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Research Article

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STUDIES ON THE PATHOGENESIS OF MENINGITIS. I. INTRATHECAL INFECTION *

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The manner in which bacterial pathogens produce infection within the subarachnoid space is poorly understood. Even means of entry, rate of growth, and mode of dissemination of bacteria are unclear. At cellular levels the action of bacteria upon the exchange of normal and abnormal metabolites between blood and cerebrospinal fluid (CSF), effect upon enzyme systems, and mechanisms of death are even more obscure. One of the difficulties in answering some of these questions stems in part from failure to have at hand a reproducible experimental model. The present experiments describe meningitis produced in dogs with a single pathogen, the pneumococcus. They evaluate the effect of the size of the bacterial inoculum, route of infection, role of bacteremia, and certain acute alterations in host resistance on the course of infection. In subsequent publications the relationship of alterations in CSF dynamics during bacteremia to the development of meningitis, mechanism of hypoglycorrhachia, and effect of antimicrobials will be described.

The first attempts to produce experimental pneumococcal meningitis were those of Lamar (1). He inoculated pneumococci into the lumbar subarachnoid space of monkeys and found remarkable similarity between simian and human infection. He also noted different degrees of susceptibility among individual animals. Bull's (2) claim that intravenous administration of pneumococci to dogs was followed by meningitis was not substantiated by Idzumi (3) and Weed, Wegeforth, Ayer and Felton (4). Idzumi found that dogs were resistant to pneumococcal infection of the meninges even when organisms were introduced directly into the subarachnoid space. Despite this, Stewart preferred dogs to rabbits because the latter were unduly susceptible to the pneumococcus and developed fatal septicemia before meningitis became apparent (5, 6). He found that dogs regularly developed meningitis after intrathecal administration of a 6-hour culture of type I pneumococci. Three forms of infection resulted: *1*) minimal exudative reaction with large numbers of bacteria in the meninges and death from overwhelming bacteremia, *2*) a large number of inflammatory cells with few bacteria, and *3*) an enormous number of pneumococci in the meninges associated with a thick layer of gelatinous, purulent exudate over the dorsal and basal surfaces of the brain. A massive accumulation of pus in the central canal of the spinal cord coupled with severe myelitis was often seen in the last group (7). With the exception of the cord lesions, Stewart's results were noteworthy for their resemblance to the infection in man.

Kolmer (8) found that intrathecal injection of type I pneumococci in rabbits resulted in a rapidly fatal meningitis, while a more indolent infection occurred when types II or III were used. This difference was not apparent in monkeys that died with fulminating meningitis and septicemia regardless of the type of pneumococcus administered. Hamburger, Clark, Biehl and Jervey (9) also noted the virulence of type I pneumococci for monkeys, all of which died after intracisternal injection of more than 1,000 organisms with production of little exudate.

These experiences amply illustrate that the development and course of experimental meningitis, even with a bacterium as specific as pneumococcus, depend on the virulence of the pathogen, the size of the inoculum, the route of administration, the type of animal used, and numerous other

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variables. It is the purpose of this report to define the role of some of these factors more clearly.

MATERIALS AND METHODS

Animals. All experiments were performed in mongrel dogs weighing between 10 and 20 kg. Animals were housed in individual cages and were permitted food and water ad libitum.

Microorganism. An encapsulated strain of type III *Diplococcus pneumoniae* was used in all experiments. The organism was plated on blood agar, its type was confirmed with specific antiserum, and a smooth colony was inoculated into a flask containing 25.0 ml of tryptose phosphate broth to which had been added 2.5 ml defibrinated rabbit blood. After incubation at 37° C for 8 hours, 0.2 ml of broth culture was distributed in Wasserman tubes containing 0.5 ml of defibrinated rabbit blood. These tubes were rapidly frozen and stored at −70° C to serve as seed cultures for all subsequent studies. To prepare a culture for infection of animals, the contents of 1 tube were thawed and added to 10.0 ml tryptose phosphate broth. The cultures were incubated at 37° C for 18 hours and 1.0 ml was diluted with 9.0 ml isotonic (0.85 per cent) NaCl.

Experimental infections. All experiments were performed in animals anesthetized with pentobarbital. In preliminary experiments 4.0 ml of 1:10 dilution of the stock culture of pneumococci was instilled directly into the cisterna magna by suboccipital puncture. This method had three distinct disadvantages: *1*) bacteremia with early death was frequent; *2*) the infection was often localized to the cervical spinal cord close to the site of inoculation; *3*) leakage of the inoculum along the needle track with abscess formation in the paraspinal musculature occurred occasionally.

Most of these difficulties were overcome by instilling organisms in the following manner. The animals were placed face down on the operating table, the scalp shaved with a straight razor and thoroughly cleansed with soap, saline, and tincture of merthiolate. Employing strictly aseptic technique, a midline incision, 2.0 cm in length, was made through the skin and muscles, the periosteum was elevated, and the bone exposed. A small hole, 2.0 mm in diameter, was then drilled in the midline, halfway between the coronal and occipital sutures. The dura was punctured with a 27 gauge needle and as soon as spinal fluid appeared, the hole was closed with a plug of sterile bone wax. A 1-inch, 27 gauge needle was then inserted through the bone wax and 1.0 ml of the dilute culture was injected. The bone wax was necessary in order to prevent leakage of the inoculum through the burr hole. The wound was closed in layers and the animal returned to its cage.

TABLE I

Relationship of inoculum to rate of infection, recovery, and duration of illness

No. bact. injected	No. animals	No. animals with infection	No. animals dead	No. animals recovered	Duration fatal illness
					hrs
10^1	4	0			
10^2	6	2	1	1	
10^3	14	12	11	1	64
10^4	9	6	6	0	88
10^5	25	20	18	2	58
10^6	9	8	8	0	40
Total	67	48	44	4	

CSF for in vitro studies. CSF was obtained daily from all infected dogs by cisternal puncture under aseptic conditions. CSF, 2.0 to 3.0 ml, was withdrawn for quantitative bacterial counts, leukocyte counts, and determination of glucose.

Bacterial counts. The total number of bacteria in the inoculum or CSF was determined by colony counts of serial tenfold dilutions, a single colony being counted as one viable unit. Defibrinated rabbit blood, 0.25 ml, was added to each pour plate to promote growth of pneumococci.

Blood cultures. Blood was obtained by femoral vein puncture and 5.0-ml samples were cultured in trypticase soy broth and thioglycollate medium.

Leukocyte counts. The total number of leukocytes in blood or CSF was determined in the standard manner. Differential counts were made in some experiments with the use of Wright's stain or methylene blue stain.

Glucose. Glucose in blood and CSF was determined with glucose oxidase (10).

Pathological studies. Brains, spinal cords, and meninges were removed intact and fixed in 10 per cent formalin. In some instances brains were hemisected and one-half promptly homogenized for quantitative bacterial studies. The other halves and all remaining brains and cords were cut coronally into five blocks at the following levels: *1*) cerebral hemispheres (through the optic chiasm), *2*) midbrain (intercollicular), *3*) pons (through the fourth ventricle and lateral recesses), *4*) upper medulla, *5*) cervical cord (C6), *6*) lumbar cord (L3). These blocks were embedded in celloidin and sectioned at 20 to 30 μ. Sections from each level were stained with hematoxylin and eosin, Giemsa stain for bacteria, Van Gieson's stain for collagen, or cresyl violet.

Criteria for infection. Only when pneumococci from CSF were recovered was infection considered to be present. In some instances, CSF cultures 24 hours after inoculation of bacteria were sterile while subsequent cultures were positive; therefore three negative CSF cultures were required before an animal was considered to be free of infection.

RESULTS

Relationship of number of bacteria inoculated to development of infection (Table I)

Instillation of 10 or 100 bacteria rarely resulted in meningitis, but over 80 per cent of animals given

1,000 or more organisms developed infection. Therefore, 10^3 organisms appeared to be the critical level above which infection occurred, and there was no difference in the rate of infection between the number of animals given 10^3 and 10^6 bacteria. Failure to produce meningitis in some animals given large doses may have been due to escape of bacteria from the subarachnoid space via the burr hole.

Rate of recovery and duration of illness (Table I)

Of 48 animals developing meningitis, 4 recovered and 44 died. The 4 survivors were never very ill and their CSF contained at most 10^4 organisms per mm^3 and 2,000 cells per mm^3. In general, the duration of the infection paralleled the size of the inoculum and the most fulminating infections occurred in the dogs given 10^6 bacteria intrathecally.

Relationship of infecting dose to number of bacteria and leukocytes in CSF (Figure 1)

The mean values for CSF bacterial and leukocyte counts in each group of animals are shown in Figure 1. Except for animals given 10^6 bacteria, there was no relationship between the infecting dose and the number of organisms in the CSF 24 hours later. The same was true for CSF leukocyte counts. Once infection had been established, however, there was always a consistent increase in bacteria in the CSF for the ensuing 48 hours. With one exception, there was a parallel increase in the number of leukocytes in the CSF.

CSF glucose.

CSF glucose was almost invariably low in infected animals. There was no correlation between the magnitude of hypoglycorrhachia and the number of bacteria and cells in the CSF. In general,

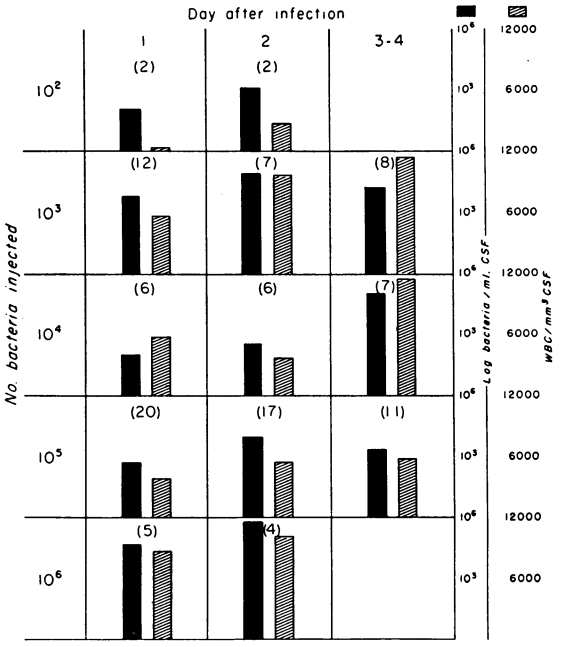

FIG. 1. RELATIONSHIP OF INFECTING DOSE TO THE NUMBER OF BACTERIA AND LEUKOCYTES IN CSF 24 TO 96 HOURS AFTER INDUCTION OF INFECTION. Numbers in parentheses are the number of dogs.

however, the animals with the large number of cells *and* bacteria displayed the greatest drop in sugar. Some of these results have been reported previously (11, 12).

Incidence of bacteremia (Table II)

Seventy-two per cent of 75 animals with pneumococcal meningitis had bacteremia at some time during the disease. Blood cultures were not positive in all animals every day, and a few animals never developed demonstrable bacteremia. It is of interest that none of the 15 animals which failed to develop infection had bacteremia. This suggests that in the animals resisting infection, organisms were probably destroyed *in situ* in the subarachnoid space.

TABLE II

Incidence of bacteremia in dogs after intrathecal injection of pneumococci

	Total	Day 1	Day 2	Day 3	Day 4
No. animals with meningitis	75	73	56	28	18
Positive blood cultures (%)	72	40	45	50	56
No. animals not developing meningitis	15				
Positive blood cultures (%)	0				

Clinical manifestations

Animals with meningitis were usually febrile, with average temperatures of 40.2° C, a rise of approximately 1.2 to 1.5° C above normal. The majority also had a marked increase in peripheral white blood cell count. The animals were lethargic and refused food. Ataxia, paralysis of the hind limbs, muscular twitchings, and terminal convulsions were prominent neurologic findings.

Morphologic observations

Injection of bacteria into the subarachnoid space of the midparietal region caused a diffuse meningitis encompassing the entire neuraxis. Grossly purulent exudate was present over the convexities of the cerebral hemispheres as well as at the base of the brain (Figure 2). Usually there was a heavy collection of pus in the cerebellopontine angles. Venous and capillary congestion was prominent in many animals. Thromboses of superficial and deep veins and of major sinuses were common.

The histological lesions of the brain could be divided into five types: *1*) exudate of the convexity and basal subarachnoid spaces of brain and cord, *2*) exudate of the ventricular system and central canal of spinal cord with associated ependymitis, *3*) vascular congestion and inflammation of the choroid plexus, *4*) major sinus and superficial and deep venous thrombophlebitis with subarachnoid bleeding, *5*) cortical granulomata and necrosis of neural tissue.

Figure 3 illustrates some of the exudative lesions. Regardless of its location, the inflammatory exudate consisted almost solely of polymorphonuclear leukocytes. Perivascular cuffing, glial nodules or diffuse inflammatory changes were found in neural tissue surrounding purulent collections in the ventricular system or in the central canal of the spinal cord.

In general, the meninges provided remarkable protection to the underlying cortex of brain or white matter of the cord. In some instances diffuse cortical granulomata were found beneath dense collections of pus in the subarachnoid space (Figure 4A). In spinal cord it was common to see purulent necrosis extending into the subjacent gray matter from the central canal (Figure 4B). In several brains the arachnoid septa served as effective barriers largely confining the infection to the subarachnoid space (Figure 5A).

In other animals venous and major sinus throm-

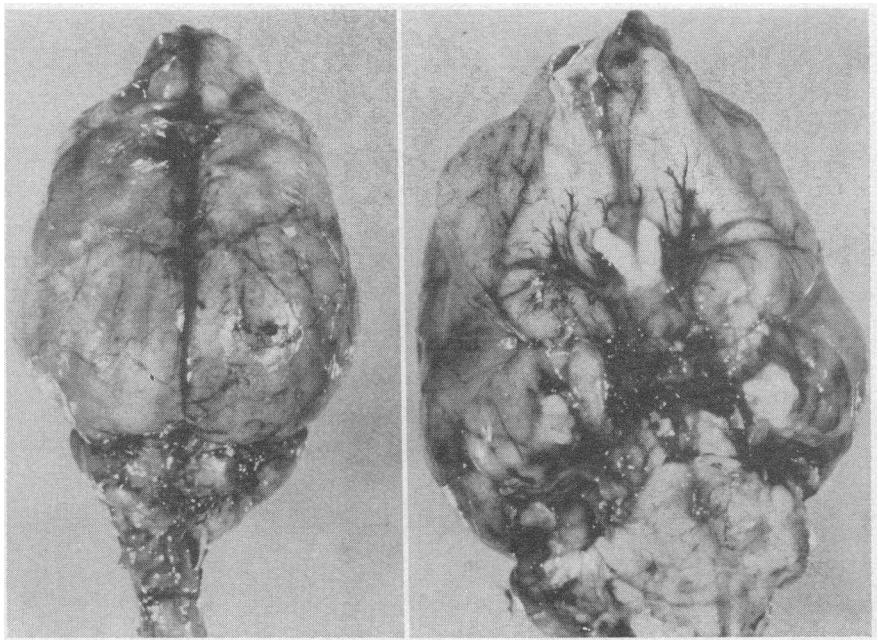

FIG. 2. DORSAL AND BASAL SURFACES OF THE BRAIN. Note collection of pus in cerebellopontine angles.

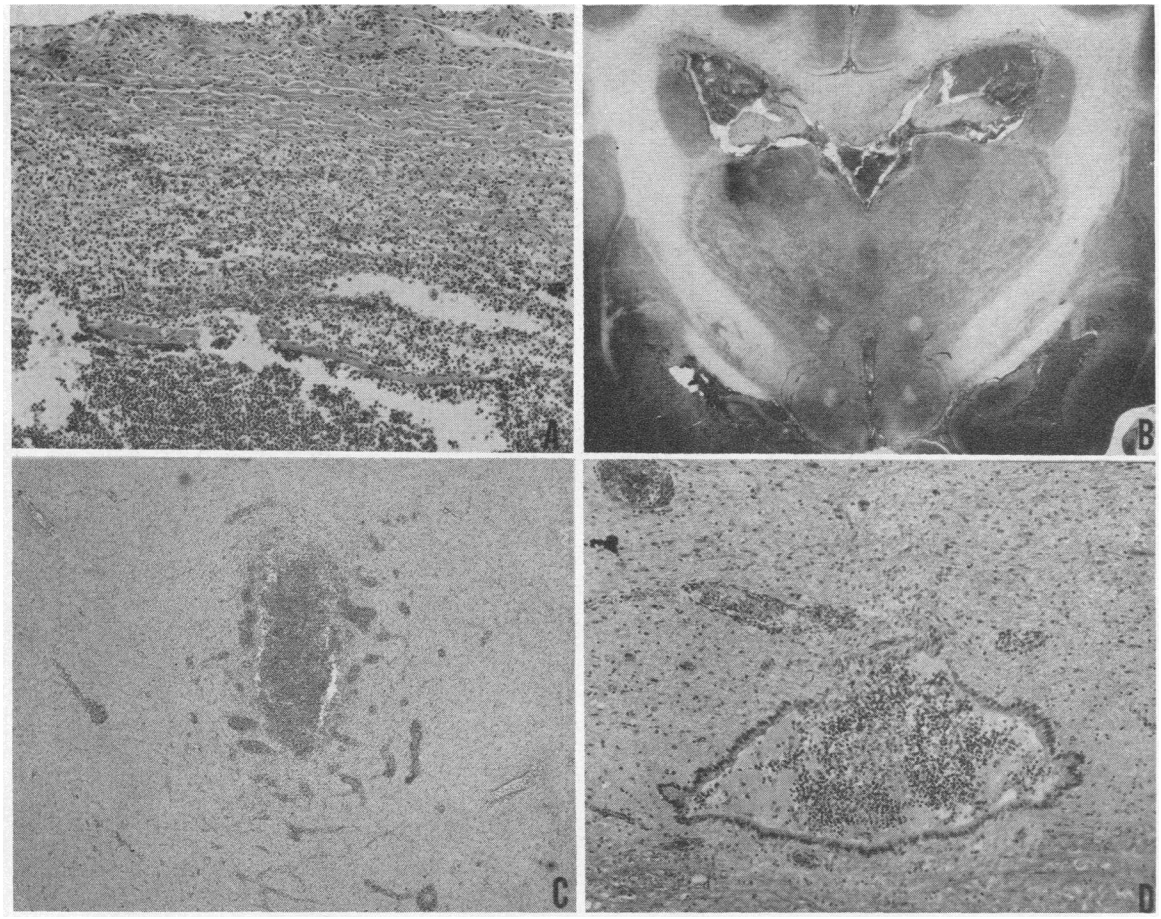

FIG. 3. EXUDATIVE MANIFESTATIONS OF MENINGITIS. **A**. Subdural empyema (hematoxylin and eosin, × 150). **B**. Coronal section through thalamus with exudate in lateral ventricles and third ventricle (cresyl violet, × 4). **C**. Aqueduct of Sylvius occluded by purulent exudate; there are also many foci of periaqueductal inflammation in the gray matter (hematoxylin and eosin, × 35). **D**. Pus in the central canal of the cervical cord (hematoxylin and eosin, × 100).

boses overshadowed the exudative reaction. Such findings were associated with hemorrhagic necrosis of the cortex and white matter drained by these venous tributaries. Figure 5B shows a sagittal sinus and superficial cortical vein thromboses with hemorrhagic necrosis of the cortex. Vascular congestion and inflammatory infiltration of the choroid plexus with polymorphonuclear cells was also common (Figure 5C).

Bacteria were present in most collections of exudate but were often difficult to find in sections because of autolysis. Cultures of pus from the surfaces of the brain were invariably positive for pneumococci, as were homogenates of brain and meninges.

Clinicopathological correlations

In 20 animals the degree of inflammation of the surface of the brain, the amount of exudate in the ventricular system, the severity of vascular congestion, and the presence of thrombosis and subarachnoid hemorrhage were correlated with the number of bacteria and leukocytes in the CSF on the day before death, and the length of the animals' survival (Figures 6–8). In animals with marked pleocytosis (greater than 6,000 leukocytes per mm^3 per CSF) and a larger number of bacteria ($> 10^7$ per ml CSF), the inflammatory reaction in the meninges covering the cortex was most severe. On the other hand, in animals with fewer cells and bacteria in the CSF, there

was little correlation between the severity of the inflammatory process in the cortical meninges and the number of cells and microorganisms. Animals surviving 72 hours all had severe meningitis, while those living for only 24 hours demonstrated little exudate (Figure 8).

In contrast to the inflammatory response on the surface of the brain, the degree of exudation in the ventricular system correlated poorly with the number of leukocytes and microorganisms in the CSF (Figures 6 and 7). Likewise, there was no relationship between the exudative response in the ventricles and the length of survival.

Mild to moderate vascular congestion had no discernible effect on CSF bacterial and leukocyte counts or duration of illness. Thrombosis and hemorrhage were notable in only 5 of 20 dogs, and in 2 were the probable cause of death within 24 hours of infection. However, 2 other animals without thrombosis and hemorrhage also died within 1 day after inoculation of bacteria.

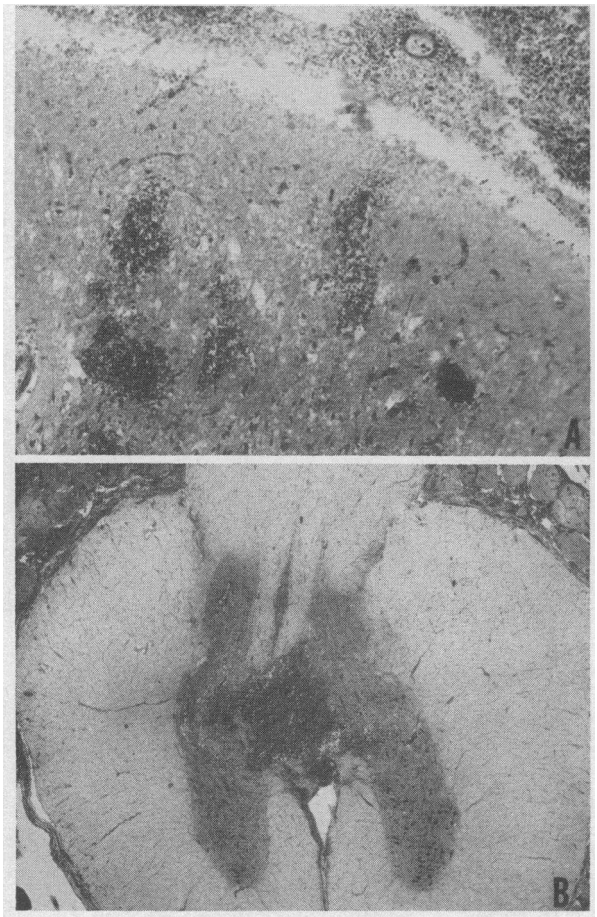

FIG. 4. INVOLVEMENT OF PARENCHYMAL TISSUE OF CNS IN MENINGITIS. **A.** Granulomatous nodules in cerebral cortex (cresyl violet, × 100). **B.** Inflammatory necrosis of gray matter in the lumbar cord. Note central canal obliterated by pus (cresyl violet, × 15).

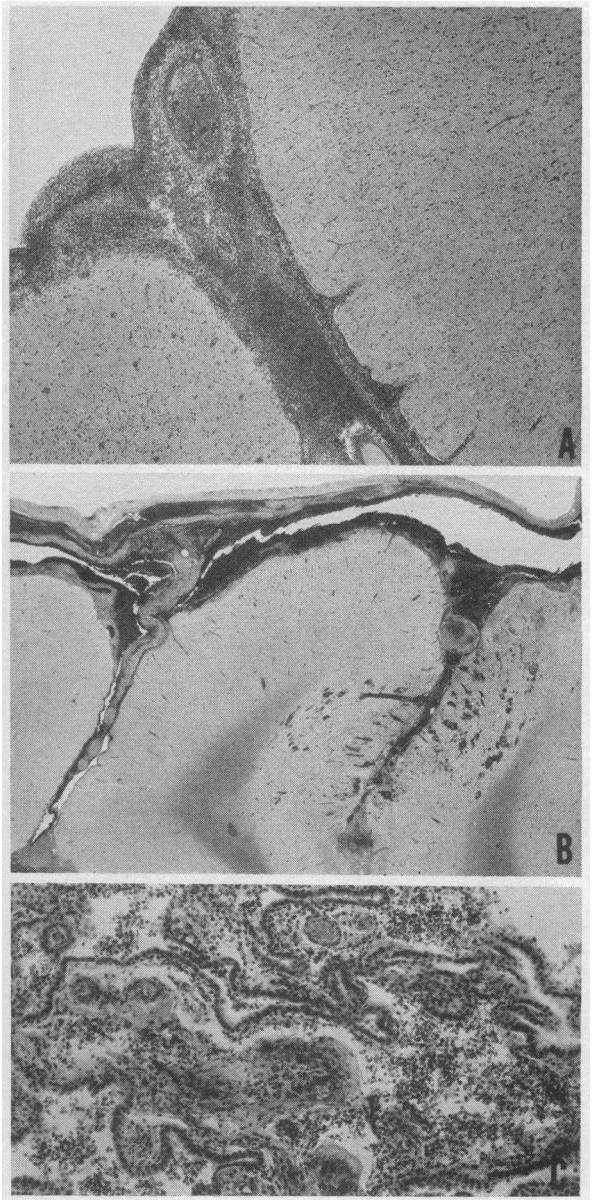

FIG. 5. VASCULAR FEATURES OF MENINGITIS. **A.** Dense meningitis over dorsal surface of brain. There is also phlebitis of superficial cortical veins and an early sagittal sinus thrombosis (hematoxylin and eosin, × 35). **B.** Thrombosis of sagittal sinus; marked congestion and hemorrhage in cortical veins (hematoxylin and eosin, × 10). **C.** Choroid plexus showing vascular congestion and infiltrate of inflammatory cells (Giemsa, × 100).

Alterations in host resistance

In order to evaluate the effect of acute alterations in host resistance on the course of the experimental infection, one group of animals was pretreated with adrenal cortical hormones to modify the inflammatory response, and a second was subjected to total body irradiation in an attempt to render the animals leukopenic.

A. Effect of adrenal cortical hormones. Groups of three dogs were given 1 mg prednisolone [1] per kg per day intramuscularly for 5 days. On the sixth day, the animals were inoculated with 10^5 pneumococci through a central burr hole and three normal controls were injected with the same inoculum at the same time. Animals were then followed with daily cisternal punctures and measurements of bacteria, leukocytes, and glucose in the CSF.

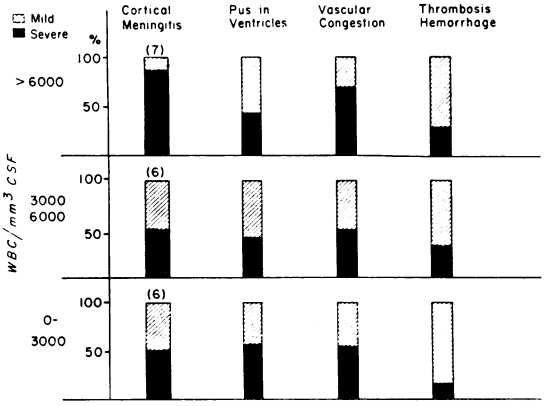

FIG. 6. RELATIONSHIP OF NUMBER OF CELLS IN CSF ON DAY BEFORE DEATH TO EXUDATION IN MENINGES AND VENTRICULAR SYSTEM, VASCULAR CONGESTION, THROMBOSIS, AND HEMORRHAGE.

Six separate experiments were performed; the composite results of the prednisolone-treated and 17 control animals are summarized in Table III. In both groups there was a progressive increase in bacteria in the CSF. The increase in leukocytes was less consistent but the highest leukocyte counts were observed on the third day of infection in the prednisolone-treated group. There was no difference in the duration of the illness, and at autopsy lesions in the prednisolone-treated dogs were indistinguishable from those of the controls. Decreases in CSF glucose were also comparable. These results indicate that in the doses employed, adrenal hormones did not modify the course of this infection.

[1] Meticortolone, intramuscular; Schering.

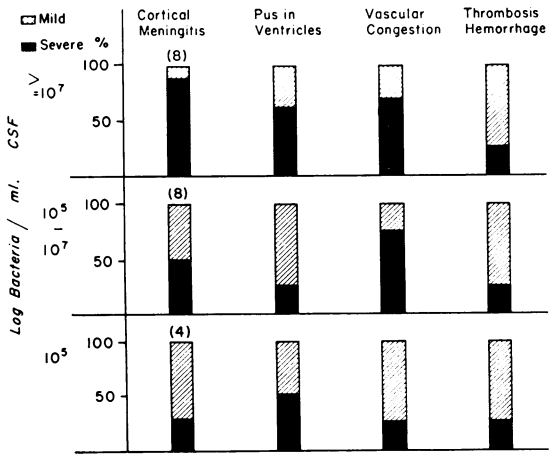

FIG. 7. RELATIONSHIP OF NUMBER OF BACTERIA IN CSF ON DAY BEFORE DEATH TO MORPHOLOGIC CHANGES IN CNS.

B. Total body irradiation. In order to assess the effect of leukopenia on the course of the experimental meningeal infection, groups of three dogs were subjected to 450 r total body irradiation. When the blood leukocyte counts had fallen to 1,000 leukocytes per mm^3 or less, animals were infected, and normal controls were inoculated with the same culture. Seventeen leukopenic and 16 control animals were available for study. All but 2 in each group received 10^5 pneumococci; the remainder were given 10^4 bacteria.

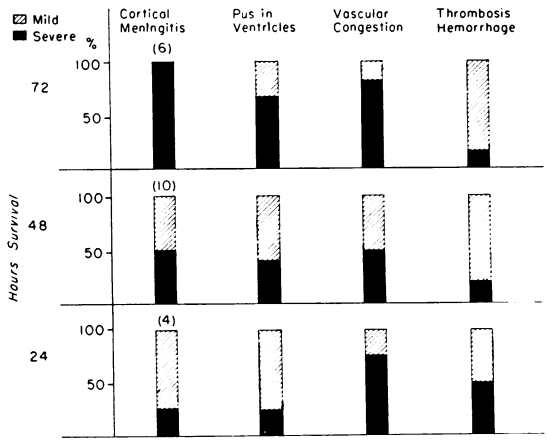

FIG. 8. CORRELATION OF MORPHOLOGIC CHANGES IN CNS AND LENGTH OF SURVIVAL OF DOGS WITH EXPERIMENTAL PNEUMOCOCCAL MENINGITIS.

TABLE III

Comparison of pneumococcal meningitis in normal dogs and in dogs treated with prednisolone

	Prednisolone-treated			Normal		
No. animals		20			20	
No. infected		18			17	
No. dead		18			17	
Hrs survived		57			58	
Hrs after infection	24	48	72	24	48	72
Log bacteria/ml CSF	2×10^3	1.6×10^4	2×10^5	10^3	8×10^3	9×10^4
WBC/mm³ CSF	5,200	3,800	8,300	5,000	5,400	5,800

The results are depicted in Table IV. In each group 1 animal recovered spontaneously; infection failed to take place in 2 leukopenic and 3 control animals. A comparable amount of bacterial multiplication in the CSF occurred in both irradiated animals and controls. However, there was a marked difference in the number of leukocytes in the CSF, and irradiated animals responded only with sparse exudation. As has been reported previously (11), leukopenic animals failed to manifest a fall in CSF sugar. Also of interest is a mean survival time of 62 hours in irradiated animals compared with one of 46 hours in controls. While the small number of animals precludes statistical analysis, the data suggest that the leukocytic exudate contributed to the earlier demise in the controls. Pathological examination of the irradiated dogs showed considerably less inflammation, but no increase in hemorrhage or thrombosis compared with the controls.

DISCUSSION

Although there was considerable variability between individual animals in the response to intrathecal instillation of pneumococci, there was some relationship between the magnitude of the inoculum and the rate of infection, duration of illness, CSF pleocytosis, and increase in bacteria within the subarachnoid space. Furthermore, with a given inoculum the infection was reasonably reproducible and involved the entire neuraxis, a distinct advantage over most previous experimental models. Despite its obvious shortcomings, not the least of which is the artificiality of the portal of entry, this experimental model should provide a standard for comparison with infections by other types of pneumococci and other bacterial species; it should also serve as a control for meningeal infections produced by other routes and as an assay for various therapeutic regimens. Several of these studies are in progress. Finally, utilization of special histochemical techniques might provide valuable information concerning the biochemical alterations in the leptomeninges during bacterial infection.

These studies provide some insight into the mechanism of death in meningitis. In a few animals mortality seemed to be clearly related to subarachnoid hemorrhage, but in the majority interference with CSF circulation subsequent to obstruction of the ventricular system by the inflammatory exudate must have resulted in functional hydrocephalus. More importantly, in the spinal cord, pus in the central canal often led to severe

TABLE IV

Pneumococcal meningitis in normal and irradiated dogs

	Irradiated			Normal		
No. animals		17			16	
No. infected		15			14	
No. dead		14			13	
No. recovered		1			1	
Hrs survived		62			46	
Hrs after infection	24	48	72	24	48	74
Log bacteria/ml CSF	1.1×10^3	7×10^3	2×10^4	9×10^2	2.4×10^4	9×10^3
WBC/mm³ CSF	250	200	300	4,800	3,900	7,200

inflammatory necrosis of the surrounding gray matter. This transverse myelitis was also described by Stewart in canine pneumococcal meningitis (7), but in his experiments may have been due to direct injection of organisms into the lumbar subarachnoid space. In the present studies, the organisms were introduced far from the site of myelitis. The lumbar spinal cord was the part of the neuraxis showing the most significant parenchymal damage. It is of considerable interest that the relatively narrow aqueduct of Sylvius was also surrounded by infiltrates of inflammatory cells with destruction of neural tissue.

Exudate covering the cortical surface of the brain may not be of much consequence in the animals' demise, and normal dogs with the most profuse exudation lived for the longest period of time. On the other hand, the data in leukopenic animals, with infections characterized by a sparse inflammatory reaction, suggest that exudate was deleterious and these animals lived even longer than normal controls. These observations might be interpreted to mean that pus in the subarachnoid space exerts its most harmful effect in the ventricular cavities and aqueduct where it is more likely to produce hydrocephalus.

SUMMARY

Type III pneumococcal meningitis was produced in dogs by the instillation of 10^3 to 10^6 organisms into the subarachnoid space through a small midline burr hole. This afforded a reproducible infection lasting 48 to 96 hours, which was characterized by a progressive increase in CSF cells and bacteria as infection progressed. Morphologically, the infection was characterized by thrombosis and hemorrhage in a few animals, but by an exudative reaction involving the meninges of the entire neuraxis as well as the ventricular system in the majority. There was some correlation between the degree of surface meningitis and the number of cells and bacteria in the CSF, but there was no correlation between the amount of exudate in the ventricles and the cells and bacteria in the CSF. The course of the infection was not altered by prednisolone given for 5 days before instillation of bacteria. Meningitis in leukopenic animals resulted in few cells in the CSF coupled with sparse exudation. These animals survived for a longer period than normal controls, suggesting that the exudative reaction in the subarachnoid space may be deleterious.

ACKNOWLEDGMENT

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