ON THE NATURE OF BACTEREMIA IN EXPERIMENTAL PNEUMOCOCCAL PNEUMONIA IN THE DOG

I. Relationship of Natural Pneumococcidal-Promoting Activity of the Serum to Blood Invasion

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Studies on bacteremia in pneumococcal lobar pneumonia have brought forward accumulating evidence to indicate that this form of pneumococcus infection in the human being is characteristically a localized disease. The older view that lobar pneumonia in its beginning stages is accompanied by a transient invasion of the blood stream is not supported by the results of blood cultures in early cases. While bacteremia may occur within the first 24 hours of the disease, the great majority of cases exhibit a sterile blood at this time (1, 2).[1] The marked alteration in prognosis occasioned by finding pneumococci in the blood stream at any period in the disease is so generally recognized that it is mentioned here only to emphasize the importance of this phenomenon about which so little is known. The conception that bacteremia indicates a breaking down of the body's defence mechanism is probably correct, since it has been observed that the more extensive the pulmonary involvement and the lower the white blood count the greater the likelihood of bacteremia (3), and that following the onset of bacteremia an increasing number of pneumococci in the blood is associated with a rapidly rising mortality. However, we have little information as to how or why pneumococci escape from the pulmonary lesion.

Of the various factors in bodily resistance, which might operate to control the liberation of pneumococci from the lung into the blood stream, that of

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[1] These observations were made before sulfonamides and antibiotics came into practically universal use. Such studies are now impossible. Despite the greatly improved prognosis of pneumococcal pneumonia due to modern treatment, further investigation of the processes and course of bacteremia continues to be of importance for a clearer understanding and more adequate management of the disease.

phagocytosis and intracellular digestion appears to be of first importance (4). Animals whose blood possesses the power to kill pneumococci are capable of localizing infection with this microorganism and exhibit a high degree of resistance to it. Studies on the blood of such animals have demonstrated that pneumococcidal activity depends solely on phagocytosis mediated by the presence of natural opsonins (5).[2] Other animal species possessing some degree of resistance to virulent pneumococci but whose blood lacks demonstrable pneumococcidal properties are nevertheless able to localize pnemuococci implanted experimentally in the lung. This mechanism has been recently shown by Wood and associates (6) to consist of a process designated by them as surface phagocytosis, which can proceed in the absence of opsonizing fluids. It would seem likely that both types of phagocytosis play a role in localizing pulmonary pneumococcal infection, at least in the highly resistant animal species.

If then, the occurrence of adequate phagocytosis of pneumococci in the pulmonary lesion acts as the principal barrier to the escape of these microorganisms from the lung into the blood stream, the measurement of penumococcidal activity in the blood of human cases of lobar pneumonia should serve as a guide to events occurring in the infected lung, since man exhibits considerable resistance to the pneumococcus. Yet our earlier studies of the natural pneumococcus-killing power of the blood during the course of lobar pneumonia in human beings revealed no constant relationship between this antipneumococcal property of the blood and the occurrence of bacteremia (7). It was found that while the onset of blood invasion was usually associated with diminution or disappearance of humoral immunity, in certain patients the blood remained sterile in the absence of demonstrable pneumococcidal activity, and an occasional one exhibited bacteremia despite the persistence of circulating immune substances. Other observers working with similar techniques have reported findings of the same general nature (8). Some quantitative information on the role played by surface phagocytosis might well throw light on these wide variations in the reaction of pneumonia patients to the invading microorganism. Furthermore the considerable number of recognizable variables present in each individual disease state would also tend to modify the body response. Not only are there marked differences between normal persons in respect to the pneumococcus-killing properties of their blood for the different types and various strains of the same type, but the disease-producing pneumococci also vary much in their virulence, certainly for animals and probably for human beings.

[2] Variations in this property of the blood have been found to depend entirely on the opsonic content of the serum. Leucocytes from susceptible animals are just as effective in engulfing and digesting adequately opsonized pneumococci as are the leucocytes of highly pneumococcus-resistant animals.

The production of experimental canine pneumonia with a single strain of pneumococcus has enabled us to study this subject in a manner not feasible in the human patient. The dog is a particularly suitable animal for this purpose since the natural antipneumococcal properties of its blood are much the same as those of the average human being and the dog's response to the presence of a pneumococcus lesion in the lung is analogous in most respects to the course of clinical lobar pneumonia (4). Furthermore, tests on hundreds of normal dogs have shown without exception the presence of pneumococcus-killing capacity in the blood and the great majority of them exhibit this property to about the same degree. The variations in the pneumococcidal activity of the dog's blood during experimental pneumonia are not unlike those occurring in clinical lobar pneumonia as reported by Terrell (9) from this laboratory.

A study of some 375 dogs with experimental pneumococcus pneumonia has brought out certain striking relationships between bacteremia, the white blood count, extent of pulmonary involvement and mortality (10), which we wish to recall briefly as a basis for the investigation, now to be reported, of the factors inhibiting or controlling bacteremia. A condensed summary of these previous observations is presented in Table I.

The number of circulating white blood cells 24 hours after the onset of the experimental disease bore a definite relationship to the occurrence of bacteremia and to outcome; later white cell counts were less significant. Dogs showing counts of more than 20,000 per c.mm. exhibited a low incidence of bacteremia and a correspondingly low mortality. With diminishing white blood cell counts both the incidence of bacteremia and death rate increased progressively until with counts of less than 2,000, 93 per cent of the dogs developed bacteremia and 91 per cent died. But if the blood remained sterile the mortality was very low even in the presence of a marked leucopenia.

The frequency with which bacteremia occurred was related to the extent of the pulmonary lesion. With the lesion confined to a single lobe bacteremia was present in only 8 per cent of 104 animals. With the progressive spread of the inflammatory process from lobe to lobe the incidence of bacteremia increased to 96 per cent in dogs with four or more lobes involved. There was a death rate of less than 1 per cent in animals with single lobe involvement in contrast to a 98 per cent mortality when four or more lobes were consolidated. If, however, the blood remained free from pneumococci the mortality was only 4 per cent in dogs with a lesion occupying one-half the lung field whereas the mortality in the bacteremic dogs with similar pulmonary involvement was 98 per cent. The white blood cell count also diminished with the extending lesions.

A further analysis of these data showed that even in the presence of a marked leucopenia and extensive pulmonary involvement the prognosis was good, provided the blood remained sterile. Out of eight animals with a white blood count of less than 5,000 and consolidation of approximately one-half the lung field only one died, whereas the death rate was 96 per cent in 57 dogs having analogous lesions and white blood counts but with bacteremia in addition.

Just as in human pneumonia the mortality rate paralleled the degree of bacteremia. The death rate in dogs showing 1 to 20 pneumococci per cc. of blood was 56 per cent, while 87 per cent of those with 100 to 800 pneumococci died, and none of the animals exhibiting a bacteremia of 1,000 colonies or more survived.

In sum the canine disease presented in general the same features exhibited by the patient suffering from lobar pneumonia.

TABLE I

Mortality in Relation to White Blood Cell Count Extent of Pulmonary Involvement and Bacteremia in 350 Dogs with Experimental Pneumococcal Pneumonia

1. White blood cell count	Incidence of bacteremia	Total mortality	Mortality	
			With bacteremia	Without bacteremia
		per cent	*per cent*	*per cent*
Over 20,000	13	7	50	0
10,000 to 20,000	24	17	70	0
5,000 " 10,000	43	34	70	8
2,000 " 5,000	61	57	88	6
Below 2,000	93	91	98	0 (3 dogs)
2. Extent of pulmonary involvement				
1/6 ± (1 lobe)	8	1	12	0
1/3 ± (2 lobes)	41	24	52	3
1/2 ± (3 ")	76	75	98	4
3/4 ± (4 " or more)	96	98	100	0 (1 dog)
3. Leucopenia and extensive pulmonary involvement	With bacteremia		Without bacteremia	
	No. of dogs	Mortality	No. of dogs	Mortality
		per cent		*per cent*
W.B.C. less than 5,000 Consolidation of 1/2 ± lung	57	96	8	12 (1 dog)

Materials and Methods

The organism employed throughout the work now to be reported was the standard strain (A_5) of Type I pneumococcus used as routine in this laboratory, grown for 16 hours in 0.5 per cent dextrose broth enriched with 2 per cent normal rabbit serum. The bacteria so cultivated are Gram-positive, possess well defined capsules which readily swell in the presence of immune rabbit serum of the homologous type, and produce smooth colonies on agar. The culture contains 1 billion or more diplococci per cc. As few as ten pneumococci kill a mouse in 24 hours, and as little as 0.00001 cc. of culture suitably placed in the lung of a dog produces lobar pneumonia (11). The organism is passed through a rabbit approximately every 6 weeks. Passage through a dog on several occasions has not perceptibly increased its virulence for this animal.

Before being used the culture was centrifuged and the bacteria resuspended in gelatin–Locke's solution. For the work *in vitro* the suspension was adjusted to contain approximately 1 billion pneumococci per cc., and serial tenfold dilutions made of this standard suspension. The dilution calculated to contain 10 microorganisms per 0.1 cc. was always plated out for actual counting.

Blood cultures in agar plates were always made from the same sample of blood used for the pneumococcidal tests. To facilitate the counting of colonies, the blood was diluted serially in cold gelatin–Locke's solution.

The natural pneumococcidal-promoting power of serum was measured by the method of Robertson and Sia (12) wherein varying dilutions of pneumococcus suspension and fixed quantities of serum and leucocytes are incubated together in sealed tubes which are rotated and oscillated in a special device (13). The materials are so assembled that in a final volume of 0.5 cc. there is 0.3 cc. of serum, 0.1 cc. of a leucocyte suspension containing 10,000 per c. mm., and 0.1 cc. of the pneumococcus suspension. Pneumococcidal-promoting activity of the serum is expressed by the maximum number of pneumococci killed. The numbers employed in these tests ranged from 10 to 10^7. The leucocytes were obtained from the pleural exudates produced in normal dogs by injection of aleuronat.

Pneumococcidal tests were also carried out in an analogous manner with 0.5 cc. quantities of defibrinated blood and whole blood kept fluid by adding 0.1 cc. of a 1 per cent heparin solution per cc. of blood. This anticoagulant in the concentration used here had no discernible inhibitory influence on the pneumococcidal action of dog blood as determined by a comparison of the rate at which pneumococci were killed in defibrinated blood with and without the addition of heparin. Nor did the heparin interfere with bacterial growth.

The presence or absence of acquired immune substances in the serum was determined by heating a portion of the serum sample to 56°C. for ½ hour, then testing its mouse protective action against infection with varying quantities of the A_5 strain of pneumococcus. The heated serum of the normal dog exhibits no protective action in mice.

EXPERIMENTAL

We have carried out a study of the pneumococcidal-promoting action of the serum and the pneumococcus-killing power of the whole blood in 86 dogs with experimental pneumonia. Thirty-one of these animals recovered and 55 died. For the determination of the pneumococcidal-promoting action of the serum samples were obtained daily throughout the course of the infection and all tested at the same time immediately after the termination of the disease.

(*a*) *Dogs Recovering from Pneumonia.*—Observations on dogs recovering from the experimental disease revealed the occurrence of wide individual variations in respect to changes in antipneumococcal humoral immunity. Examples of the several different types of change are shown in Table II.

The majority of the 31 recovering dogs behaved as did 1-02S and 1-19S. The blood remained sterile and the pneumococcidal-promoting titre of the serum persisted essentially unchanged. Seven animals showed a slight transient bacteremia. In only one instance, 1-54S, was this accompanied by disappearance of the demonstrable natural immune properties. Several dogs exhibited a bacteremia of 80 to 800 pneumococci per cc. of blood. In one of these (1-50S) the titre of immune substances remained unchanged, despite a prolonged bacteremia reaching a height of 175 colonies on the 4th day. In two other animals the immune properties of the serum disappeared during the height of the blood invasion, but reappeared 2 to 3

TABLE II

The Relationship between Natural Immune Substances and Bacteremia in Dogs Recovering from Experimental Pneumococcal Pneumonia

Dog No.	Dose and site of infection		Before infection	Days of disease									Duration of disease
				1	2	3	4	5	6	7	8	22	
													days
1-02S	0.01 cc.	Bld. cult. col.		0	0								2
	L.L.	Nat. imm. sub.* . .	10^5	10^4	10^3								
		Acq. imm. sub.‡ . .			0								
		W.B.C., *thousands*	22.2	10.6	13.8								
1-19S	1 cc.	Bld. cult. col.		0	0	0	0						4
	R. L.	Nat. imm. sub. . . .	10^5	10^4	10^5	10^5	10^4						
		Acq. imm. sub. . . .					0						
		W.B.C., *thousands*	10.9	3.7	11.4	10.3	14.4						
1-50S	0.6 cc.	Bld. cult. col.		36	23	43	175	24	0				6–7
		Nat. imm. sub. . . .	10^5	10^6	10^5	10^5	10^5	10^5					
		Acq. imm. sub. . . .							0				
		W.B.C., *thousands*	17.9	5.1	13	20.4	18.6	19.8	28				
9-1S	0.01 cc.	Bld. cult. col.		0	80	57	78	0	0				6
	R. L.	Nat. imm. sub. . . .	10^4	10^4	10^4	0	0	10^4	10^5	10^4			
		Acq. imm. sub. . . .						0		0			
		W.B.C., *thousands*	22.4	31.6	33	29.8	30	38.8	29	35			
1-08S	0.02 cc.	Bld. cult. col.		1	10	800	113	3	2	3	0		8
	R. L.	Nat. imm. sub. . . .	10^4				0	10^2	10^2	10^5	10^6	10^6	
		Acq. imm. sub. . . .								0	0	10^3	
		W.B.C., *thousands*	18.4	6.8	10.3	15.4	8.7	20.5	12.9	12	28.3		
V	2 cc.	Bld. cult. col.		16	0	0	0	0	0				7
	R. L.	Nat. imm. sub. . . .	10^4	10^3	10^4	10^4	10^5	10^5	10^6				
		Acq. imm. sub. . . .											
		W.B.C., *thousands*											
1-84S	1 cc.	Bld. cult. col.		4	2	0							3
	R. L.	Nat. imm. sub. . . .	10^5	10^4	10^2	10^3							
		Acq. imm. sub. . . .				0							
		W.B.C., *thousands*	22.5	15.6	22.4	30							
1-54S	0.02 cc.	Bld. cult. col.		3	2	0							3
	R. L.	Nat. imm. sub. . . .	10^5	0	0	10							
		Acq. imm. sub. . . .				0							
		W.B.C., *thousands*	25.5	7.6	24.3	14							

Natural immune substances measured by number of pneumococci killed in serum-leucocyte mixture:

10^3 = 1,000
10^4 = 10,000
10^5 = 100,000

* Natural pneumococcidal-promoting action of serum.

‡ Serum heated to 56°C. for ½ hour then tested for its mouse-protective properties.

TABLE III

The Relationship between Pneumococcidal-Promoting Properties of Serum and Bacteremia in Dogs Dying from Experimental Pneumococcal Pneumonia

Dog No.	Dose and site of infection		Before infection	Days of disease							Outcome
				1	2	3	4	5	6	7	
3-01S	5 cc.	Bld. cult. col.		19							Died 21
	R. L.	Nat. imm. sub.	10^5	10^4							hrs.
		W.B.C., *thousands*	18.2	12.3							
2-45S	1 cc.	Bld. cult. col.		210							Died 26
	R. L.	Nat. imm. sub.	10^6	10^6							hrs.
		W.B.C., *thousands*	11.6	2.05							
1-52S	0.6 cc.	Bld. cult. col.		1,000's							Died 24
	R. L.	Nat. imm. sub.	10^6	0							hrs.
		W.B.C., *thousands*	18.3	2.15							
2-02S	1 cc.	Bld. cult. col.		32	1,000's						Died 3
	R. L.	Nat. imm. sub.	10^4	10^3	0						days
		W.B.C., *thousands*	7.8	5.3	1.65						
1-32S	0.6 cc.	Bld. cult. col.		34	78	55					Died 3–4
	R. L.	Nat. imm. sub.	10^5	10^5	10^3	10_2					days
		W.B.C., *thousands*	6.15	2.35	9.7	14.3					
IV	3 cc.	Bld. cult. col.		0	50	18	0	0			Died 6th
	R. L.	Nat. imm. sub.	10^6	10^5	10^6	10^5	10^5	10^5			day.
		W.B.C., *thousands*									Heart bld., 0
1-26S	1 cc.	Bld. cult. col.		0	48	400	30	1,000's	1,000's		Moribund
	R. L.	Nat. imm. sub.	10^3	10^3	10	10^4	10	10^4	10^3		6th day
		W.B.C., *thousands*	13.4	1.5	3.5	5.6	4.3	9.9	15.6		killed
2-0	3 cc.	Bld. cult. col.		60	90	76	90	280	1,000's		Died 7th
		Nat. imm. sub.	10^5	10^5	10^3	10^4	10^5	10^5	10^4		day
		W.B.C., *thousands*									
1-28S	0.6 cc.	Bld. cult. col.		85	7	187	1,000's	1,000's	1,000's		Died 6th
	R. L.	Nat. imm. sub.	10^5	0	0	0	0	0			day
		W.B.C., *thousands*	6.3	12.8	7.8	12.3	13.8	16.1	4.45		
5-8S	0.2 cc.	Bld. cult. col.		40	800	200	—	1,000's	1,000's		Died 7th
	L. L.	Nat. imm. sub.	10^3	10^4	0	0	0	0	0		day
		W.B.C., *thousands*	14.6	21.7	36.6	19.6	4.9	17.8	5.0		

1,000's indicates more than 2,000 colonies per cc. of blood.

days before recovery. It is of interest to note that the recurrence of pneumococcidal-promoting properties of the serum in these animals 9-1S and 1-08S represented a reappearance of the natural immune bodies and not the development of newly acquired ones since the heat-inactivated serum at this time failed to show mouse protective action. It was only after several days subsequent to recovery that such acquired immune properties were detected.

(*b*) *Dogs Dying from Pneumonia.*—All but one of the dogs in which the disease terminated fatally exhibited bacteremia. The several types of reaction observed in this group of animals are shown in Tables III and IV.

A number of dogs died within 2 days of a fulminating infection. The blood of about half of these showed retention of pneumococcidal-promoting power 20 to 24 hours after the inception of the infection, despite the presence of circulating pneumococci in considerable numbers. However, when bacteremia was extreme, the immune properties disappeared (1-52S). In dogs surviving from 3 to 7 days three general types of response were observed. First, a limited bacteremia with retention of immune bodies, examples of which are dogs 1-32S and IV (Table III). Second, marked bacteremia with persistence of humoral immunity; 1-26S and 2-0. This phenomenon was observed earlier by Terrell (9). Third, marked bacteremia with loss of pneumococcidal-promoting power of the serum; 2-02S, 1-28S, and 5-8S. It is of especial interest to note in these two last mentioned dogs (Table III), that despite the loss

TABLE IV

Sequence of Bacteremia and Loss of Natural Immune Substances

Dog. No.	Dose and site		Before infection	Hours and days of disease			Outcome
				5 hrs.	24 hrs.	2 days	
2-57S	5 cc.	Bld. cult. col.....		8	16	2,080	Died 3 days
	R. L.	Nat. imm. sub.....	10^4	10^4	10	0	
		W.B.C., *thousands*..	18.6	25.2	1.2	2.4	
2-87S	1 cc.	Bld. cult. col.....		1	8	1,000's	Died 5 days
	R. L.	Nat. imm. sub.....	10^5	10^4	10	0	
		W.B.C., *thousands*..	14.4	22.8	2.1	7.2	
2-69S	3 cc.	Bld. cult. col.....		12	1,000's		Died 30 hrs.
	L. L.	Nat. imm. sub.....	10^5	10^5	0		
		W.B.C., *thousands*..	15.2	12.1	0.3		
1-34T	1 cc.	Bld. cult. col.....		18	1,000's	1,000's	Died 3 days
	L. L.	Nat. imm. sub.....	10^5	10^5	0	0	
		W.B.C., *thousands*..	8.1	13.6	3.2	1.3	
2-06T	1 cc.	Bld. cult. col.....			81	54	Died 4 days
	L. L.	Nat. imm. sub.....	10^5		10^2	0	
		W.B.C., *thousands*..	8.2		8.4	8.8	

of demonstrable circulating antibodies the bacteremia of the first 3 days was of limited degree. This would suggest either that some factor other than the pneumococcus-killing power of the blood was operating to hold the bacteremia in check or that there was a residual concentration of natural immune bodies sufficient for this function even undetectable by the methods employed.

Sequential Relationship between Bacteremia and Loss of Pneumococcidal-Promoting Properties of Serum

Whenever we were fortunate in securing our blood samples at the optimum time, bacteremia was, with a single exception, found to precede the disappearance of pneumococcidal-promoting properties of the serum. Numerous examples

of this sequence are shown in Table IV and in Tables II and III (dogs 9-1S, 2-02S, and 5-8S). While not infrequently a blood sample exhibited what appeared to indicate simultaneous occurrence of bacteremia and loss of immune properties, yet it seems probable that another sample taken sometime during the preceding 24 hours would have shown changes analogous to those found in dogs 2-69S and 1-34T at 5 and 24 hours. The one exception noted above occurred in a dog which exhibited before infection an unusually low pneumococcidal-promoting activity of the serum. At the end of 24 hours neither immune properties nor bacteria were demonstrable in the blood. Blood culture the following day revealed a bacteremia.

Bacteremia in the Early Stages of the Disease

In order to determine how frequently bacteremia occurred early in the course of experimental infection the first blood cultures were made 5 to 6 hours after the initiation of the lesion in 94 dogs. The majority of these animals were infected with an inoculum which results in a mortality of 50 per cent or more. This time interval was chosen because in the average infection the lesion is growing rapidly after the lapse of 6 hours and shows a pronounced peripheral zone of edema-filled alveoli. Thirty-three or approximately one-third of the dogs exhibited a bacteremia at 6 hours. In all but 8 of these animals the blood invasion increased and the disease terminated in death. Of the 8 instances of early bacteremia in which the blood later became sterile, only two showed a disappearance of the blood invasion by 24 hours. Thus, in a series of almost 100 experimental pneumonias, early transient bacteremia was observed but rarely.

Functional Activity of the Leucocytes during the Disease

While the foregoing observations provide abundant and unquestionable evidence for the presence of free antigen (pneumococci) and antibody in the blood of many bacteremic dogs, the data thus far discussed provide no information as to whether such blood is capable of destroying the microorganisms. Earlier work would suggest that the phagocytic and digestive functions of the blood leucocytes are not impaired during infection even in the presence of marked bacteremia. However, it was felt that more complete information on this subject was desirable, so a number of tests of the functional activity of the leucocytes in bacteremic blood were carried out. The leucocytes were washed free from pneumococci and their own serum and were mixed with sera as indicated in Table V. It was found that the leucocytes from the blood of dogs showing even thousands of colonies and late in the course of the disease were quite as active, if not more so, than normal dog leucocytes in comparable numbers. Similar tests carried out in pneumonia patients yielded analogous results.

Pneumococcus-Killing Power of Whole Blood—Heparinized and Defibrinated

The decisive test to determine whether or not bacteremic blood exhibiting pneumococcidal-promoting properties in the serum was capable of destroying

TABLE V

Functional Activity of Leucocytes

		(*a*) Bacteremic dogs	
Dog 1-26S	30 colonies per cc.; 4th day of disease	Normal serum + bacteremic* leucocytes	= 10^5 pneumococci killed
		" " + normal "	= 10^3 pneumococci killed
		Bacteremic* " + " "	= 10 pneumococci killed
	1,000's colonies per cc., 6th day of disease	Normal serum + bacteremic leucocytes	= 10^4 pneumococci killed
		" " + normal "	= 10^3 pneumococci killed
		Bacteremic " + " "	= 10^2 pneumococci killed
Dog. 1-39S	35 colonies per cc.; ? day of disease	Normal serum + bacteremic leucocytes	= 10^4 pneumococci killed
		" " + normal "	= 10^3 pneumococci killed
		Bacteremic " + " "	= 0 pneumococci killed
		(*b*) Bacteremic case of human lobar pneumonia	
	990 colonies per cc.; 11th day of disease	Normal serum + bacteremic leucocytes	= 10^4 pneumococci killed
		" " + normal "	= 10^5 pneumococci killed
		Bacteremic " + " "	= 0 pneumococci killed
		" " + bacteremic "	= 0 pneumococci killed

Numbers of leucocytes kept constant in each test.

* Serum or leucocytes of bacteremic blood.

the contained microorganisms, consisted in adding heparin to, or defibrinating, samples of such blood to prevent clotting and incubating them in the same manner as the serum-leucocyte mixtures. The results of many such tests, an example of which is shown in Table VI, were striking. Blood containing hundreds to thousands of pneumococci was able to sterilize itself within 3 to 24

hours, provided an adequate number of leucocytes was present (more than 4,000 per c.mm.).[3] Furthermore, this action proceeded with astonishing rapidity.

One blood sample from dog 1-82T containing 10,000 pneumococci per cc., at the end of 15 minutes had reduced the number to 5,000 and thereafter cultures at frequent intervals showed rapid diminution in the number of pneumococci, until at 8 hours there were only 6 colonies left. The previous day the blood of the same animal not only cleared itself completely of 1,380 pneumococci within 3 hours, but also killed 10,000 added microorganisms.

On the other hand, bacteremic blood showing an early loss of antipneumococcal immune substances was incapable of sterilizing itself. Very occasional

TABLE VI

Pneumococcidal Action of Bacteremic Heparinized Blood

Dog No.	Day of disease	No. of pneumococci in 1 cc. of blood	Pneumococcidal-promoting action of serum	W.B.C.	Complete clearing of blood	*In vitro* tests on 1 cc. blood samples Reduction in No. of pneumococci		Outcome of disease
						From	To	
1-82T	3rd	1,380	10^5	10,600	+*	1,380	10 in 1 hr. 0 " 3 hrs.	
	4th	10,000	—‡	6,900	0	10,000	5,000 in 15 min. 3,000 " 30 " 800 " 1 hr. 80 " 2 hrs. 20 " 4 " 6 " 8 "	Died 5 days

* Cleared additional 10,000 pneumococci.

‡ Not done.

exceptions to this finding occurred in which bacteremic blood without demonstrable pneumococcidal-promoting properties in the serum, did sterilize itself. This phenomenon will be dealt with in the second paper of this series. Defibrination was found to be less satisfactory than the use of heparin since the number of leucocytes in leucopenic bloods was often considerably reduced by the former procedure.

That certain of these same phenomena occur in the human being with lobar pneumonia has been shown by Sutliff and Rhoades (8), who found that the bacteremic blood of some patients was capable not only of sterilizing itself *in vitro* but also could destroy many added pneumococci.

[3] In three animals with bacteremia of 8 to 25 colonies per cc. the blood cleared itself in the presence of a white blood count of only 2,200.

Interpretation

The problem of interpreting these observations appears at first sight very complex. However, the situation is clarified to a considerable degree by taking into account the time element in the reactions involved. Our tests give us a picture of a single instant in a constantly changing process. In an attempt to depict this process in a simplified form, we offer the accompanying diagram which shows the lung on one side of the blood stream and the general body tissues on the other (Fig. 1). In bacteremic states there is, in all probability a continuous liberation of pneumococci into the blood. Even with an adequate mechanism for disposing of these circulating microorganisms they probably

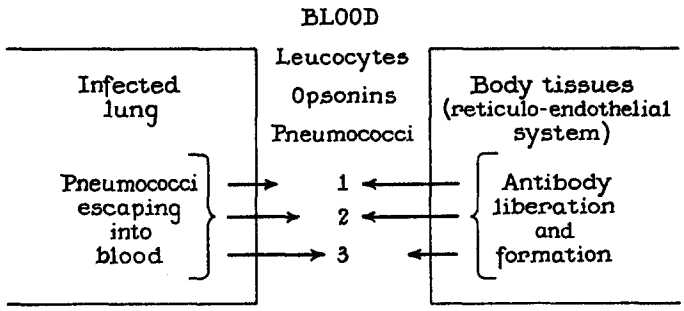

FIG. 1. Diagrammatic conception of varying relationships between circulating pneumococci and natural antibodies.

1 = limited bacteremia with persistence of circulating antibodies.
2 = marked " " " " " " .
3 = extreme " " disappearance of " " .

Length of arrows indicates magnitude of pneumococcus or antibody liberation into blood stream.

are not engulfed immediately by the phagocytes and destroyed, as this function requires time. Meanwhile more pneumococci are being poured into the blood. To keep pace with the absorption of the opsonins by the pneumococci, new opsonins must be elaborated. This process may go on very rapidly, as was found in several of our experimental animals in which the natural immune bodies reappeared in normal concentrations after being absent for some days.

It seems not unlikely that individual variations in the rapidity of formation of immune substances may account at least in part for the wide differences observed in this response to bacteremia. If only a moderate number of pneumococci are being liberated into the blood and the renewal of circulating immune bodies is kept up at a good rate, the bacteremia will be of minimal degree. With a greater outpouring of microorganisms into the blood, persistence of circulating immune substances may occur, provided the antibody formation goes on actively. Under such conditions, including a sufficient number of blood

phagocytes, the pneumococci may be destroyed almost as fast as they are liberated and a certain balance is maintained whereby the degree of bacteremia is limited. When very large numbers of pneumococci escape into the blood and the production of antibody is less than normal or reduced as a result of the infectious process, the immune bodies disappear and bacteremia may become extreme. Discussion of other non-measurable factors which presumably play a role in the control of pneumococcemia in the dog will be taken up in the following paper.

SUMMARY

In a study of the relationship of natural antipneumococcal immune substances to the incidence and course of bacteremia in dogs with experimental pneumococcus pneumonia the following findings came to light: (1) In non-bacteremic animals, natural immune substances, as measured by the pneumococcidal-promoting action of the serum, continue to be present in relatively undiminished concentration throughout the course of the infection. (2) With the advent of bacteremia these immune properties of the blood tend to decrease or disappear, depending on the degree of bacteremia and the length of the disease course, but in certain instances they persist despite the presence of large numbers of circulating pneumococci. (3) Disappearance of natural immune substances from the blood during bacteremia is followed by their reappearance upon cessation of the bacteremia. (4) Bacteremic blood containing antipneumococcal immune substances and a sufficient quantity of leucocytes is capable of destroying *in vitro* relatively large numbers of pneumococci and will often sterilize itself. (5) The sequence of bacteremia first, then diminution and disappearance of humoral immunity excludes this antipneumococcal action of the blood as being the principal inhibitor of blood invasion.

These observations have been interpreted as indicating that the bacteremic state consists of a constant escape of pneumococci from the pulmonary lesion and an attempt on the part of the body to compensate for the depletion of circulating immune substances resulting from their progressive immobilization by the pneumococci and their products. Thus, the loss or retention of humoral immune substances in the presence of bacteremia would appear to depend on the rate at which the body can provide new supplies of antibodies and on the number of pneumococci being discharged into the circulation. While the pneumococcidal action of the blood may not be sufficient to prevent the occurrence of bacteremia our study provides ample evidence that it exerts a potent restraining effect on the increase in numbers of pneumococci in the circulation.

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