THE RELATIONSHIP OF INFECTING DOSAGE, LEUCOCYTIC RESPONSE, BACTEREMIA, AND EXTENT OF PULMONARY INVOLVEMENT TO THE OUTCOME OF EXPERIMENTAL LOBAR PNEUMONIA IN THE DOG

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Since our original description of pneumococcus lobar pneumonia in the dog published in 1933 (1), we have made a large number of observations on this experimental disease. The findings we wish to present here deal with certain factors which influence the course and outcome of the infection. The most important of these is the size and distribution of the infecting dose. Our earlier studies indicated that within a limited range of dosage a definite relationship existed between the amount of culture inoculum and the outcome of the disease. It was found that inocula containing 0.03 cc. or less of culture uncommonly produced death. With doses ranging from 0.04 cc. to 0.1 cc. the mortality rose progressively from 25 per cent to approximately 60 per cent. However, a further increase in the size of the infecting dose even to 0.6 cc. or 1 cc. of culture, always suspended in 1 cc. of the starch broth medium, resulted in no higher death rate (Table I). This problem has been investigated further and by varying the methods of initiating the infection as detailed below we have been able to secure many additional data on the relationship of dosage to outcome.

In the initial studies it was also recognized that early leucopenia, bacteremia and extensive pulmonary involvement were unfavorable prognostic signs, but the fewness of the data did not permit a quantitative correlation between these manifestations of the body's reaction and eventual recovery or death. The present observations on dogs undergoing their primary infection have provided material for the more satisfactory elucidation of such relationships.

Methods and Materials

The technique of producing experimental pneumococcus lobar pneumonia in the dog has been described in an earlier communication (1). Briefly the infecting dose consisting of a 16 hour pneumococcus culture separated from the culture fluid, was mixed with 1 cc. of a 6 per cent starch broth medium and injected through a radio-opaque catheter into a terminal bronchus with the dog under the fluoroscope. We have used the same strain of Type I (A^5) pneumococcus throughout. It has been passed through a rabbit approximately every 6 weeks and maintains its virulence for mice; ten pneumococci kill a mouse in 24 hours. The dogs used were of various breeds and ranged in size from 8 to 16 kilos. Within this range we have not detected any significant variation in response to a given dose. Smaller dogs, however, appear to stand large doses of culture less well and very large dogs seem to be more resistant. The dogs were debarked and kept for 2 weeks before infection. During this time they were given cod liver oil with their food and usually gained weight. Only young dogs in good condition with a normal temperature (below 103°F.) were used. A white blood count was made before infection and at 24 hours, x-ray, blood culture and a white count were taken. These two latter observations were repeated daily in most instances. In a number of cases daily x-rays were made, in others every other day. By this means, the maximum extent of the pulmonary involvement was probably rarely missed.

The length of the course of the disease was estimated as nearly as possible in terms of elapsed hours since its inception. In many instances, however, in which the dog died during the night or showed a normal morning temperature, an error of ± 7 to 8 hours was unavoidable.

The degree of pulmonary involvement in the recovering dogs was estimated in terms of the fraction of the total lung field occupied by the x-ray shadow. This method has been found to be more useful than attempting to designate lobes involved from the x-ray appearance. While a comparison of films taken just before death with the lesions found at autopsy has shown that usually one can tell from the x-ray which lobes are involved, there occur not infrequently variations in the size and shape of the several lobes which alter the x-ray shadow considerably. The tabulated pulmonary involvement in the animals which succumbed to infection represents the extent of the lesion found at autopsy. This was usually more than seen in the last x-ray taken because of late spread of the process. However, when death occurred shortly after the final x-ray there was a surprisingly close correspondence between the amount of lung involved as indicated by the film and the actual extent of the lesion seen at post mortem.

EXPERIMENTAL

The Growth of Pneumococci in the Starch-Broth Medium

In an attempt to explain the lack of correspondence between progressively increasing doses of pneumococci and rising mortality rate beyond a certain level, a study was made of the rate of multiplication of pneumococci in the 6 per cent

starch-broth medium used for inoculation. Varying quantities of pneumococci, centrifuged and resuspended in 0.1 per cent gelatin Locke's solution at pH 7.4, were thoroughly mixed in the starch medium and incubated at 37°C. At frequent intervals up to 6 hours samples were removed and plated out for colony counts. Where the initial seeding amounted to less than 0.1 cc. of culture per cc. of starch, the pneumococci were found to multiply rapidly for 4 hours, after which time the numbers tended to diminish. An initial seeding of 0.1 cc. of culture per cc. of starch resulted in little or no further growth of pneumococci. With heavier seedings there was not only no increase in the number of pneumococci, but an actual diminution was found after 2 hours of incubation, which progressed during the period of observation.[1]

Since starch can be seen to persist in the lesion of experimental pneumonia for from 3 to 6 hours after its inception, it seems probable that for a limited period of time many of the pneumococci in the lung are subject to the same conditions as those present in the test tube experiments.

The Effect on Mortality of Increasing the Amount of Starch-Broth Medium

In the light of the growth behavior of pneumococci in the starch-broth medium it seemed not improbable that one method of enhancing the effective dose of pneumococci would be to increase the volume of the starch inoculum, injected either into a single site or in multiple sites in the lung. For the purpose of comparison a dose which produced a low mortality with the 1 cc. starch inoculum was selected. The difference in mortality resulting from this alteration in the method in implanting the infecting dose was striking. It was found that 0.01 to 0.02 cc. of culture, suspended in 3 to 6 cc. of starch-broth medium and injected in one to three sites resulted in a mortality of 65 per cent, whereas the same dose in 1 cc. of the medium caused death in only 9 per cent of the dogs (Tables I, II and III). Furthermore, 0.001 cc. of culture, which when suspended in 1 cc. of starch resulted regularly in recovery and in two instances out of ten failed to cause infection, when contained in 6 cc. of the medium produced

[1] Dr. T. E. Friedemann in a personal communication explains this phenomenon as due to the rapid consumption of sugar available in the medium which stops growth of the microorganisms and brings about conditions favorable to their autolysis.

death in one of six dogs. With increasing doses the death rate gradually rose to 80 per cent with 0.6 cc. of culture (Table II). The

TABLE I

Summary of Dosage and Outcome in Dogs Infected with Pneumococci Suspended in 1 Cc. of the Starch Mixture

Infecting dose of culture	Number of dogs	Occurrence of bacteremia		Mortality			
				With bacteremia		Total	
		Number of dogs	Per cent	Number of dogs	Per cent	Number of dogs	Per cent
cc.							
1.0	6	3	50	3	100	3	50
0.25–0.6	25	19	76	14	74	15	60
0.04–0.1	38	15	40	12	80	16	42
0.01–0.03	60	11	18	4	36	5	8
0.001*	10	0	0	0	0	0	0
0.000001*–0.0001	3	0	0	0	0	0	0

Total number of dogs = 142.

For the sake of comparison of 1 cc. and 6 cc. starch inocula the different doses are grouped as shown in table.

* Two dogs given 0.001 cc. and one given 0.000001 cc. showed no signs of infection.

TABLE II

*Summary of Dosage and Outcome in Dogs Infected with Pneumococci Suspended in 6 Cc. of the Starch Mixture**

Infecting dose of culture	Number of dogs	Occurrence of bacteremia		Mortality			
				With bacteremia		Total	
		Number of dogs	Per cent	Number of dogs	Per cent	Number of dogs	Per cent
cc.							
0.6	15	12	80	12	100	12	80
0.2 –0.3	11	8	73	8	100	8	73
0.05–0.1	8	6	75	6	100	6	75
0.01–0.02	43	29	67	25	86	28	65
0.001	6	1	16	1	100	1	16

Total number of dogs = 83.

* A few of these dogs received a 3 to 4 cc. starch inoculum.

dosage was not carried beyond this point since it was felt that single inocula of more than 3 cc. might cause pressure trauma, and further-

more it is not easy to implant the infecting dose into more than three separate sites. 0.1 cc. of culture per cc. of starch was the upper limit of the concentration of pneumococci employed for infection. While the mortality per cent would probably be altered by a larger number of animals in most of the dosage series, the difference between the 1 cc. and the 6 cc. starch inocula is quite definite.

The Comparative Effect of Volume and Distribution of the Starch Inoculum with a Constant Infecting Dose of Pneumococci

In order to determine whether the greatly increased mortality produced by the use of a large starch-broth inoculum (as compared with

TABLE III

Effect of Volume and Distribution of Starch Inoculum on Outcome of Infection with a Constant Infecting Dose (0.01 to 0.02 Cc. of Culture)

Volume and distribution of starch-broth inoculum	Number of dogs	Average number of white blood cells at 24 hrs.		Occurrence of bacteremia		Mortality				Average duration of disease in dogs dying
						With bacteremia		Total		
		Dogs dying	Dogs surviving	Number of dogs	Per cent	Number of dogs	Per cent	Number of dogs	Per cent	
										hrs.
1 cc. in 1 site	57	2,200	16,200	10	18	4	**40**	5	**9**	92
3–6 cc. in 1 site	8	6,000	8,000	6	75	5	**83**	5	**62**	97
3–6 cc. in 3 sites in 1 lobe	17	2,540	6,500	10	60	9	**90**	11	**65**	57
4–6 cc. in 2 sites in 1 lobe	10	1,270	8,900	7	70	5	**71**	6	**60**	33
4–6 cc. in 2 sites one in each lower lobe	8	3,400	8,800	6	75	6	**100**	6	**75**	51

the 1 cc. amount) was due to the more extensive area of lung tissue initially infected or resulted principally from the production of multiple foci of infection, observations on the effect of varying the volume and distribution of the inoculum were made as shown in Table III. The great majority of animals listed under 3 to 6 cc. starch inoculum received 6 cc. of the starch medium. There did not appear to be much difference between the use of 3 cc. and 6 cc. although in the small series infected with a 3 cc. inoculum the mortality seemed to be a little lower. Since it was not feasible to carry out a large series

of tests comparing the relative effects of these two inocula, our efforts were principally directed to obtaining information on the influence of focal distribution of the infecting dose. The resulting mortality from 0.01 to 0.02 cc. of culture suspended in 6 cc. of starch was about the same, 60 to 65 per cent, whether the total dose was injected into a single site or divided between two or three sites on the same lobe. Dogs receiving bilateral infections showed a somewhat higher mortality, but a considerably larger series would have to be secured before we could be sure of this fact. While the data are admittedly few in number they do suggest that the difference in mortality between the 1 cc. and the 6 cc. starch inocula, is to be accounted for principally by the total amount of lung tissue initially infected. In harmony with this inference is the finding of extensive pulmonary involvement by the end of 18 to 24 hours in most of the dogs injected with the larger starch mass. Eighteen of the 43 dogs so infected showed involvement of ½ of more of the total lung field within 24 hours of the inception of the disease. All these animals died. Only six dogs showed the lesion confined to a single lobe at the end of this time. Whereas among the 57 animals receiving the same dose (0.01 to 0.02 cc.) in 1 cc. of starch, 47 exhibited involvement of a single lobe or less at 24 hours.

The Production of Lobar Pneumonia by the Injection of Pneumococci Suspended in Gelatin-Locke's Solution Followed by Mucin

In order to undertake certain problems which have arisen during our studies on experimental pneumonia, such as determining the degree of immunity resulting from an attack of the disease, estimation of the value of therapeutic procedures, etc., it has become increasingly important to produce if possible an infection which would be uniformly fatal in the normal animal. Our extensive observations on dogs indicate that this species exhibits great individual variability in resistance to pneumococcus infection. Even with the maximum dose of starch-suspended pneumococci which we felt was desirable to employ, *i.e.* 0.6 cc., 20 per cent of the infected dogs survived (Table II).

The simplest method of increasing the effective concentration of pneumococci locally was found to consist in the injection of microorganisms suspended in a physiological solution such as 0.1 per cent gelatin-Locke's followed by mucin to prevent their expulsion through the bronchus (2). The pneumococci were sepa-

rated from their culture fluid by centrifugation in order to eliminate the variable factor of the soluble specific carbohydrate and resuspended in 1 cc. gelatin-Locke's solution, pH 7.4. If the amount of culture exceeded 1 cc. it was suspended in a quantity of solution equivalent to the volume of the culture. Sterile animal mucin was prepared by the method of Nungester and Jourdonais (3) and made up with a degree of viscosity which just permitted its suction through a 16 gauge needle into a 20 or 30 cc. syringe. Before implantation of the infecting dose the head end of the board was elevated 10 inches to prevent escape of the injected fluid. With slow injection under very little pressure as much as 5 cc. of fluid could be given with no apparent trauma. Before moving the catheter, 1 cc. of air was injected to expel the fluid and this was followed by 2 cc. of mucin, 1 cc. to fill

TABLE IV

Summary of Dosage and Outcome in Dogs Infected with Pneumococci Suspended in Gelatin-Locke's Solution Followed by Mucin

Infecting dose of culture	Number of dogs	Occurrence of bacteremia		Mortality			
				With bacteremia		Total	
		Number of dogs	Per cent	Number of dogs	Per cent	Number of dogs	Per cent
cc.							
5	10	10	100	10	100	10	100
3	10	10	100	10	100	10	100
1	29	24	83	24	100	24	83
0.25–0.5	8	7	88	6	86	6	75

Total number of dogs = 57.

the catheter and 1 cc. to plug the bronchus at the injection site. The catheter was then withdrawn about an inch and another cc. of mucin injected. This was followed by a third cc. after withdrawing the catheter about the same distance a second time.

The results of infection produced in this manner are shown in Table IV. Of eight dogs infected with 0.25 cc. to 0.5 cc. of culture six died, a mortality not much different from the same dose given in starch. However, when the dose was increased to 1 cc. the death rate increased. Only five out of 29 dogs survived, a mortality of 83 per cent. With doses of 3 to 5 cc. all the dogs died, usually within 24 hours. What effect the mucin may have in enhancing the invasiveness of the pneumococci as found by Nungester and Jourdonais (3) in the rat we have not determined. Our results would suggest that this action of the mucin plays a less conspicuous rôle in the dog than it does with the

rat, and that the number of pneumococci is the determining factor in outcome.

The Course of Pneumonia in Dogs Infected during Warm Weather

In the early phases of the study of experimental canine pneumonia observations on a few animals infected in the summer led to the impression that the dog's resistance to infection was higher during warm

TABLE V

Summary of Dosage and Outcome in Dogs Infected during Warm Weather

Summer of years	Infecting dose of culture	Amount of starch inoculum	Number of dogs	Occurrence of bacteremia		Mortality		Mortality of dogs infected in cool weather	
				Number of dogs	Per cent	Number of dogs	Per cent	Per cent	Number of dogs
	cc.	*cc.*							
1932	0.25	1	8	2	25	2	25	64	14*
1934									
1936†	0.25	1	3	3	100	3	100	64	14*
1937	0.3	6	6	6	100	6	100	71	7
	0.02–0.03	1	8	7	84	6	75	8	50‡
	0.001	1	18	5	28	6	33	0	10
1938	0.02	1	11	0	0	0	0	8	50‡

Total number of dogs = 54.

* Same series.

† Number of dogs equally divided between 2 years.

‡ Same series.

weather than it was in the cool and cold months of the year. Since that time several series of summer infections have been carried out with the results shown in Table V. During the summers of 1936 and 1937 the mortality among 32 dogs was considerably higher than that of animals infected with similar doses during the winter months when conditions of temperature and humidity were practically constant at 70–75°F. and 30 to 35 per cent humidity. On the other hand, infections in the summer of 1938 were unusually mild. We have no explanation to offer for these variations in the severity of the experimental disease occurring in different years. No significant differences in temperature or humidity were observed between sum-

mers, nor does it seem likely that change in virulence of the infecting microorganism is responsible. While we cannot exclude alterations in virulence of this strain of pneumococcus for the dog no fluctuations in its virulence for mice and rabbits have been observed. We wish merely to point out at this time these observed seasonal variations of the dog's response to experimental pneumococcus infection of the lung. Of possible significance may be the finding that 20 out of 21 summer infected dogs with bacteremia, died.

Relationships between White Count, Bacteremia and Mortality

A large number of observations on the fluctuation of the number of circulating white blood cells during the course of experimental pneumonia in the dog revealed the fact that the count made at 24 hours after the inception of infection was of greater prognostic import than were those made at subsequent time intervals. When leucopenia occurred it was almost always found to be maximum at 24 hours. Even in animals showing a profound lowering of the white blood cells at this time, there was a tendency toward a later increase provided the animal lived more than 36 hours. The number of circulating leucocytes in the supposedly normal dog has been found to vary between 5,000 and 25,000. 70 per cent of the white blood counts on 400 normal dogs ranged between 10,000 and 18,000, and 80 per cent between 8,000 and 20,000. Just where to place the upper limit of normal we do not know. However, when the count has exceeded 25,000 we have considered that the animal was probably not normal and have not used such a dog until its count fell below this figure.

With small doses of culture in 1 cc. of starch which usually resulted in the involvement of only a single lobe, depression of the white blood cells below 5,000 was infrequently observed. The majority of such infections was characterized by relative leucocytosis at 24 hours, which ranged from 15,000 to 40,000 white blood cells. The leucocytosis was usually maintained during the course of the infection, then fell rapidly to normal with recovery. When the data on 300 dogs were arranged as shown in Table VI a striking relationship between the numbers of circulating white blood cells and the outcome of the disease was revealed. Of 45 dogs showing a count of more than 20,000 at 24 hours after the inception of infection only three or 7 per cent died. 70 animals with counts between 10,000 and 20,000 showed a mortality

of 17 per cent. As the white count decreased, the mortality rose progressively to over 90 per cent in dogs with a count of less than 2,000.

Another conspicuous relationship shown in the table is that existing between the incidence of bacteremia and the number of leucocytes. Bacteremia was detected least often in dogs with a white count of more than 20,000, only 13 per cent; but as the numbers of white blood cells diminished, invasion of the blood stream became more and more common until it was present in 91 per cent of dogs with a count of less

TABLE VI

*Relationship between White Count, Bacteremia and Mortality in Experimental Canine Pneumonia**

White count 24 hrs. after infection†	No. of dogs	Occurrence of bacteremia				Mortality					Average duration of disease in dogs dying
		At 24 hrs.		Eventual‡		With bacteremia		Without bacteremia	Total		
		No. of dogs	Per cent	No. of dogs	Per cent	No. of dogs	Per cent	Per cent	No. of dogs	Per cent	
											hrs.
Over 20,000	45	3	7	6	13	3	50	0	3	7	87
10,000–20,000	70	11	15	17	24	12	70	0	12	17	64
5,000–10,000	44	12	27	19	43	13	70	8	15	34	117
2,000– 5,000	84	42	50	51	61	45	88	6	48	57	55
Below 2,000	57	33	58	53	93	52	98	0	52	91	50

Total number of dogs = 300.

* Dogs infected with varying doses of pneumococci Type I, and with varying amounts of starch inoculum.

† Dogs dying before 24 hours not included because of lack of data.

‡ Includes all dogs showing bacteremia at any time during disease.

than 2,000. All but one of 33 dogs showing bacteremia and a white count of less than 2,000 at 24 hours died. The death rate among non-bacteremic dogs was low even in the leucopenia group; 6 per cent in dogs with white counts of 2,000 to 5,000 as contrasted with an 88 per cent mortality among bacteremic animals. Of the five survivors among 57 dogs with white counts of less than 2,000 only one showed invasion of the blood stream.

Relationship between Degree of Bacteremia and Mortality

As might be expected, the greater the degree of bacteremia the higher the mortality rate (Table VII, column 7). Furthermore, dogs

showing blood invasion at 24 hours were more likely to die than were those which developed bacteremia at a later stage in the disease. This difference is most striking among those animals showing a relatively slight blood invasion of 1 to 20 colonies per cc. 74 per cent of those with pneumococci in the blood at 24 hours died, while the mortality in the dogs developing bacteremia subsequent to this time was only 56 per cent. Bacteremias of more than 100 colonies at 24 hours were always fatal whereas three out of eighteen animals in which the

TABLE VII

*Relationship of Degree of Bacteremia to Outcome of Infection**

Colonies of pneumococci per 1 cc. of blood	Outcome based on bacteremia at 24 hrs.			Outcome based on maximum bacteremia recorded during life		
	Number of dogs	Mortality		Number of dogs	Mortality	
		Number of dogs	Per cent		Number of dogs	Per cent
None	223	43	20	192	20† (8)	10† (4)
1–20 colonies	54	40	74	39	22	56
21–100 "	11	9	82	11	9	82
101–1,000 "	13	13	100	23	20	87
Over 1,000 "	30	30	100	65	65	100

Total dogs = 331.

Difference between number of dogs at 24 hours and at maximum at any level due to large number of dogs showing increasing number of colonies after 24 hours.

* As recorded during life only.

† Of these 20 dogs, 12 showed positive blood culture of undetermined degree at autopsy, although none were found during life. The corrected non-bacteremic death rate would then be only 4 per cent.

numbers of pneumococci in the blood gradually increased to a maximum of 101 to 800 colonies, survived. When the number of bacteria in the blood exceeded 1,000 colonies death invariably ensued, although some of the animals lived for several days with this condition.

Relationship between Extent of Pulmonary Involvement and Outcome, Bacteremia and White Blood Count

A striking correlation was found to exist between the extent of the pulmonary involvement and the death rate (Table VIII, column 10).

Of 104 dogs showing a lesion confined to approximately 1/6 of the total lung field (1 lobe or less) and no complications, only one died, a mortality of less than 1 per cent. Two other dogs died with a single lobe involved but one had empyema, and the other, pericarditis. With involvement of 1/3 of the lung field (usually 2 lobes) the death rate in 66 dogs was 24 per cent. Lesions comprising about half of the lung resulted in a much higher mortality, 75 per cent in 106 animals, while

TABLE VIII

Relationship between Extent of Lung Involvement and Outcome, White Blood Count and Bacteremia

Fraction of total lung field involved (x-ray or autopsy)	Number of dogs	Average white count at 24 hrs. after infection		Occurrence of bacteremia		Mortality				Average duration of disease in dogs dying
						With bacteremia		Total		
		Dogs dying	Dogs surviving	Number of dogs	Per cent	Number of dogs	Per cent	Number of dogs	Per cent	
										hrs.
Empyema and/or pericarditis	25	6,000	—	25	100	25	**100**	25	**100**	123
3/4±	47	3,560	5,650 (1 dog)	45	96	45	**100**	46	**98**	51
1/2±	106	2,250	6,700	81	76	79	**98**	80	**75**	46
1/3±	66	3,800	10,100	27	41	14	**52**	16	**24**	59
1/6±	104	300 (1 dog)	17,800	8	8	1	**12**	1	**1**	30 (1 dog)

Total number of dogs = 348.

the death rate in animals with involvement of 3/4 of the lung tissue was almost 100 per cent; only one out of 42 such dogs survived. The mortality in dogs with bilateral lesions did not appear to be higher than that occurring in animals showing a similar extent of consolidation on one side.[2] The development of empyema with or without pericarditis was always followed by death.

As might be surmised from the parallelism between death rate and increasing pulmonary involvement, the incidence of bacteremia was

[2] This statement applies only to those animals in which the pathological process spread to the opposite side during the course of the infection.

found to rise progressively with the expanding lesion (Table VIII, column 6); from 8 per cent in dogs with single lobe consolidations to 96 per cent in those with ¾ of the lung field involved.

The average white blood counts at 24 hours were highest in the dogs with the smallest lesions. Among the fatally terminating group the highest counts were found in those animals dying with empyema. This finding is not inconsistent with the observed relationship between extent of pulmonary involvement and white blood count, since quite

TABLE IX

Relationship of Infecting Dosage to Extent of Eventual Lung Involvement in Dogs Infected with Pneumococci Suspended in Starch Inoculum

Amount and distribution of starch inoculum	Infecting dose of culture	Number of dogs	Average extent of eventual lung involvement (Fraction of lung field)	Mortality	
				Number of dogs	Per cent
	cc.				
1 cc. of starch in one site	1	6	1/2	3	50
	0.25–0.6	25	1/2	15	60
	0.04–0.1	38	1/3+	16	42
	0.01–0.03	60	1/4	5	8
	0.001 or less	13	1/4−	0	0
6 cc. of starch in single and multiple sites	0.01–0.02	43	1/2	28	65

a number of these animals showed a limited lesion at the end of 24 hours. It will be noted in Table VIII that dogs with empyema or pericarditis exhibited the longest duration of the fatally terminating infections.

Relationship between Size of Infecting Dose and Extent of Lung Involvement

Since the mortality rate was found to be influenced by both dosage and extent of pulmonary involvement, the inference would be that increasing the infecting dose results in more widespread pneumonia. That such a direct relationship exists, is shown in Table IX. This effect of dosage is brought out more clearly in the dogs infected with a 1 cc. starch inoculum than is the case with the larger amounts of starch.

Infecting doses not exceeding 0.001 cc. resulted in pulmonary involvement of less than ¼ the lung field and no mortality. Doses of 0.01 to 0.03 produced slightly larger lesions and an attendant 8 per cent mortality. With larger amounts of culture the average extent of the involvement increased to ½ the lung field and the mortality rose to over 60 per cent.

It will be noted in the table that an infecting dose of 0.01 cc. to 0.02 cc., when suspended in 6 cc. of starch, produced twice the extent of pulmonary involvement resulting from the same dose injected in 1 cc. of starch and eight times the mortality.

Mixed Infections

Contaminating microorganisms were uncommonly found in the diseased lungs when the autopsy was performed within 2 to 3 hours after death. However, if the dog had died sometime during the night bacteria other than pneumococci were not infrequently grown from cultures of the pulmonary lesions. Gram-negative bacilli of the *bronchisepticus* group were the most frequent contaminants encountered. Staphylococci, Gram-positive rods, and streptococci, both hemolytic and non-hemolytic, were also found. The heart's blood seldom showed the presence of secondary invaders, although several animals died with hemolytic streptococcus bacteremia.

DISCUSSION

The results of the present study indicate that the relationship of dosage to outcome in experimental pneumonia in the dog is in general the same as that of pneumococcus infection in other sites and in other animals, *i.e.*, infections ranging in severity from uniform recovery or low mortality to constant fatal outcome could be produced by varying the amount of culture injected into the lung. However, infection in this locus (at least in the dog) can be produced with very much smaller quantities of pneumococcus culture than is required for the initiation of lesions in the subcutaneous tissues or blood stream. No attempt was made to determine the minimum infecting dose in view of the wide individual variations in antipneumococcal resistance exhibited by the dog. The explanation of the striking effect on mortality produced by simply increasing the amount of the starch in which the

pneumococci are suspended is not altogether clear. It seems not improbable that the chief difference between the large and small starch inocula lies in the number of alveoli into which the infecting dose is implanted. With the larger amounts of starch a greater proportion of pneumococci are placed in the most advantageous position to produce infection and fewer are lost by elimination through the bronchi. Other effects of a widespread early inflammatory reaction may also play a part in accentuating the severity of the disease process.

Examination of the data on number of circulating leucocytes, bacteremia and extent of pulmonary involvement brings out the fact that in many instances the outcome of the disease can be predicted with a fair degree of probability by the end of 24 hours. Evidence of the body's success or failure to control the infection is most clearly expressed by marked changes in the white blood count at this time. With counts of more than 20,000 the mortality was very low, only three out of 45 dogs died. Absence of bacteremia and a lesion confined to a single lobe at this early period are of less prognostic importance since invasion of the blood stream and extension of the lesion may occur at a later period, as was the case in the three fatalities in this group. Equally indicative of the outcome is a profound leucopenia. 91 per cent of 57 dogs with 24 hour white counts of less than 2,000 died. Only 58 per cent of these animals showed a bacteremia at this stage. The presence of marked bacteremia or a pulmonary lesion occupying ½ the lung field at 24 hours also pointed to an unfavorable prognosis. Many of the dogs showing either one or both these conditions are included in the group of 57 with less than 2,000 white blood cells.

After the first 24 hours of the disease the most significant indication of outcome is obtained from blood culture. Even in the presence of a leucopenia and extensive consolidation the animal may survive provided the blood remains sterile. In the group of dogs showing early white blood counts of less than 5,000 the mortality with blood invasion was fifteen times that of dogs without bacteremia. With involvement of approximately one-half the lung field the death rate in bacteremic animals was twenty-four times that of the non-bacteremic.

Two of the three non-bacteremic survivors in the group with less than 2,000 white blood cells at 24 hours showed consolidation of one-half the lung.

A detailed comparison of these observations in dogs with similar changes occurring in clinical lobar pneumonia is not possible since analogous data on the human disease do not exist. One of the principal differences between this study of experimental pneumonia in the dog and that of any group of human cases lies in the fact that the canine disease represents the dog's reaction to a single strain of pneumococcus of relatively unchanging virulence. From what we know of the widely varying response of laboratory animals to infection with different strains and types of pneumococci we may assume that the pathogenicity of the strain producing the pneumonia will affect the reaction of the human patient to a considerable degree. Nothing is known concerning the possible relationship of dosage to inception or outcome of pneumonia in the human being.[3] Furthermore, there is relatively little recorded laboratory data on the very early stages of clinical pneumonia.

Aside from these considerations there are certain quite definite similarities in the courses of the human and canine diseases. These are most strikingly apparent in the relationship of extending pulmonary lesion and bacteremia to mortality (5–7). The most complete study of human lobar pneumonia for purposes of comparison with our observations is that by Tilghman and Finland (8). These authors found that the mortality rose progressively from 35 per cent with consolidation of a single lobe to 77 per cent with four or more lobes involved and the incidence of bacteremia increased with the extension of the lung lesion. In cases showing a white count of less than 5,000 the incidence of bacteremia was much higher and the mortality considerably greater than when the count was 10,000 to 25,000. The difference in mortality between bacteremic and non-bacteremic patients was not as marked as was found in canine pneumonia. Neither does a high white count early in the human case carry such a good prognosis as it appears to do in the dog. Extra-

[3] This point has been discussed in a previous publication (4).

pulmonary complications are less diversified in the dog, being confined principally to empyema and pericarditis. Endocarditis was not observed.

The chief difference between the two diseases is that of duration. The average length of the disease course in recovering dogs was a little over 3 days. In the fatal infections the duration appeared to depend largely on the size of the infecting dose. With doses producing a mortality of 60 per cent or over, the average was less than 2 days and a half, while with doses resulting in a mortality of 20 per cent or less, the disease lasted on an average of almost 4 days.

SUMMARY

The present study comprises observations on the course and outcome of experimentally produced Type I pneumococcus lobar pneumonia in dogs. It was found that within certain limits of dosage the outcome of the disease was closely related to the amount of culture and the volume of the starch inoculum employed. The much higher mortality rate resulting from simply increasing the volume of starch in which the pneumococci were suspended, appeared to be due chiefly to the greater area of lung tissue initially involved. Certain striking relationships were observed to exist between the leucocytic response, extent of pulmonary involvement, bacteremia and outcome. Marked changes in the number of white blood cells at end of the first 24 hours of the disease provided more valuable prognostic information than either the blood culture or the size of the lesion. Dogs showing high white counts at this stage seldom succumbed, while absence of leucocytosis and leucopenia were associated with a mortality which increased with the diminishing numbers of circulating white blood cells. Over 90 per cent of the animals with counts of less than 2,000 cells per c. mm. died. The extent of the pulmonary lesion was likewise observed to bear a direct relationship to death rate. With the lesion confined to a single lobe the mortality was only 1 per cent. Spread to other lobes was accompanied by a sharply rising death rate until it reached 98 per cent in dogs with three-fourths the lung field involved. Increasing occurrence of bacteremia paralleled both the decreasing white counts and the extending pulmonary lesions and subsequent to the 24 hour stage of the disease the presence or absence of pneumococci

in the blood appears to be of more significance, in relation to outcome, than either of these two other factors. Even in the presence of leucopenia and an extensive lesion the animal might survive provided the blood remained sterile.

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