning of observation, approximately 22 hours after blood inoculation, there were more pneumococci in the lymph than in the blood. The injection of antiserum sterilized the blood for a period of almost 2 hours but had no effect on the number of organisms in the lymph, which remained practically stationary and then increased.

Relative Quantities of Antibody in Blood and Lymph in Infected Rabbits

In the experiments involving the use of antiserum, titrations of the agglutinin content of lymph and in some cases that of the blood serum were carried out. Within 10 minutes after injection of 8 cc. of Antipneumococcus Type III rabbit serum (see page 855), the agglutinin

titre of which was approximately 1:200, that of the rabbit's blood serum rose to 1:32 and did not increase further. No agglutination occurred in any of the lymph samples in 1:2 dilution. It is of interest that the presence of soluble antigenic substance in the blood serum

TABLE I

*Serological Reactions of Blood Serum and Lymph of a Rabbit Injected Intravenously with Antipneumococcus Type III Horse Serum**

Samplings of blood and lymph	Time of sampling	Agglutinin titre vs. Pneumococcus III†	Precipitinogen titre (horse serum) vs. anti-horse rabbit serum‡	Precipitinogen titre (specific soluble pneumococcus antigen)	Remarks
	hrs. min.				
Blood 1	0 00	0	0	1:8	
" 2	0 25	Not done	Not done	Not done	
" 3	0 50	1:128	1:128	" "	15 cc. Antipneumococcus
" 4	1 12	1:128	1:128	" "	III horse serum given
" 5	2 27	1:128	Not done	" "	intravenously 2 min.
" 6	2 55	1:128	" "	" "	before taking blood sample 3
" 7	3 25	1:128	" "	" "	
" 8	3 56	1:128	" "	" "	
" 9	4 22	1:128	1:32	1:8	
Lymph 1	0 10	0	0	1:8	
" 2	0 30	0	0	Not done	
" 3	0 55	0	0	" "	Lymph turbid; ring test
" 4	1 15	1:8	1:32	" "	difficult to read, in case
" 5	2 30	Not done	1:32	" "	of specific soluble pneu-
" 6	3 00	1:32	1:32	" "	mococcus antigen
" 7	3 30	Not done	1:32	" "	
" 8	4 00	1:32	1:32	" "	
" 9	4 30	Not done	1:32	1:8	

* 15 cc. of the antiserum were injected.

† Agglutinin titre of Antipneumococcus Type III horse serum injected was 1:512.

‡ Precipitin titre of anti-horse rabbit serum against falling dilutions of horse serum was 1:1,000.

could be demonstrated to a titre of 1:8 using Antipneumococcus Type III horse serum as antibody.

In the experiment recorded above in which 10 cc. of Antipneumococcus Type III horse serum exhibiting an agglutinin titre of 1:128 was

injected, no agglutinins were detected in any of the lymph samples, but the presence of the horse serum in low concentration in the lymph was revealed by titration with anti-horse rabbit serum. The agglutinin content of the blood serum was not determined. 15 cc. of an Anti-pneumococcus Type III horse serum having an agglutinin titre of 1:512 were injected in a second experiment in all other respects similar to the foregoing. Agglutinins were found in the lymph to a titre of 1:32 and in the blood to 1:128. Nevertheless, while the organisms occurring in large numbers in the blood previous to administration of serum were almost completely eliminated, the numbers in the lymph, which were nearly equivalent to those in the blood, were at first slightly reduced and then began to increase. Again free soluble pneumococcus antigen was shown to coexist in the blood serum along with a considerable amount of agglutinin. A summary of the serological observations in the case of this rabbit is presented in Table I.

From these results taken as a whole it is clear that when blood and lymph infection with Pneumococcus Type III occurs, a very large quantity of an antiserum containing a high titre of agglutinins is required before these antibodies become demonstrable in the lymph. This volume of serum has been estimated to represent about one-seventh of the blood volume of the rabbit. If the quantity of either heterologous or homologous antiserum is less and the agglutinin titre lower, antibody fails to appear in the lymph. Since even when antibody does enter the lymph, the organisms are not eliminated or significantly reduced in number, it is suggested that a second factor, possibly one involving phagocytic cells is absent or inoperative.

DISCUSSION

The import of these experiments is apparent, and from the point of view of therapy, disturbing. It is first of all clear that somehow or other microorganisms in the blood pass through the capillary walls into the tissue fluid and through the walls of the lymph capillaries to enter the lymph stream. This passage requires neither motility on the part of the organisms nor phagocytosis, and it is accomplished rapidly. Our experiments have dealt with thoracic duct lymph alone and the major part of this lymph, indeed in the quiescent anesthetized animal practically all of it, has origin in the highly permeable capil-

laries of the abdominal region, rather than in the capillaries of the skin, subcutaneous tissues, and muscles.   No data are available for the lymph from these regions.   If bacteria were present in such lymph, their growth would undoubtedly be less inhibited by antibody action than in lymph from the abdominal region, owing to the fact that the less permeable blood capillaries of the periphery permit but slight passage of antibodies (1).   In the presence of a bacteremia, apparently one cannot expect to sterilize the lymph by a single intravenous injection of antiserum.   If the injection sterilizes the blood, organisms will begin at once to enter from the lymph and removal from the blood will depend upon a sustained concentration of antibodies due to a very large initial injection or to subsequent small injections.

The problem of effecting the removal of the pneumococci in the lymph itself might possibly be attacked by some measure leading to an increase in the permeability of the blood capillaries, thus raising the antibody content of the lymph.   The organization, however, of the lymphatic system both in the multiplicity of vessels and in the complexity of the nodes makes it difficult to think that lymph antibody content and lymph flow can be increased sufficiently to bring about sterilization, but it is of course worth while to make the attempt experimentally.   Moreover, if antibody were increased in some manner, it is exceedingly doubtful whether its presence alone would be sufficient for the efficient elimination of the organisms in the lymph, for in one instance mentioned above a considerable quantity failed to produce any but a transitory diminution in the number of pneumococci.   We believe, therefore, that the adjuvant action of one or more additional factors, most probably cellular in nature, is requisite.

It must be emphasized that the foregoing remarks are based upon observations made during only the 4 hours subsequent to the administration of antiserum.   It is entirely possible that after this time mobilization of auxiliary defensive mechanisms may take place, resulting in the destruction of the organisms in the lymphatic area.

SUMMARY

1. Rabbits injected intravenously with a large dose of a virulent Type III Pneumococcus develop a bacteremia, and within an hour organisms may be cultivated from the thoracic duct lymph.   The

rapidity with which entrance into the lymph occurs appears to be correlated with the size of the dose injected.

2. The organisms may become more numerous in the lymph than in the blood.

3. If homologous or heterologous antisera are injected, the blood may be sterilized, but though the organisms may be lessened in the lymph, sterilization at least within 4 hours is not secured, and in the intact animal living organisms must continue to enter the blood with the thoracic duct lymph.

4. In infected rabbits after intravenous injection of considerable quantities of antisera containing moderate amounts of agglutinin, no antibody appears in the thoracic duct lymph although the presence of horse serum may be detected.

The injection of a very large quantity of antiserum containing a high titre of agglutinin is followed by the penetration of antibody into the lymph. This, however, has failed to sterilize the lymph or to permanently affect the rate of multiplication of the pneumococci.

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