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ROLE OF THE LYMPHATICS IN THE PATHOGENESIS OF ANTHRAX*

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Several investigators have confirmed the participation of the lymphatic system in anthrax infection (Trnka et al, 1956; Henderson et al, 1956; Ross, 1957; Widdicombe et al, 1956; Malek et al, 1959). In this work we quantitated the change of bacilli in the thoracic lymph, right lymph, and the blood following challenge with anthrax spores and during the development of a fatal septicemia in order (1) to gain more information on the role of the lymphatics in pathogenesis of anthrax, (2) to test the hypothesis on extravascular circulation of bacilli proposed by us (Lincoln et al, 1960), (3) to test for the removal of spores from the lung epithelium into the lymphatics and/or blood stream immediately after challenge or during the infectious process of the disease, and (4) to determine whether detection of systemic anthrax might be made earlier by observation of bacilli in the lymph than by observation of the blood. The routes of challenge were intradermal, intraperitoneal, intravenous, gastrointestinal, and respiratory.

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* In conducting the research reported herein the investigators adhered to "Principles of Laboratory Animal Care" as established by the National Society of Medical Research.

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METHODS

Cannulation and sampling procedures.—Cannulation of the right and/or thoracic lymphatic duct of the rhesus monkey was done by the method of Hodges and Rhian (1962). Bisection of the sternum exposed the thoracic and right lymphatic ducts and the jugular veins. Cannulation of the jugular vein allowed a cannula to be placed in the right auricle of the heart. Cannulae were led under the skin out of the body on top of the head. A simpler operation was used to cannulate the jugular vein when lymphatic cannulation was not desired.

Before recovery from anesthesia the monkey was placed upright in a holding chair to prevent the cannula from being disturbed. Lymph flowed continuously and was collected in a calibrated test tube. The blood cannula and an injectable solution of saline, glucose, or protein were connected to a 3-way stopcock. Blood was sampled by inserting a syringe on the third outlet of the stopcock. After a blood sample was taken, the cannula was immediately back-washed with the sterile solution. Between sampling periods the system was kept sterile by inserting a cotton stopper saturated with phenol in the syringe outlet and by wrapping the stopcock in gauze saturated with phenol. Glucose and protein hydrolysate were given at a rate of 100 to 200 ml per 8-hour period (depending on the size of the monkey) to prevent dehydration and hypoproteinemia of ani-

mals in which lymph cannulae had been placed.

This method of taking samples allowed blood to be collected by 1 person on any schedule without touching or exciting the animal. Although there was some restraint of the animal, lymph flow was continuous. Neither the surgical wound nor the rest of the animal became contaminated, and the wound never became infected with anthrax from aerosol or contact sources. The volume of lymph flow was recorded at 1 or 2-hour intervals, and bacterial counts were done on the total volume of lymph collected during the sampling interval. Bacterial counts were made on the blood at indicated intervals. Monkeys used in cannulation experiments weighed at least 12 pounds and most of them weighed 16 to 20 pounds. Virtually all animals were conditioned for several months before use in experiments. A postoperative period of 24 hours was used throughout these experiments.

Quantitation of anthrax bacilli.—The quantitative estimation of bacilli per ml of blood or lymph was done by the conventional dilution plate count of 1 ml of blood or 0.4 ml of lymph and by direct observation of bacilli in smears of 0.03 ml of blood spread over a 1-cm square surface. By observation of the number of bacilli in some definite number of microscopic fields, usually 144, the number of bacilli per ml was estimated rapidly by reference to the standard curve. Dilution plates made at the same time as slides could not be read until 12 hours after plating, but by using the direct count the change in the blood or lymph was quantitatively estimated before the next sample was taken.

Aerosol exposure technique.—When challenge was by the aerosol route, the monkeys were exposed in an air-tight

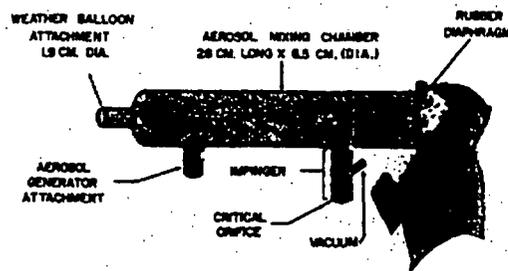


FIGURE 1.—Aerosol exposure chamber.

cage to an aerosol of spores of the M1b strain. The air pressure inside the cage was made negative to the outside by attachment to a ventilated hood system. The aerosol was generated in a Plexiglas mixing chamber (figure 1) placed over the monkey's nose. A rubber diaphragm which made a complete seal when placed over the monkey's mouth and nose was mounted at one end of the cylinder, and a Darex weather balloon (W. R. Grace and Co., Cambridge, Mass.) was attached to the opposite end to collect the excess aerosol.

A cloud of anthrax spores was aerosolized into the chamber by a nebulizer (Vaponefrin Standard Nebulizer, Vaponefrin Co., Portland, Oregon) mounted on the side of the mixing chamber. The nebulizer was operated with an air flow rate of 16 liters per minute, and the liquid spore suspension was disseminated from the nebulizer at a rate of 0.08 ml per minute. The cloud was sampled with an impinger during the entire exposure period at the rate of 7.2 liters of air per minute.

After exposure, the cylinder was removed and the collecting fluid in the impinger was plated on tryptose agar for determining the concentration of viable spores per ml. The volume of air per minute inhaled by the monkey was calculated by the Guyton (1947) formula. The calculated number of viable spores inhaled by the monkey

was obtained by multiplying the number of viable spores in a liter of aerosol by the liters of air breathed during a 4-minute exposure. The procedure for aerosol exposure as well as the cannulation operation has been described on film by the U. S. Army Biological Laboratories (1962).

Other challenge methods.—Intradermal (ID) challenge was over the region of the posterior tibia so that drainage to the popliteal lymph node occurred. Intraperitoneal (IP) inoculation was conventional. Intravenous (IV) challenge was through the venous cannula into the right auricle of the heart. The gastrointestinal challenge was made by means of a K-31 sterile infant feeding tube (Pharmaseal Laboratories, Glendale, Calif.) inserted into the stomach. After challenge, the tube was rinsed with water and withdrawn.

Generalization of data.—Certain generalizations have been made in this report to allow both presentation of data and visualization of the dynamic progressive nature of this disease. Following challenge with enough spores to cause anthrax infection, a sequence of events occurs in the lymphatic system and blood stream. These events are (1) initial observation of bacilli in the blood or lymph, (2) intermittent or constant presence of bacilli, (3) number of bacilli per ml of lymph or blood, (4) rate of change in the number of bacilli observed, and (5) death of the host. We have called the sequence of events "critical points" and we show these critical points diagrammatically in figure 2. The tables of this report present the experimental results following this schema. Data also are grouped according to type of cannulation and route of challenge.

In interpreting the data presented in this report it must be recognized that

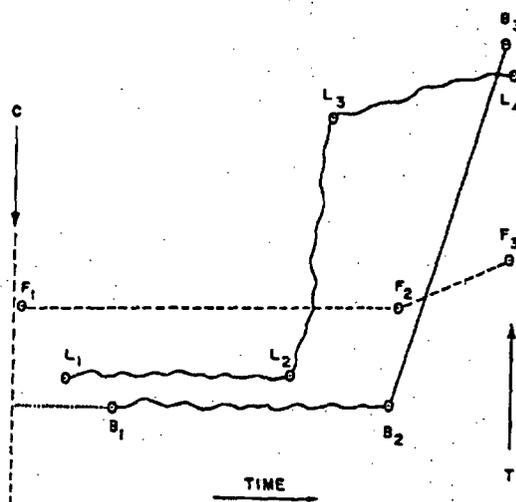


FIGURE 2.—Schema illustrating critical points in the dynamic course of anthrax infection in a host. C: time of challenge; T: time of death; F: rate of lymph flow (F_1 to F_2 : rate constant; F_2 to F_3 : rate increasing); L: organisms in lymph (L_1 : initial detection; L_2 : critical point initiating rapid rise to near maximum number, L_2 to L_3 ; L_3 : critical point initiating period of near static growth, L_3 to L_4 ; L_4 : number of organisms at death); B: organisms in blood (B_1 : initial detection; B_2 : initiation of septicemia; B_3 : number of organisms in terminal blood; B_2 to B_3 : period of logarithmic increase of bacilli in blood, i.e., septicemia).

individual variation occurs. A plot of the actual data is a series of individual points tending to fall either above or below the general regression observed between successive critical points.

RESULTS

Lymph flow rates and output of bacilli.—The anatomical variability of the right lymphatic duct experienced by Widdicombe et al (1956) and Hughes et al (1956) in their studies with rabbits was also found to occur in the rhesus monkey. Only 10% of the monkeys had a common right lymphatic duct that entered the external jugular vein and could be cannulated. Ligation of a common duct was not practical in the remainder because the duct was bi-

furcated, too small, or located too deeply in the chest to be isolated by probing. It is considered that in general the right lymphatic duct drains the major portion (about 75%) of the lungs, while the thoracic duct drains the remainder of the lungs and some of the abdominal viscera. For those animals successfully cannulated, the lymph flow, the number of bacilli collected in thoracic and right lymph, and the number of bacilli in the blood are given in table 1.

In most cases lymph flowed throughout the course of disease until death. In the data presented, the lymph cannula became blocked in only 3 of the 21 animals. Based on the flow rates of those animals whose lymph flowed throughout the disease (table 1), both thoracic and right lymph flow rate was constant until the septicemic stage, at which point the thoracic lymph flow increased, while the right lymph flow decreased, usually with blockage at the afferent orifice of the cannula. This increased flow rate was 77% in aerosol-challenged hosts and less in those challenged by other routes. After the bacilli had built up in the lymph, blockage appeared due to stoppage by bacterial growth and by fibrin or cellular precipitates rather than to a physiological response, because the stoppage of flow occurred between 2 sampling periods and was not a gradual change occurring over several hours. An initially high flow rate tended to continue throughout the life of the animal. There was nonrandom selection of hosts, in that all animals in which a right lymphatic duct was placed were challenged by the aerosol route.

The dynamics of initiating bacillary growth in the lymph following aerosol challenge differs from that following ID, IV, and probably IP routes of challenge (figure 3). In the animals challenged by aerosol the number of

TABLE 1.—Average lymph flow and number of bacilli collected in thoracic and right lymph during anthrax infection in the rhesus monkey

Route of challenge	Number of animals observed	Average weight, pounds	Stage of disease (critical point)	Flow, ml per hour				Lymph (average values)				Blood, average organisms per ml $\times 10^6$
				Thoracic		Right		Thoracic		Right		
				Organisms per ml	Organisms per ml	Organisms per ml	Organisms per ml	Organisms per ml	Organisms per ml	Organisms per ml	Organisms per ml	
Intradermal	1	14	Before septicemia (F ₁ -F ₂)	5.6	3 $\times 10^7$	0	0	2 $\times 10^8$	0	0	0.11	
			During septicemia (F ₂ -F ₃)	6.2	1 $\times 10^8$	0	0	6 $\times 10^8$	0	0.000001		
Intravenous	2	14	Before septicemia (F ₁ -F ₂)	6.8	0	0	0	1 $\times 10^8$	0	0	7.62	
			During septicemia (F ₂ -F ₃)	8.3	0	0	0	1 $\times 10^8$	0	0	1.6	
Intraperitoneal	1	12	Before septicemia (F ₁ -F ₂)	10.8	9 $\times 10^7$	0	0	1 $\times 10^8$	0	0	0	
			During septicemia (F ₂ -F ₃)	11.2	1 $\times 10^8$	0	0	1 $\times 10^8$	0	0	0.48	
Aerosol	2	15.5	Before septicemia (F ₁ -F ₂)	7.6	1 $\times 10^8$	0	0	1 $\times 10^8$	0	0	0	
			During septicemia (F ₂ -F ₃)	13.5	2 $\times 10^8$	0	0	3 $\times 10^8$	0	0	0	
	3	14	Before septicemia (F ₁ -F ₂)	3.4	0	0	0	0	0	0	0	
			During septicemia (F ₂ -F ₃)	2.5	0	0	0	0	0	0	0	
11	11	Before septicemia (F ₁ -F ₂)	10.4	2 $\times 10^8$	0	0	3 $\times 10^8$	0	0	0		
		During septicemia (F ₂ -F ₃)	10.9	0.1	0	0	2 $\times 10^8$	1 $\times 10^7$	1 $\times 10^7$	0.64		

* See figure 2.

† No organisms observed.

‡ One animal was not positive.

§ Plotted in figure 3.

TABLE 2.—Association of critical points as presented in figure 2 for monkeys challenged by the aerosol route

Monkey number	Dose $\times 10^6$	Type of cannulation	Initial observation (B _i , L _i) of bacteria		Critical point in growth curve of bacteria in blood (B _c) or lymph (L _c)		Critical point (L _d): period of static growth in lymph		Death of host and number of bacilli (B _d , L _d)	
			Hours after challenge	Organisms per ml	Hours after challenge	Organisms per ml	Hours after challenge	Organisms per ml	Hours after challenge	Organisms per ml
1	4	V*	57	8×10^6	57	7×10^6	140	6×10^8	140	6×10^8
2	5.6	V	72	1×10^6	74	2×10^6	94	4×10^8	94	4×10^8
3	5	V	100	2×10^6	104	3×10^6	147	4×10^8	147	4×10^8
4	5	V	56	3×10^6	58	9×10^6	64	2×10^8	64	2×10^8
5	20	V	13	5×10^6	30	6×10^7	50	3×10^8	50	3×10^8
6	6	V	15	1×10^6	33	1×10^6	47	3×10^8	47	3×10^8
7	5	T	48	9×10^6	48	9×10^6	57	2×10^8	57	2×10^8
8	3	V	20	3×10^7	2		25	4×10^8	25	4×10^8
9	3	V	26	8×10^7	36	1×10^6	58	3×10^8	58	3×10^8
10	0.5	R	34	3×10^7	34	3×10^7	47	4×10^8	47	4×10^8
11	0.1	T	26	1×10^8	26	1×10^8	44	6×10^8	44	6×10^8
12	1	V	50	4×10^8	50	4×10^8	70	9×10^8	70	9×10^8

* Heart cannulation via the jugular vein.
 † Thoracic lymph duct cannulation.
 ‡ Death occurred before static state.
 § Right lymph duct cannulation.
 ¶ No build-up of organisms.
 †† Lymph from thoracic duct stopped flowing 31 hours after challenge.

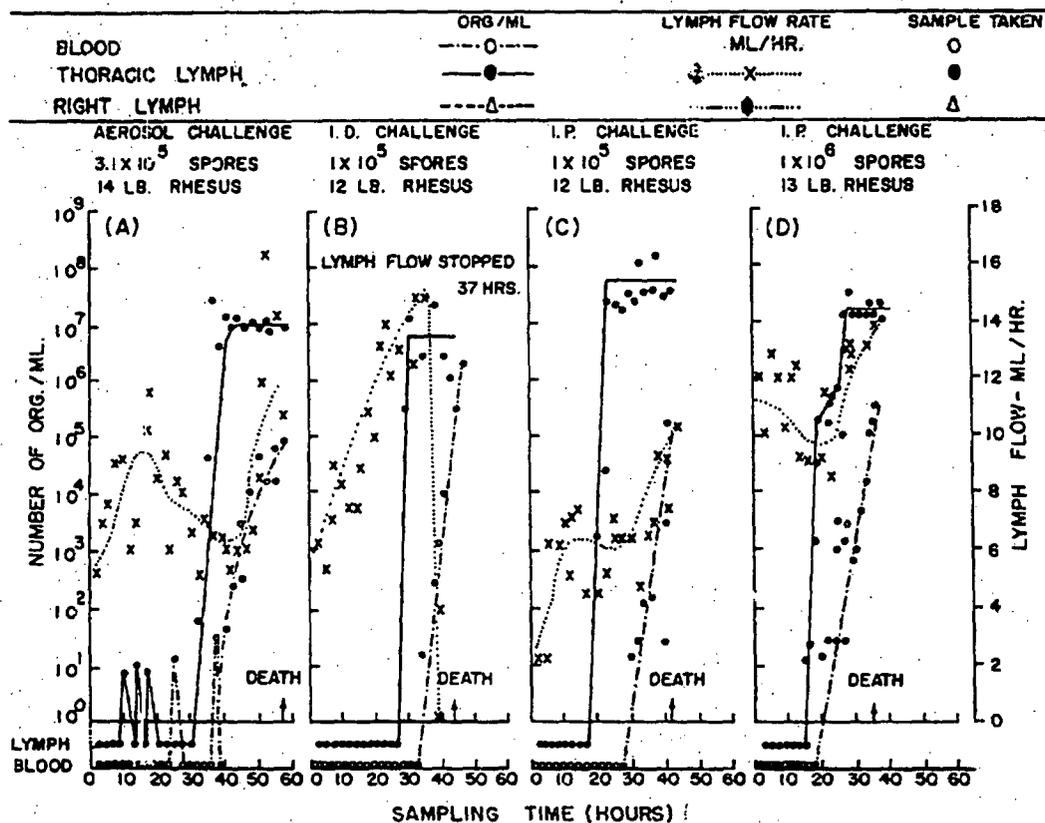


FIGURE 3.—Lymph flow rates and bacilli per ml of lymph or blood of individual rhesus monkeys infected with anthrax. Monkeys A to D.

bacteria per ml of lymph increased slowly, reaching a static maximum during the septicemic phase of disease, while following ID and probably other routes of challenge the number of organisms per ml of lymph increased from zero to near maximum during a single sampling period of 1 or 2 hours. By all routes of challenge a static maximum occurs, as shown in figure 3.

The average number of bacilli per ml in the lymph and the total number of bacilli (mean number of bacilli per ml times average flow rate per hour) collected in the lymph before and during anthrax septicemia are given in table 1. We found that the concentration of organisms in the lymph was on the average equal to or greater than the concentration of bacilli in the blood.

Growth of anthrax bacilli in the lymph appears independent of the route of challenge.

Critical points and quantitation of anthrax established by aerosol, ID, IP, and IV challenge.—Data on 12 monkeys in which inhalation anthrax was established are reported in table 2. All animals had venous cannulae, while 7 had thoracic and/or right lymph ducts. The dose varied between 0.1 and 20×10^6 spores, with most of the animals receiving about 500,000 spores. The variability of individual animals in the responses recorded as time to death, numbers of organisms per ml of blood at death, or hours organisms were observed in blood or lymph is representative of that observed following this route of challenge. Time to death

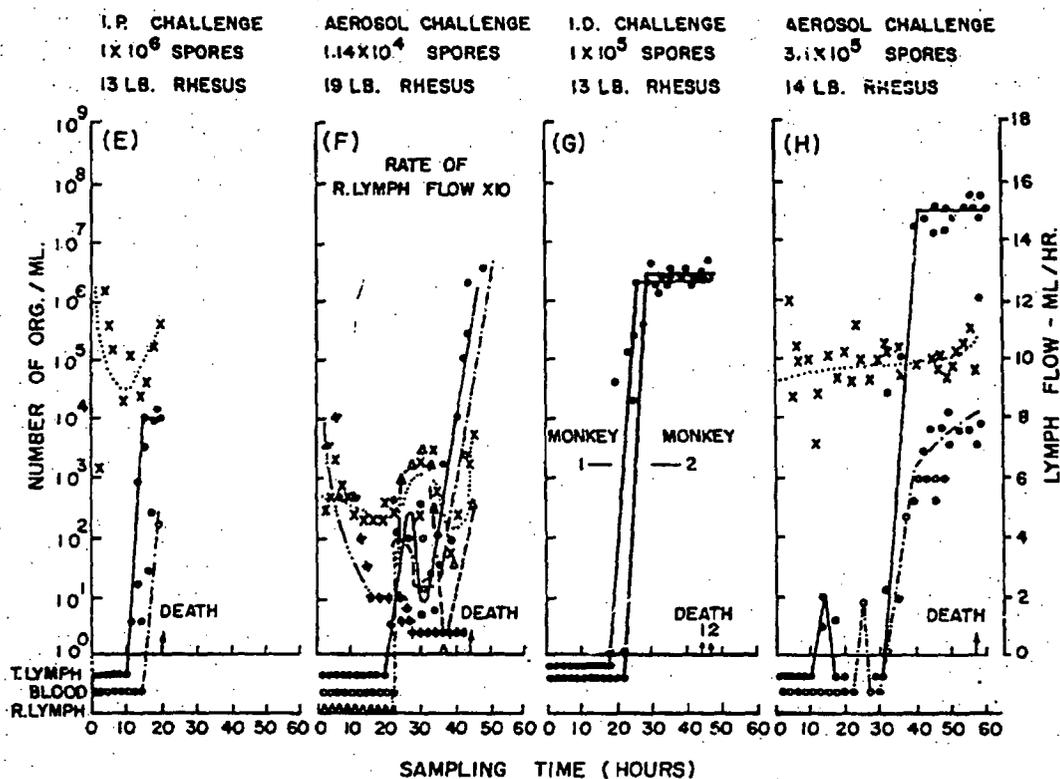


FIGURE 3 (CONTINUED).—Monkeys E to H. Symbols as for monkeys A to D.

varied between 25 and 147 hours. Animal no. 8 was unusual in dying early with no buildup of organisms in the blood. These data show that once bacteria were observed in the blood (bacteremia) progressive septicemic growth usually occurred within a few hours. The septicemic period averaged 30% of the interval between dose and death.

When anthrax was established by ID challenge with 100,000 spores (table 3), time to death ranged from 32 to 59 hours. All 10 animals developed a high number of organisms in the blood, and the septicemic period averaged 40% of the time interval between dose to death.

The data for 8 monkeys (of which 2 had thoracic cannulae placed) challenged by the IP route are given in table 4. Unusually long times to death were observed for 2 of these animals.

The septicemic period averaged 28% of the interval between dose and death.

In table 5 the data from 15 monkeys challenged IV are given. Doses tended to be relatively high or low. When the dose was 300,000 spores or less, the blood was sterile at the 15-minute sampling period. When the dose was 10,000,000 spores, the blood was positive for the first 2 hours and then sterile until hours later when a septicemia was observed. At doses of 1×10^8 and above the blood remained positive throughout the course of disease. The time to death varied between 20 and 142 hours. When the dose was low a septicemia developed before bacilli were observed in the lymph; when the dose was high the lymph became positive practically simultaneously with the inception of septicemia.

Challenge by the gastrointestinal

TABLE 3.—Association of critical points as presented in figure 2 for monkeys challenged by the intradermal route

Monkey number	Dose $\times 10^6$	Type of cannulation	Initial observation (B ₁ , L ₁) of bacteria				Critical point in growth curve of bacteria in blood (B ₂) or lymph (L ₂)				Critical point (L ₃): period of static growth in lymph				Death of host and number of bacilli (B ₃ , L ₃)			
			Hours after challenge		Organisms per ml		Hours after challenge		Organisms per ml		Hours after challenge		Organisms per ml		Hours after challenge		Organisms per ml	
			Blood	Lymph	Blood	Lymph	Blood	Lymph	Blood	Lymph	Blood	Lymph	Blood	Lymph	Blood	Lymph	Blood	Lymph
1	1	V*	45		3×10^7		47		9×10^8									
2	1	V	33		4×10^6		32		3×10^5									
3	1	V	32		3×10^6		37		8×10^5									
4	1	V	37		8×10^5		24		2×10^6									
5	1	V	24		2×10^6		30		2×10^5									
6	1	V	30		2×10^5		38		7×10^3									
7	1	T†	38		6×10^5		34		6×10^5									
8	1	V	34		2×10^5		28		2×10^5									
9	1	T	28		6×10^6		27		6×10^5									
10	1	V	26		3×10^5		20		3×10^5									
		T	30		1×10^7		5		1×10^6									
		T	20		1×10^6		20		1×10^6									

* Heart cannulation via jugular vein.
 † Thoracic lymph duct cannulation.
 ‡ Lymph stopped flowing 21 hours before death.
 § Lymph stopped flowing 6 hours before death.
 ¶ Death occurred before static state.
 †† No build-up of organisms.

TABLE 4.—Association of critical points as presented in figure 2 for monkeys challenged by the intraperitoneal route

Monkey number	Dose $\times 10^6$	Type of cannulation	Initial observation (B ₁ , L ₁) of bacteria				Critical point in growth curve of bacteria in blood (B ₂) or lymph (L ₂)				Critical point (L ₃): period of static growth in lymph				Death of host and number of bacilli (B ₄ , L ₄)			
			Hours after challenge		Organisms per ml		Hours after challenge		Organisms per ml		Hours after challenge		Organisms per ml		Hours after challenge		Organisms per ml	
			Blood	Lymph	Blood	Lymph	Blood	Lymph	Blood	Lymph	Blood	Lymph	Blood	Lymph	Blood	Lymph	Blood	Lymph
1	1	V*	66		1×10^8		66		1×10^8				76		2×10^8			
2	1	V	42		1×10^8		48		8×10^8				55		8×10^7			
3	1	V	252		1×10^7		7		3×10^7				253		9×10^8			
4	1	V	1		5×10^7		25		3×10^7				86		1×10^8			
5	116	V	0.08		2×10^7		10		5×10^7				42		8×10^8			
6	928	V	2		1×10^7		84		5×10^7				128		1×10^8			
7	10	V ††	18	11	4×10^7	5×10^8	11	11			5×10^8		20		2×10^7		2×10^7	
8	11	V †	20	1	9×10^7	5×10^8	20	16			8×10^7		36		3×10^7		1×10^7	

* Heart cannulation via the jugular vein.
 † No build-up of organisms.
 †† Thoracic lymph duct cannulation.

route.—Spores deposited on the ciliated surfaces of the lung bronchials constitute 65 to 75% of the retained spores from an aerosol challenge, and most of these spores pass into the stomach within a few hours (Harper and Morton, 1953). Two monkeys each were challenged with doses of 10^2 , 10^4 , 10^6 and 10^8 spores, respectively, in order to determine whether spores deposited on ciliated surfaces and passing into the enteric region might cause anthrax infection. All monkeys challenged enterically survived, regardless of dose. These results indicate that spores deposited from the aerosol on ciliated membranes likely do not often initiate infection.

DISCUSSION

Role of the lymphatics in anthrax infection following aerosol challenge.—The specific data, as generalized by the schema given in figure 2, do not conflict with the observations of Ross (1957) that entry of bacilli from the lung is via the lymphatics. Bacilli were not observed in the blood (B₁) prior to their observation in the lymph (L₁), as might be expected if bacilli penetrated the capillaries directly from the lung alveoli. Although tests were made for heat-resistant spores, none were found in either the blood or lymph at any time. However, this test admittedly does not tell the state of the bacterial cell when it enters the body. We found no evidence for the direct entry of anthrax spores or bacilli from the lung alveoli into the blood stream.

The lymphatic system allows a significant exchange of bacilli between the lymphatics and the blood. If this conclusion were not true, then, as noted by Widdicombe et al (1956), cannulation of both the thoracic and right lymphatic ducts would prevent or at least delay the establishment of anthrax if the lymphatics were the only route of

TABLE 5.—Association of critical points as presented in figure 2 for monkeys challenged by the intravenous route

Monkey number	Dose X tips	Type of cannulation	Initial observation (B ₁ , L ₁) of bacteria				Critical point in growth curve of bacteria in blood (B ₂) or lymph (L ₂)				Critical point (L ₃): period of static growth in lymph				Death of host and number of bacilli (B ₄ , L ₄)			
			Hours after challenge		Organisms per ml		Hours after challenge		Organisms per ml		Hours after challenge		Organisms per ml		Hours after challenge		Organisms per ml	
			Blood	Lymph	Blood	Lymph	Blood	Lymph	Blood	Lymph	Blood	Lymph	Blood	Lymph	Blood	Lymph	Blood	Lymph
1	1	V*	56		2X10 ⁸		64		2X10 ⁴		98		6X10 ⁸					
2	10	V	1		3X10 ⁸		60		2X10 ⁷		142		4X10 ⁸					
3	100	V	0.5		6X10 ⁸		2		7X10 ⁸		48		3X10 ⁸					
4	114	V	1		1X10 ⁸		27		1X10 ⁸		35		5X10 ⁸					
5	1000	V	0.5		5X10 ⁸		21		4X10 ⁸		67		7X10 ⁸					
6	1140	V	1		1X10 ⁸		29		3X10 ⁸		60		2X10 ⁸					
7	1180, 1000	V	1		3X10 ⁸		28		7X10 ⁸		46		2X10 ⁸					
8	570, 1000	V	1		2X10 ⁸						38		†					
9	0.1	V, T†	20		1X10 ⁸		19		1X10 ⁷		27		1X10 ⁷					
10	0.1	V, T	0.5		4X10 ⁷						23		2X10 ⁷					
11	0.1	V, T	32		3X10 ⁸						32		3X10 ⁸					
12	3	V, T	10	22	1X10 ⁸	1X10 ⁸	18		4X10 ⁸		31		4X10 ⁷					
13	1000	V, T	10	14	7X10 ⁷	5X10 ⁷	10		7X10 ⁷		20		3X10 ⁷				4X10 ⁸	
14	1000	V, T	30	14	6X10 ⁸	1X10 ⁸	31	31	1X10 ⁸	9X10 ⁸	40		2X10 ⁸				1X10 ⁷	
15	1000	V, T	26	24	1X10 ⁸	5X10 ⁸	26	26	1X10 ⁸	5X10 ⁸	32		2X10 ⁷				5X10 ⁷	

* Heart cannulation via jugular vein.
 † Not done.
 ‡ Thoracic lymph duct cannulation.
 § No organisms observed.
 ¶ Death occurred before static state.

entry from alveoli into the body. We were able to obtain only 1 animal (table 2, monkey number 11; figure 3F) in which both the right and thoracic ducts were cannulated and in which lymph flowed freely throughout the course of disease. Anthrax was established by respiratory challenge, and time to death (B_3) was not noticeably changed by the cannulation. Thus, it appears that significant lympho-venous connections exist. Additional evidence for believing there are lympho-venous connections, possibly pathological in the sense of Malek et al (1959), occurred following stoppage of lymph flow (table 3, monkeys 7 and 8). In these cases, the number of bacilli per ml in the blood (B_2 to B_3) increased approximately 1 log, reflecting an increased number of bacilli entering the blood through the pathological lympho-venous channels in the lymph nodes. Particularly with those animals challenged by the ID or IP routes, the animals having only venous cannulae tended to have a higher number of bacteria per ml of blood when initially observed than did those having lymph cannulae.

If the lymphatics are the route of entry of anthrax organisms into the body, the first lymph node or nodes on the lymphatics draining the lungs is challenged during any unit of time by a few bacilli, possibly dormant spores, germinated spores, or newly developed vegetative bacilli; these cells would be unencapsulated. The primary challenge continues until the lung is cleared, a process that with anthrax requires weeks, or until death from anthrax. Encapsulated bacilli that are relatively resistant to phagocytosis result with the further growth of virulent anthrax cells. Once the lymph nodes are overcome, tremendous numbers of bacilli pour into the blood to be distributed throughout the body. With few excep-

tions, once the septicemia (B_2) was observed the number of bacilli increased logarithmically (B_2 to B_3) with 1 doubling (apparent generation time) per 48 minutes. The concentration of bacteria in the blood of uncannulated (control) monkeys at death (B_3) averaged $10^{6.3}$ ($10^{5.5}$ to $10^{7.2}$) bacilli per ml (Lincoln et al, 1964).

Toxins can be demonstrated in the terminal blood of most monkeys and in all species of animals dying of anthrax thus far tested. With uncannulated monkeys we find a negative correlation between time of death and both the concentration of toxins in the terminal blood and the number of bacilli per ml at death. We suggest that a time-concentration effect of these toxins on the phagocytic system may be a primary factor in initiating the terminal septicemia (B_2) (Lincoln et al, 1964).

Role of lymphatics in anthrax infection following ID or IP challenge.—When anthrax is established after IP or ID challenge, it appears from our data as well as the data of others that the regional lymph nodes draining the site receiving the spores become infected. Depending on dose, bacilli may or may not appear in the thoracic lymph (L_1) immediately after challenge. A period of incubation then occurs in which no bacilli are collected in the thoracic lymph. Shortly before the terminal septicemia (B_2), the thoracic lymph becomes and remains positive (L_2 to L_4) for bacteria which enter the blood stream through the thoracic duct. Our data agree with the observations of other workers, such as Schultz et al (1938), that the lymph cultures are always positive before the blood cultures. Once the bacteria move into the lymphatics, there is little or no difference in the dynamics of infection by any of the routes of challenge, aerosol, ID, IP and, as discussed in the next

paragraph, IV (tables 2, 3, 4 and 5), respectively.

Extravascular circulation of bacilli.—The IV route of challenge (table 5) furnished critical information on possible extravascular circulation of bacilli through the lymphatics that was not furnished by other routes of challenge. Following challenge by all routes except IV, bacteria were detected in the thoracic lymph (L_1) some hours before a septicemia (B_2) was observed. Since the IV route of challenge failed to show this phenomenon when challenge was at low doses that consistently established anthrax, (monkeys 9, 10, 11; table 5), the critical experiment to verify that extravascular circulation of anthrax bacilli through the lymphatic would be a feature of infection was not supported by these data. It seems probable that anthrax cells with a volume of at least $4 \mu^3$ may be too large to follow this route of circulation, and tests with other particulate materials of smaller size (such as viruses) may show extravascular circulation to be a feature of some infections.

Dynamics of growth in the lymphatics.—As noted by most workers, the reticuloendothelial system is a very efficient, although not perfect, collector of anthrax bacilli. The ability of the vascular bed to clear the blood of an initial dose up to 100 million spores has been noted. Also noted is the fact that an incipient bacteremia, except for the period immediately prior to the septicemia, is not a feature of anthrax in the monkey following any route of challenge. The increase of organisms in the thoracic lymph following aerosol challenge was gradual in contrast to the observation of organisms in the thoracic lymph at a several log elevation following ID challenge. In the latter case the increase in numbers was too rapid to be explained by simple growth in the

lymph comparable to growth in the blood. This observation seems best explained as retention by the lymph node until it is neutralized in some manner, allowing the retained bacilli to flow to the next lymph node or into the blood stream.

Once free flow of bacilli occurs, growth dynamics within the thoracic lymph were those of a continuous culture system, as would occur if a relatively constant amount of liquid entered and the same amount were removed from the lymphatic system per unit of time. The dynamics would be affected if rate of flow, generation time of the bacillus, or resistance of the host changed markedly. Although lymph flow increased after septicemia, growth of the bacilli was essentially in equilibrium with the milieu and flow. Therefore, the number of bacilli per ml of effluent was also relatively constant. Trnka et al (1956) also observed in sheep a rapid build up of bacilli in the efferent lymph to a steady state level that continued throughout the course of disease. The 2 observations appear to be parallel. These data make it appear probable that the architecture of the lymph node and lymphatic vessels is not changed appreciably by infection. This observation is in agreement with that of Berdjis et al (1962) who noted little or no disturbance of architectural pattern of the lymph nodes even though they might contain anthrax bacilli. However, in a later report Berdjis et al (1963) noted that greater damage to the lymph nodes was observed after infection by the cutaneous route than occurred following respiratory challenge.

Early detection of systemic anthrax.—Experimental cannulation of the lymphatics is a feasible method of detecting generalized anthrax earlier than by observation of a septicemia. However, considering the seriousness of the opera-

tion, the possibility that cannulae cannot be placed, and the biological variability of hosts, cannulation is not considered a practical method of early detection.

General comments.—Although the exact amount varied with the individual animal, a large volume of lymph was removed from cannulated animals, particularly those with a thoracic cannula. We attempted to compensate for the attended protein and mineral loss by administering protein hydrolysate and saline. Nevertheless, a change in the physiological balance of the host attributable to the lymph removed must have occurred. Cannulated animals tended to die before similarly challenged noncannulated animals. This earlier death occurred in spite of the fact that great numbers of bacilli and large amounts of toxin were removed from the host with the lymph and did not enter the blood stream as would occur in an animal without a lymphatic cannula.

In certain cannulation experiments our colleagues in the U. S. Army Medical Unit, Fort Detrick, Maryland, administered human plasma at the same rate lymph was being removed from the body of monkeys. Their technique probably would maintain physiological balance better than ours.

SUMMARY

Early identification of anthrax infection was attempted by means of a surgical technique allowing cannulation of both the lymphatic and blood systems in the rhesus monkey. This technique permitted observation and quantitation of organisms in the lymphatics and blood stream at any given time during the infection without disturbing the animal. Although the establishment of anthrax can be detected several hours before a septicemia, the seriousness of the surgical

technique and the variability of individual animals precludes routine use of this technique in diagnosis of anthrax.

After challenge by all routes (intra-peritoneal, intradermal, and aerosol) other than intravenous, bacilli were detected in the thoracic lymph approximately 12 hours before detection in the blood. The rate of thoracic lymph flow was constant until septicemia and then tended to increase in rate of flow, suggesting that the architecture of the lymph node and vessels was not changed appreciably by infection. The dynamics of bacillary growth in the thoracic lymph were comparable to those in a continuous culture system at equilibrium.

Anthrax was established while the right and thoracic lymph was drained from the animal, indicating that there was a significant exchange of bacilli directly from the lymphatics to the blood. Once the regional lymph nodes draining the region of challenge became infected there was little or no difference in the dynamics of infection. Anthrax was not established following gastrointestinal challenge of 8 monkeys.

An average of 80 million bacilli were generated in the infected lymphatics and poured into the blood over an 8-hour period before a septicemia occurred. The concentration of organisms in the lymph was on the average equal to or greater than the concentration of bacilli in the blood. Growth in the lymph appeared independent of route of challenge. Extravascular circulation (i.e., from blood to lymph) of anthrax bacilli appears not to occur.

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