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Experimental Pneumococcal Meningitis I: A Rabbit Model (38149)

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Prior to 1930, experimental models of pneumococcal meningitis employing monkeys and cats were described (1–3), but in recent years only a dog model has been reported in detail (4, 5). Experimental infection in rabbits heretofore has been unsuccessful (6).

The present study was undertaken to develop and characterize a reproducible, inexpensive and convenient model of pnueumococcal meningitis in rabbits. Such a model can be used in the study of basic processes responsible for the morbidity and mortality associated with bacterial meningitis.

Materials and Methods. Animals and method of inducing meningitis. New Zealand white rabbits weighing between 2.5 and 3.2 kg were employed. All rabbits were anesthetized with pentobarbital given intravenously, and in 22 animals (Group I), 0.5 ml of cerebrospinal fluid (CSF) was removed from the basal cistern according to the technique of Chalmars and Wurtman (7). Within 2 min of cisternal puncture, 1 ml of a saline suspension containing 10^7 colony forming units/ml (cfu/ml) of pneumococci was injected into a marginal ear vein. Five min later, blood for quantitative culture was obtained from the opposite ear. An additional 53 rabbits (Group II) were infected similarly, except that the CSF withdrawn from the cistern was replaced with 0.5 ml of a sterile 0.125% suspension of mucin (Sigma Chemical Co., St. Louis, Mo.)in Ringer's lactate adjusted to pH 7.34. All animals were followed to spontaneous death; 2 animals in Group II died from excess anesthesia and were excluded from subsequent analyses. CSF and blood were obtained at 24–48 hr intervals.

Six control animals served as non-infected mucin controls. Each received 0.5 ml of a 0.125% suspension of mucin intracisternally, and was sacrificed 72 hr later. Blood and CSF were obtained at 24 hr intervals. A normal animal served as a control for histological purposes.

Microorganism. A strain of type III *Diplococcus pneumoniae* previously employed to induce meningitis in dogs (4, 5), was passed intraperitoneally in mice, and used to induce meningitis in rabbits by intracisternal inoculation four times before aliquots were stored at −70° in trypticase soy (TSY) broth containing 3% defibrinated sheep blood. As needed, aliquots were thawed, inoculated on to sheep blood agar (SBA) and incubated overnight at 37 in 5% CO_2. TSY broth was inoculated from these cultures and incubated overnight. The broth then was centrifuged and the supernatant discarded. Pneumococci were resuspended in 0.15 *M* NaCl to obtain $1.0\text{-}1.7 \times 10^7$ cfu/ml. Pneumococci recovered from the CSF of infected animals were isolated on SBA and used for subsequent experiments.

Laboratory and autopsy studies. Quantitative cultures of blood and CSF were obtained by making serial dilutions in 0.15 *M* NaCl. Leukocyte counts in blood and CSF were determined in a standard manner. Glucose concentration in the serum and CSF was measured by the ferricyanide method (Technicon Autoanalyzer, Ardsley, NY).

Post mortem examination of all animals with meningitis was performed. Brains from 18 rabbits, including 5 animals from Group I, 11 from Group II, and 2 mucin controls were submitted for histological examination. Thoracic and abdominal viscera from 6 rabbits in Group II also were examined microscopically. Formalin fixed tissue was embedded in paraffin, sectioned and stained with hematoxylin and eosin.

Results. Rates of infection. Rabbits were considered to have meningitis when they had

CSF pleocytosis, positive culture of the CSF, and clinical findings indicative of CNS infection 24 hr after inoculation. Accordingly, 12 of 22 (54.5%) rabbits in Group I developed meningitis, and 42 of 51 (83.9%) of Group II animals became infected ($P = .025$, X^2). All the animals with meningitis, except 2 from Group II, died of their infection.

Clinical course of meningitis. The duration of survival was 64.0 ± 20.8 hr (mean ± S.D.) and 80.2 ± 20.1 hr for animals in Groups I and II respectively ($P < .001$, t test). Six of the 12 animals with meningitis in Group I survived less than 48 hr.

Animals in both groups lost approximately 0.115 kg or body weight each day they survived with meningitis. The maximum temperature response, similar in both groups, was recorded 24–48 hr after the induction of bacteremia, and was 41.1 ± .39. Temperatures routinely fell prior to death, and the mean recorded temperature of animals 4 hours before death was significantly less than that recorded 12 to 24 hours before demise, i.e., 36.9 ± 1.2 vs. 40.8 ± .39 ($P < .02$, t test). The 6 mucin controls had no fever 24 and 72 hr after injection of mucin.

Within 24 hr after meningitis was induced, rabbits became inactive, and refused water and food. Nuchal spasm or rigidity was present in all animals with fatal infection, and cerebellar and pyramidal tract signs usually appeared if they survived 60 hr or more. Convulsions, opisthotonus, and a decreasing level of consciousness were characteristic agonal events.

Laboratory findings in meningitis. The number of organisms and leukocytes in the CSF increased daily in both groups (Table I). However, the mean counts at 24 hr were significantly greater in the animals of Group II. The mean leukocyte count in the CSF of the mucin control animals at 24 hr was 825/mm^3 ± 802 (S.D.), and at 72 hr it was 78/mm^3 ± 24. Hypoglycorrachia, defined as a CSF/serum glucose ratio 2 standard deviations below the initial value, developed in 44.4% of Group I and 39.3% of Group II rabbits. The CSF glucose did not fall in mucin controls, or in animals which failed to develop meningitis. Peripheral leukocyte counts were usually elevated in infected animals.

Influence of bacteremia on development and course of meningitis. The failure of 9 mucin treated rabbits to develop meningitis may have resulted from insufficient numbers of bacteria in the bloodstream 6–7 min after cisternal puncture, since the mean colony counts of noninfected and infected Group II counterparts was 10^3 and 10^5 cfu/ml respectively

TABLE I. Comparison of CSF Bacterial and Leukocyte Counts in Group I and Group II Animals with Pneumococcal Meningitis.

	CSF bacterial counts (cfu/ml) following infection			
	24 hr	48 hr	72 hr	96 hr
Group I mean	7.7×10^3	2.6×10^5	7.0×10^6	–
range	$10^1 - 10^5$(9)[a]	$10^3 - 10^6$ ((9)	$10^6 - 10^7$ (4)	– (2)
Group II mean	2.2×10^5	1.9×10^6	6.8×10^6	2.9×10^7
range	$10^4 - 10^9$ (9)	$10^4 - 10^9$ (17)	$10^5 - 10^9$ (18)	$10^5 - 10^9$ (6)
P[b]	< .025	> .05	> .05	
	CSF leukocyte counts (mm^3) following infection			
	24 hr	48 hr	72 hr	96 hr
Group I mean	109	936	1810	–
S.D.	± 101 (9)	± 980 (6)	± 590 (5)	– (1)
Group II mean	1199	1382	3849	6242
S.D.	± 933 (9)	± 1170 (11)	± 7000 (17)	± 4350 (9)
P[b]	< .025	> .05	> .05	

[a] Number of animals studied (In some instances same animal studied at 24–96 hr).

[b] Mann-Whitney U test.

($P < .001$, t test). A similar difference was not observed in Group I animals where the mean 5 min culture of both noninfected and infected rabbits was 10^5.

When the magnitude of bacteremia 40 to 48 hr after induction of meningitis was in the range of 10^4 cfu/ml, the animals died within a few hours. When the colony count was around 10^2, the animals usually survived 72–98 hr. Overall, the number of bacteria in the bloodstream fell progressively from a mean of 10^5 cfu/ml at 24 hr to a mean of 10^1 in rabbits surviving 96 hr.

Pathological findings. The CNS pathology of the animals within the two groups was indistinguishable. Brain weights were identical, and congestion of the superficial vessels and opacification of the leptomeninges was invariably observed. Grossly evident exudate covered the cerebellum dorsally, and the brainstem ventrally up to the level of the optic chiasm. In microscopic sections, subarachnoid fibrin and cellular exudate extended from olfactory lobe to medulla, but was thickest ventrally over the brainstem and dorsally over the cerebellum, and occasionally exudate extended into the Virchow-Robin spaces. No significant concentration of exudate was noted at the site of cisternal puncture. Brains from animals surviving 72 hr or more, in addition to having greater meningeal exudate, contained areas of superficial infiltration of the parenchyma, but frank abscess formation was found in only 1. Two specimens exhibited intraventricular exudate, but ventricular dilatation was not observed. Mild edema of the neuropile was observed, but there was no rarefication. Neuronal changes and gliosis were not present. Brains from mucin controls were normal except for the presence of vacuolization in the cells of the choroid plexus.

Gross and microscopic examination of viscera was generally unremarkable. Histological sections of lung often showed areas of microatelectasis. Fibrin thrombi in small pulmonary vessels, a finding reported previously in rabbits with pneumococcal sepsis (8), were rarely seen, but peribronchial and perivascular cuffing with inflammatory cells was frequently noted. Focal myofiber necrosis was seen in the myocardium of 2 animals, and unilateral pyelonephritis in another. Fatty metamorphosis of the liver was present in every case.

Discussion. An animal model of experimental bacterial meningitis must fulfill several criteria before its suitability can be determined. These include clinical evidence of CNS infection, CSF pleocytosis, and persistently positive bacterial cultures of the CSF. Progressive CNS infection should be fatal in a high proportion of untreated animals, but they should survive long enough for meaningful studies to be conducted. Finally, pathological studies should show predominant meningeal inflammation and other histologic features similar to those seen in human disease.

The rabbit model described in this report meets each of these criteria. In addition, the method of inducing infection is preferable to the more frequently used alternative of injecting 10^5 of 10^6 organisms directly into the cistern. The fact that minor trauma to the meninges during bacteremia produces meningitis was reported previously (3, 5), but the mechanism involved is unknown. Pathologic studies of the rabbit model show that inflammation is distributed along the base of the brain and over the cerebral and cerebellar hemispheres rather than at the site of cisternal puncture. This argues against the hypothesis that with this technique organisms only gain access to the meninges through the defect created by the procedure (4, 5).

Injection of low concentrations of sterile mucin intracisternally increases the number of animals which developed meningitis, and produces higher bacterial and leukocyte counts in the CSF at 24 hr. The latter is probably a reflection of the pleocytosis induced by mucin alone, despite the fact that pathologic studies fail to show inflammatory infiltration of the meninges in mucin controls. The reason for the significantly longer survival of mucin treated animals is not known. There may have been some loss of virulence of the organism after repeated passages, but the strain of pneumococcus employed and the infecting inoculum was the same throughout the study.

Finally, the model described is convenient and inexpensive. Large numbers of animals can be studied, and theories regarding the basic processes responsible for morbidity and mortality in bacterial meningitis can be formulated and refined before testing them in

more expensive primate models or in man.

Summary. A model of experimental pneumococcal meningitis in rabbits is described. Animals were infected by intravenous inoculation of 10^7 cfu of type III pneumococci immediately after withdrawal of 0.5 ml of CSF from the basal cistern. Pneumococcal meningitis, defined as CSF pleocytosis, positive cultures of the CSF, and clinical findings indicative of CNS infection 24 hr after inoculation, occurred in 54.5% of rabbits infected in this way. A higher proportion of animals, 83.9% developed meningitis if 0.5 ml of a 0.125% suspension of sterile mucin was injected into the cistern at the time of the initial tap ($P = 0.025, x^2$). All but 2 of 54 animals with meningitis died spontaneously; the duration of survival of animals which did not receive mucin was 64.0 ± 20.8 hr (mean ± S.D.) while the animals receiving mucin survived 80.2 ± 20.1 hr ($P < .001$, t test). Mucin did not otherwise alter the clinical course of animals with meningitis, and the CNS pathology was indistinguishable within the two groups, and gross and microscopic findings were similar to those of human infection.

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