THE PASSAGE OF RABBIT VIRULENT TYPE III PNEUMOCOCCI FROM THE RESPIRATORY TRACT OF RABBITS INTO THE LYMPHATICS AND BLOOD

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Previous experiments (1, 2) have shown that if rabbits were given large intravenous injections of Type III pneumococci, strain SV (Tillett, 3), or if given blood infections by small intravenous injections, the organisms could be cultivated from thoracic duct, cervical, and leg lymphatics and that intravenous treatment with antisera, while often effective in immediate sterilization of the blood, did not produce the same favorable result in the lymph. The bearing of these experiments on the serological treatment of such infections was quite obvious. It was also shown (4) that a variety of visible particles readily made their way from blood to lymph. It thus became a matter of interest to see whether pneumococci placed upon the uninjured mucosa of the respiratory tract succeeded in penetrating the tissues and reaching lymphatics. Four groups of experiments were performed with rabbits, the rabbit virulent Type III Pneumococcus, strain SV, being used in all. The experiments were:

1. Cannulation of thoracic duct and trachea. Culture instilled in trachea.

2. Cannulation of a cervical lymphatic and trachea. Esophagus tied. Culture instilled intranasally.

3. Intravenous injection of antipneumococcus horse serum. Cannulation of thoracic duct and trachea. Culture instilled in trachea.

4. Intravenous injection of antipneumococcus horse serum. Can-

nulation of a cervical lymphatic and trachea. Esophagus tied. Culture instilled intranasally.

Material and Methods

With but few exceptions, albino rabbits were used throughout the experiments. The animals, averaging 2 kilos, were anesthetized by the intravenous administration of a 5 per cent sodium pentobarbital (nembutal) solution, beginning with an initial dose of 35 mg. per kilo of body weight and adding small amounts subsequently in order to keep the anesthesia uniform.

The cervical lymphatics and thoracic ducts were exposed and cannulated as previously described (1). In order to be sure that thoracic duct lymph was not contaminated by blood entering the duct through small veins, a circumstance not infrequent in the rabbit, red cell counts were made in all cases, and in the experiments given the red cell content of the thoracic duct lymph never was above normal limits so that it is certain no gross contamination with blood occurred. In the group of animals receiving intranasal instillations the esophagus was tied and the trachea cannulated in order to prevent the animal from swallowing or inhaling the instilled culture. The right jugular vein and carotid artery were exposed for the taking of blood samples.

Two methods were employed for the culture of blood samples. One consisted in streaking 0.5 cc. of arterial and venous blood over plates, the other in adding 0.5 cc. of freshly drawn blood to melted nutrient agar as plates were poured. During the early experiments blood was withdrawn every 10 minutes. In later experiments it was withdrawn at half hour intervals.

Lymph for culture was collected as it accumulated. A portion of a sample was at once streaked on blood agar plates, a second portion smeared on a slide, a third portion centrifuged and the sediment used in making smears, and a final portion was used for broth cultures. At the end of the experiment, tissues from the exposed areas were fixed for microscopic study, including the accompanying regional lymph nodes.

The organisms employed were derived from an SV strain of Type III pneumococci which was known to cause the death of rabbits following the intradermal injection of 0.001 cc. of a 16 hour blood broth culture, and from time to time the organisms were passed through rabbits to assure their virulence. The respiratory tract of each animal received the centrifuged concentrate from 20 cc. of a blood broth culture which had been incubated for 16 hours. The organisms were resuspended in 2 cc. of the supernatant broth. Difficulty in retaining a full dose given intranasally was occasionally encountered. In order to prevent such temporary overloading, the organisms were administered in two doses, one at the beginning of the experiment and the remainder 2 hours later.

Films made directly from lymph and those from concentrated lymph sediment were stained with Wright's blood stain and by Gram's method.

In the third and fourth group of observations antiserum was administered

intravenously 2½ to 3 hours before the organisms were instilled in the nose and in the trachea. The antiserum used had an agglutinating titre of 1:400. The number of organisms recovered from lymph samples is recorded roughly in from one to four plus signs for each half hour period.

RESULTS

Tables I and II show that the organisms were first usually demonstrated in the thoracic duct and cervical lymph in the second half hour specimen. The number of cases in which the lymph became positive increased up to the fourth half hour period when the lymph in the majority of the animals had become positive. In two animals (Nos. 13 and 18) organisms did not appear during the experiment. Variation in the number of organisms recovered at intervals after they were first demonstrated was at times definitely associated with increased and decreased flow of lymph during that period, usually due to clots forming at the tip of the cannula.

It is apparent from Table I that pneumococci in fluid suspension placed in the trachea and frequently reaching the alveoli, as observed at autopsy, made their way into the thoracic duct lymph quite rapidly. In order to do this, lung lymphatics must be traversed and lymph nodes passed at the root of the lung. Similarly (Table II) organisms instilled intranasally soon began to appear in the cervical lymph. In this latter case there must have been penetration of the nasal mucous membrane and passage of at least one cervical lymph node before the point of cannulation was reached low in the neck. The blood rarely became positive even after intratracheal instillation, and in the intranasal group the blood was practically free from organisms during all experiments. Table III, covering 10 rabbits which received antiserum and then, after several hours, pneumococci intratracheally, shows a few positive results for organisms in thoracic duct lymph, but evidently the antiserum has had some effect in controlling lymph infection. In Table I, presenting comparable data for 19 rabbits similarly infected but unprotected by antiserum, there are 16 in which the thoracic duct lymph became positive, whereas in Table III, composed of data upon 10 animals given antiserum, only 4 showed organisms in the thoracic duct lymph. In a similar manner it is apparent that antiserum has been of assistance in connection

TABLE I

No.	Weight	Temperature	Red blood cells per c.mm. lymph	0.5 hr.		1.0 hr.		1.5 hr.		2.0 hr.		2.5 hr.		3.0 hr.		3.5 hr.		4.0 hr.	
				T	B	T	B	T	B	T	B	T	B	T	B	T	B	T	B
	kg.	°C.																	
1	2.2	38.0	600	+	++++	+	++++	+	++++	++	+++	++	++++	++	++++	++	++++	++	++++
2	1.8	38.5	None	0	0	0	0	0	0	0	0	+	0	++	0	+	0	Died	
3	1.9	39.0	None	0	0	+	0	+++	0	++	0	++	0	+++	0	+++	0	++++	0
4	2.0	39.0	100	0	0	0	0	++	0	+	0	++	+	++	+	++	0	+	+
5	1.7	40.0	None	0	0	0	0	0	0	0	0	++	0	++	0	++	0	++	0
6	2.0	39.5	None	0	0	0	0	0	0	0	0	+	0	+	0	0	0	++	+
7	2.0	40.0	200	0	0	0	0	+	0	++	0	+	+	0	0	++	0	++	0
8	2.1	39.0	None	0	0	0	0	+	0	+	0	Died							
9	2.0	39.5	300	0	0	0	0	0	0	+	0	++	0	+	0				
10	1.8	40.0	None	0	0	+	0	0	0	++	0	++	+	+	0	++	+	++	0
11	1.6	39.5	200	0	0	0	0	0	0	0	0	+	0	0	0	+	0	++	0
12	2.0	40.0	200	0	0	0	0	+	0	0	0	+	0	++	0	++	0	++	0
13	2.8	39.0	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14	1.4	40.0	200	0	0	0	0	Died											
15	2.0	39.2	200	0	0	+	0	+	0	+	+	++	+	0	0	0	0	+	+
16	4.6	40.1	None	0	0	0	0	0	0	+	0	+	0	+	0	+	0		
17	2.0	40.0	100	0	0	0	0	0	0	0	0	+	0	+	0	+	0	+	0
18	2.3	40.0	200	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
19	2.3	41.0	None	0	0	0	0	0	0	+	0	+	+	Died					

This table shows the results of instilling 2 cc. of rabbit virulent Type III Pneumococcus culture into the trachea of normal rabbits. +, 1 to 5 organisms per half hour sample; ++, 5 to 15 organisms; +++, 15 to 30 organisms; ++++, 30 and above organisms. T, thoracic duct lymph; B, blood.

TABLE II

No.	Weight	Temperature	0.5 hr.		1.0 hr.		1.5 hr.		2.0 hr.		2.5 hr.		3.0 hr.		3.5 hr.		4.0 hr.		
			C	B	C	B	C	B	C	B	C	B	C	B	C	B	C	B	
	kg.	*°C.*																	
20	1.9	39.0	0	0	0	0	++	0	+	0	0	0	+	0	++	0	++	0	
21	2.6	40.0	0	0	++	0	0	0	++	0	0	0	+	0	+	+	+	0	Flow scant
22	1.6	36.0	0	0	0	0	0	0	0	0	0	0	+	0			+	0	Flow scant
23	2.7	37.2	0	0	0	0	0	0	+	0	++	0	+	0	+	0	++	0	
24	2.0	38.0	0	0	0	0	0	0	+	0									
25	2.2	38.2	0	0	+	0	0	0	0	0	0	0	0	0		0		0	Lymphatic dilated above node
26	2.2	38.1	0	0	0	0	+	0	0	0	++	0	++	0	+	0	0	0	
27	2.1	38.0	0	0	0	0	0	0	+	0	Died								
28	2.0	37.7	0	0	0	0	0	0	0	0	+	0	Died						
29	2.0	38.5	0	0	0	0	0	0	0	0	+	0	+	0	+	+	0	0	Flow scant

This table shows the results of instilling 2 to 3 cc. of rabbit virulent Type III Pneumococcus culture into the nose of normal rabbits. +, 1 to 5 organisms per half hour sample; ++, 5 to 15 organisms. C, cervical lymph; B, blood.

TABLE III

No.	Weight	Temperature	Red blood cells per c. mm. lymph	Time between antiserum injection and instillation of organisms		0.5 hr.		1.0 hr.		1.5 hr.		2.0 hr.		2.5 hr.		3.0 hr.		3.5 hr.		4.0 hr.	
						T	B	T	B	T	B	T	B	T	B	T	B	T	B	T	B
	kg.	*°C.*		*hrs.*	*min.*																
30	2.2	38.0	200	3	20	0	0	+	0	+	+	+	0	+	0	0	0	0	0	0	0
31	2.4	37.5	200	3	00	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
32	1.7	38.4	None	2	20	++	++	++	+++	++	+++	++	++	++	+	+	++	++	+++	+	++
33	1.9	37.8	400	2	35	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
34	3.7	39.1	None	2	50	0	0	+	0	+	0	0	0	+	0	0	0	0	0	0	0
35	2.6	39.4	None	3	00	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
36	2.7	37.8	None	3	10	0	0	0	0	+	0	+	0	0	0	0	0	0	0	0	0
37	2.2	38.1	200	2	55	0	0	0	0	0	0	0	0	0	+	0	0	0	0	0	0
38	3.3	38.0	100	3	00	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
39	2.1	38.2	None	3	00	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

This table shows the result of instilling 2 cc. of rabbit virulent Type III Pneumococcus culture into the trachea of rabbits which had received previously 3 to 5 cc. of antipneumococcus horse serum intravenously. +, 1 to 5 organisms per half hour sample; ++, 5 to 15 organisms; +++, 15 to 30 organisms; ++++, 30 and above organisms. T, thoracic duct lymph; B, blood.

TABLE IV

No.	Weight	Temperature	0.5 hr.		1.0 hr.		1.5 hr.		2.0 hr.		2.5 hr.		3.0 hr.		3.5 hr.		4.0 hr.	
			C	B	C	B	C	B	C	B	C	B	C	B	C	B	C	B
	kg.	*°C.*																
40	2.1		0	0	0	0	0	0	0	0	Died							
41	2.7		0	0	0	0	0	0	Died									
42	2.1	38.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
43	2.3	40.0	0	0	0	0	+	0	0	0	0	0	0	0	0	0	0	0
44	2.3	39.0	0	0	0	0	0	0	++	0	+	0	+	0	No flow			
45	2.1	38.0	0	0	0	0	0	+	+	0	0	0	0	0	0	0	0	0
46	1.9	40.0	0	0	0	0	0	0	0	0	Died							
47	1.6	40.0	0	0	0	0	0	0	+	0	++	0	+	0	0	0	+	0
48	2.3	41.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
49	2.0	39.0	++	0	++	+++	+	0	++	+++	++	0	+++	++++	++	0	+++	++++
50	1.9	40.0	0	0	0	0	+	+	0	0	+	0	0	+	0	0	0	0
51	1.8	40.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
52	1.8	40.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
53	1.8	39.0	0	0	0	0	+	0	+	0	0	0	0	0	0	0	0	0

This table shows the results of instilling 2 cc. of rabbit virulent Type III Pneumococcus culture into the nose of rabbits which had received 5 cc. of antipneumococcus horse serum previously. +, 1 to 5 organisms per half hour sample; ++, 5 to 15 organisms; +++, 15 to 30 organisms; ++++, 30 and above organisms. C, cervical lymph; B, blood.

with the appearance of pneumococci in the cervical lymph after intranasal instillation. Table II covers 10 animals given intranasal instillations without antecedent antiserum. All showed organisms in the cervical lymph. In Table IV, out of 14 animals similarly treated but receiving antiserum, there were 7 in which organisms were cultivated from the cervical lymph.

DISCUSSION

The routes taken by viruses and microorganisms which are deposited initially upon some part of the respiratory tract have been the subject of much experiment and speculation. Thus in the case of poliomyelitis and epidemic cerebrospinal meningitis it is thought that organisms from the olfactory mucosa in some way pass through the cribriform plate and into the cranium. It has been suggested that in pneumonic infections organisms enter the lungs *via* submucous lymphatics in the trachea, which they somehow or other reach from the surface of the upper respiratory passages. For organisms actually in the depths of the lungs, absorption has been credited both to the lymphatics and to the blood. Burt, Tuttle, and Cannon (5) found, in experiments designed to test local immunity, that by introducing Type I pneumococci with a metal catheter into the trachea of rabbits, organisms appeared in the blood of normal controls of subcutaneously and intravenously immunized animals, but none were found during the 60 minute period in those animals immunized by the intratracheal route. Because of the early entrance into the blood it was believed that organisms passed directly into the blood stream. In subsequent work (6) these authors investigated the problem further. The experiments were made on dogs, using *Bacillus prodigiosus*, *Staphylococcus aureus*, and hemolytic streptococci. They found that the streptococci passed into the blood stream and only rarely into the lymphatics. The other two organisms were not detected in the blood or the lymph. Stillman (7, 8), using spray methods of exposing rabbits to virulent pneumococci, found that with few exceptions septicemias were delayed for more than 6 hours and in some instances for several days. In exceptional cases the organisms were recovered during the first hours after spraying.

In the present group of experiments, pneumococci highly virulent

for the rabbit placed upon the mucous membranes of the respiratory tract were found to have entered lymphatic vessels. Microscopic examination of lymph films made before and after centrifugalization revealed no organisms in the few macrophages and the rare polymorphonuclear leucocytes found. Most of the cells present belonged to the group of lymphocytes. The small number of organisms discovered in these films were lying free between the cells. Phagocytic cells thus did not appear to play a rôle in the passage of the organisms from the mucous surfaces to the lymph vessels.

The length of time required for the organisms to enter lymphatics cannot be determined from these experiments. Undoubtedly the actual time required for such entrance was shorter than indicated in the tables in which their appearance in the efferent lymphatics was recorded. The mechanical obstruction of the cannulas by clots which occurred at times had a retarding effect on the rate of lymph flow and the number of organisms which could pass. Any obstruction at the point of cannulation must also increase the opportunity for retention of pneumococci by the lymph nodes through which they must pass.

In preliminary experiments, small doses of organisms were instilled. In such tests the organisms failed to make their appearance during the 4 hour period of the experiment. Employing a larger dose, with but two exceptions organisms made their appearance during the 4 hour test period in the animals which had not received antiserum. In the same way previous work (1) showed that organisms given intravenously could not be demonstrated in the lymph stream during the $4\frac{1}{2}$ hour test when the infecting dose was small. After 20 hours, employing the small dose, they were invariably present in the lymph and tended to increase. When large doses were administered, they could be demonstrated in the lymph within 1 hour. The lag period had thus been greatly shortened. In the present experiments, the failure or delay in the appearance of the organisms in the efferent lymph is thought to be due to the barrier at the surface and also to the retention of organisms in the intervening lymph nodes. The latter factor is borne out by the histological studies to be reported later.

In the 24 experiments in which antiserum was given intravenously

prior to the instillation of organisms in the nose or in the trachea, it is reasonable to believe that the administration of this serum was responsible for the negative cultures of lymph in the animals whose lymph showed a relatively high antiserum titre. Those cases in which there were positive lymph cultures at the onset and which subsequently became negative, coupled with a fair titre of antiserum in the lymph, show more strikingly the beneficial effect of antiserum. It is more difficult to account for the three animals upon which the antiserum did not appear to have any effect. But one of these showed no antiserum in the lymph in any of the specimens examined. Previous work (1, 2) has indicated that it is difficult to obtain concentrations of antisubstances in the lymph of rabbits capable of nullifying infection permanently, but it did not yield instances in which no antisubstances were detectable in lymph.

The penetration of draining lymphatics by organisms placed upon the surface of the nasopharyngeal mucous membrane raises a number of questions. First of all, does one find in control specimens of cervical lymph taken prior to intranasal instillation any of the organisms commonly present upon the nasal mucosa? The answer with very rare exceptions in our experience is "No." On one occasion the cervical lymph of a monkey contained a streptococcus and a pneumococcus, neither of which is a probable contaminant. But this experience stands alone in many experiments upon different species. Furthermore, if one repeats the experiments recounted above, using a non-virulent organism, the lymph remains sterile. The same thing is true when the nasopharyngeal mucosa is flooded with graphite particles of bacterial dimensions. No graphite is found in the lymph. It cannot, of course, be said with certainty that organisms nonvirulent for the host fail to make the passage to the lymph, but if they do reach this destination they have become incapable of growth or they would have been detected in cultures. By analogy, with the failure of graphite particles to enter the lymph we think that organisms incapable of multiplying in the host do not survive far past the penetration of the mucous membrane if they even leave the surface at all.

Upon the mechanism of penetration of virulent organisms we have nothing to offer. With tracheal cannulation and the esophagus tied,

there is practically no motion of the nasopharyngeal membrane such as might accompany breathing or swallowing and, as has been pointed out, phagocytosis is not necessary. In the case of the instillations into the lungs, it is more than possible that non-virulent or even dead organisms may reach the lymph stream since graphite particles and particles of other sorts penetrate within an hour to the tracheobronchial lymph nodes. Once there and lacking power to multiply vigorously, it would again be difficult for organisms to go further and reach the cannula collecting thoracic duct lymph.

SUMMARY

1. Rabbit virulent Type III pneumococci when instilled into the nose or trachea were recovered from the lymphatics draining the area involved in the lymph collected during a subsequent 4 hour period. Their detection rarely failed, and not infrequently was possible at the end of the 1st hour.

2. The organisms practically invariably appeared first in the lymphatics and subsequently in a few cases were recovered from the blood during the 4 hour test period.

3. The intravenous administration of antiserum 2½ to 3 hours before the instillation of organisms decreased the number of animals whose lymph or blood became positive and the total length of time in which organisms were recovered in lymph from the efferent lymphatics during the test period.

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