LOCAL SPECIFIC THERAPY OF EXPERIMENTAL PNEUMOCOCCAL MENINGITIS.

II. THE PRODUCTION, PATHOLOGY, AND TREATMENT OF TYPE I PNEUMOCOCCAL MENINGITIS IN DOGS.

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PLATES 16 TO 21.

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In the previous paper (1) the production, pathology, and attempted treatment of Type I pneumococcal meningitis of rabbits were discussed. Reasons were presented explaining why the rabbit is a very unfavorable animal for the study of this disease, which may be briefly summarized as follows: In a normal rabbit, septicemia, following intracisternal infection with Type I pneumococci, is invariable, rapid, and massive, and septicemic death occurs before there is any reactive localization in the meninges; consequently, to produce meningeal localization, complete or partial, a previous immunizing treatment is necessary. Following this treatment, however, there is a considerable variation in the intensity of the meningeal infection, making it impossible to gauge properly the dosage of organisms which will produce fatal disease, but which will not immediately overwhelm the animal's resistance. Even if the disease intensity is properly regulated, treatment is unsuccessful on account of both the limited working space and the widespread localization of organisms in regions impossible to reach either by immune serum or lavage.

In view of these facts, the rabbit was abandoned as an experimental animal and recourse was had to young dogs. The dog is, as we know, relatively resistant to systemic pneumococcal infection, although marked variations even here are encountered. In the meninges, however, a type of disease may be produced which progresses toward fatal termination, if cultures of suitable virulence are used, and one which quite closely approximates pathologically the human pneumococcal...
meningitis. To produce rapid progressive meningitis with any degree of assurance that the disease will assume a fairly characteristic course from day to day, the infecting dose must be reasonably standardized. In the present study the dosage of organisms has been accurately counted by means of a Petroff-Hausser bacterial counter. Not only should the numbers of infecting pneumococci be known, but the culture should be standardized both as to hours of growth and as to quantity of growth. A culture should be chosen which is well along on the upward limb of the growth curve. We have employed 6 hour cultures. It must be a culture which has given a good growth. There is a marked difference between the degree of 18–24 hour infection produced by a given number of pneumococci from a 6 hour plain broth culture which has produced a low total growth, i.e., a growth ranging from 150–225 million per cc., and that produced by the same number of organisms from a 6 hour 0.2 per cent dextrose broth culture which is producing a growth of upwards of 700 million per cc. Unless these differences are recognized, conclusions are of little value. Use of late (24 hour) cultures may lead to very deceptive results. Even with these cultural conditions standardized, there is an uncontrollable individual factor which influences the rate of establishment of progressive disease; every animal is an individual and must be so handled. Whereas we hesitate to interpret the anatomic and therapeutic results of others, we are inclined to believe that the findings of Idzumi (2) were the result of the use of a culture of low virulence. Idzumi studied pneumococcal meningitis in dogs. This investigator used enormous doses of organisms, from 1–2 cc. of a suspension secured by centrifuging 10 cc. of a 24 hour culture of low mouse virulence. Although the dogs died, the pathologic changes were scarcely more than those of hyperemia. In our own study the disease has been regularly produced with approximately 1 cc. of a 1:100 dilution of 6 hour culture. It would seem that Kolmer's (3) successful therapy, i.e., lavage from one or both lateral ventricles to the cisterna magna with physiological saline or Ringer's solution, cannot be interpreted as the result of other than feeble infection with cultures well beyond the growth peak and full of non-viable organisms. The lavage was most incomplete, since the entire cord, base, and convexity were untouched by the saline. Kolmer reports, however, that at the time of lavage
the spinal fluids were always purulent and contained myriads of pneumococci, making it more difficult for one to understand how lavage from one lateral ventricle to the cisterna magna could have appreciably influenced the process, since in our own experience the other ventricle would always have been an untouched focus in a disease of the intensity he describes. Bull (4) observed meningitis developing in the course of pneumococcal septicemia in dogs. His organism was not typed. Bull noted some spontaneous recoveries.

EXPERIMENTAL.

Apparently in control dogs, infected intracisternally, death may occur in one or more of three ways. A dog may develop a fairly rapid proliferation of pneumococci in the meninges with a constant large feeding of organisms into the blood stream, and die of bacteremia. If a culture is of low potency due to poor growth conditions, a meningeal process of such intensity may result that a very marked cellular reactional process occurs, with pneumococci present in relatively small numbers. In such cases death appears to be "reactional." Thirdly, the proliferation of organisms within the meninges may attain enormous proportions, and brain and cord may be covered with a thick, gelatinous layer, consisting mainly of heavily encapsulated pneumococci, but with relatively moderate cellular reaction. In the second and third instances, death may be due, in part at least, to the mechanical obstruction of the meningeal spaces. The actual mechanism of death from pneumococcus infection is always rather obscure. Three protocols may be introduced to illustrate these points.

Dog 12.—Female hound; weight 5.5 kilos. March 23, 1927, 4.10 p.m., morphine gr. 1/5; ether; cistern puncture; clear fluid. Injected 6,500,000 Type I pneumococci (6 hour dextrose broth culture, growth 1,100,000,000 per cc.). Good recovery. 19 hours later, lethargic, weak, irritable, sicker than usual at this stage. 23 hours, morphine gr. 1/5; death in 10 minutes. Autopsy: Brain and cord injected, edematous; cisternal fluid cloudy; marked grayish yellow opacity along sulci of convexity and at base. Smears from the usual regions show on an average 20–30 pneumococci per oil immersion field and 30–60 cells, mostly polymorphonuclears. Viscera negative. Culture from heart's blood gave massive confluent growth. A death mainly due to septicemia. Microscopically, however, there was a marked diffuse fibrinopurulent leptomeningitis.

Dog 3.—Female hound; weight 7.5 kilos; February 9, 1927, 4.20 p.m., ether,
cistern puncture, clear fluid. Injected 15,000,000 Type I pneumococci (7 hour plain broth culture giving poor growth). Good recovery.

23 hours—temperature 103°. Sluggish; refuses food.
43 hours—temperature 100.2°. Unchanged; 100 cc. saline intraperitoneally.
67 hours—temperature 103°. Sleeps most of time; able to stand; 75 cc. saline intraperitoneally.
93 hours—temperature 102.2°. Able to stand leaning against cage; weak; stiffness of neck; 75 cc. saline.

5th day—temperature 102°. Very irritable.
6th day—temperature 103.2°. Irritable; chills; tremors.
7th day—temperature 99.8°. Prostrate; hypersensitive; tremors; incontinence of urine and feces; 100 cc. intraperitoneal saline.

8th day—dead. Autopsy: Upon opening spinal cord and cranial cavity, meninges are opaque and pinkish yellow; purpuric blotches in region of medulla. Cord dura is very tense; on slitting it, thick, grayish or greenish yellow pus exudes; this is generalized in distribution, but most abundant in lower medullary, upper cervical, and region of lumbar enlargement. Smears show thick masses of polymorphonuclear leucocytes and rare endothelials. Only occasional diplococci, all extracellular. Over the cerebellar vermis and adjacent portions of cerebellar hemispheres is thick pus. A smear shows a similar picture to that from cord. The base of the medulla and the pons is coated with thick, greenish yellow pus (Fig. 1). Much less exudate over convexity, and pneumococci are rare. The lateral ventricles are dilated and filled with thick pus, and the walls appear eroded. In the ventricular pus cocci are more numerous, but still do not reach the usual proportions; are free and in small agglutinated clusters. Essentially no phagocytosis. Heart's blood cultures are negative.

Summary: Infection with culture giving poor growth; delayed death; enormous reactive process; few pneumococci at autopsy. Suggests a "reactional" death. Histologically a very marked meningitis, superficial encephalitis and myelitis.

Dog 7.—Male fox terrier; weight 6 kilos. March 9, 1927, 4:05 p.m., ether; cistern puncture; clear fluid. Injected 7,000,000 Type I pneumococci (6 hour dextrose broth culture, growth 700 million per cc.). Good recovery.

18 hours—temperature 103.5°. Stands, wags tail, refuses food and water.
42 hours—temperature 103.4°. Semiprostrate; 100 cc. intraperitoneal saline.
66 hours—very irritable; unable to stand.
90 hours—dead. Autopsy: Fourth ventricle distended with thick, gelatinous material; cord meninges and meninges over convexity and base tense, opaque, filled with similar yellowish gelatinous deposit. Lateral ventricles contain thick pus. Smears from all regions show numerous pus cells, but an incredible number of pneumococci (Fig. 2), the latter really making up most of the gelatinous material. Essentially no phagocytosis. Both cerebrospinal fluid and heart's blood give abundant growth.
At this point, the observation should be emphasized that smears taken with a loop from different regions of brain or cord meninges give rather deceptive results so far as numbers of exudative cells are concerned. Probably this is due to the fact that cells lie in fibrin meshes wherein they are held. Pneumococci may be picked up more easily and smears, so far as numbers of bacteria go, check fairly well with microscopic findings in sections.

Therapeutic Experiments.

After a few trials of the culture to determine a proper infecting dose, attempts were instituted to treat the animals. The first series consisted of three dogs, two treated and one control. The culture used was that described in the protocol of Dog 3 (above). This culture was of low virulence, producing a prolonged process in the control animal. The infecting dose consisted in 2,000,000 organisms per kilo of body weight; animals were injected intracisternally. It so happened that both treated dogs made uneventful recoveries, save that one had a residual deafness. These animals were treated at an early period in the work, really before we had had much experience in puncturing the cistern and lumbar subarachnoid space, and the daily progress of the disease was not sufficiently followed, nor was the condition of the fluid of one of the treated animals determined previous to treatment. The fluid of the other animal showed but 4–6 cells per oil immersion field, and but 4 diplococci in the entire smear; the culture was nevertheless positive. Treatments were in one instance with Felton’s 3000 unit antibody solution, and in the other with unconcentrated antipneumococcus serum. The details of these treatments are not included, since from the behavior of the culture and of the control animal, we feel inclined to disregard this experiment. Suffice it to say that no subsequent test with really virulent culture has promised any duplication of this favorable result. The following protocol summarizes a more systematic study of the effect of treatment, and is introduced to show the behavior of a treated dog for which Dog 7, the animal described above in illustration of the massive pneumococcal overgrowth type of disease, was the control.
Dog 6.—Female bull terrier; weight 5 kilos. March 9, 1927, 3.50 p.m., ether; cistern puncture; clear fluid. Injected 7,000,000 Type I pneumococci (6 hour dextrose broth culture; growth 700 million per cc.). Good recovery.

18 hours—temperature 104.7°. Stands, wags tail, refuses food and water.

19 hours—ether; cistern puncture; lumbar puncture; lavage from lumbar to cistern with 20 cc. warm saline, followed by 15 cc. antipneumococcus serum; 5 cc. 800 unit antibody solution intravenously. Cisternal fluid opalescent; looks like a suspension of cocci. Smears of this fluid as follows:

Before lavage—cells 20–30 per oil immersion field; myriads of cocci.

After 15 cc. NaCl lavage—cells rare; cocci about 80 per o. i. f.

After 20 cc. NaCl lavage—cells rare; cocci about 60 per o. i. f.

24 hours—temperature 103°; ether; cistern puncture; seropurulent fluid; drainage. Injected 5 cc. antipneumococcus serum. Smear: numerous pus cells; cocci largely agglutinated and phagocyted; still numerous, but less than at the end of the previous lavage. 75 cc. intraperitoneal saline. Culture: confluent growth.

42 hours—temperature 101.6°. Weak, sluggish, able to stand. Ether, combined puncture; cloudy yellowish fluid; 20 cc. saline lavage, followed by 10 cc. antipneumococcus serum. Smear: before lavage, polymorphonuclears 30–150 per o. i. f.; cocci not increased over previous smear. After completing lavage, both cells and organisms very rare. Culture: confluent growth.

66 hours—temperature 101.7°. Refuses to stand; lethargic; shortly after coming to laboratory a generalized convulsion with salivation and gnashing. Series of bloody stools. Ether; combined puncture; cells 3–60 per oil immersion field; cocci diminished; many look swollen; bacillary; coccoid. 10 cc. antipneumococcus serum.

90 hours—dead. Autopsy: Cord grossly normal; vessels in region of cerebellum and medulla injected; posterior convexity injected; fibrinopurulent material over anterior convexity and base. Thin seropurulent material in lateral ventricles. Smears—cistern—cells 125 per o. i. f.; cocci largely phagocyted. Cord—cells 50 per o. i. f.; cocci very scanty. Anterior convexity—cells 200 per o. i. f.; cocci diffuse with only slight phagocytosis. Lateral ventricles—cells 30–40 per o. i. f.; cocci diffuse with marked phagocytosis. Heart's blood culture—no growth. Viscera: acute intussusception (the cause of death). Microscopically the cord showed relatively little exudate, but the latter was very abundant over the convexity and cerebellum and in the fourth ventricle.

Summary: A very marked initial growth of cocci; clearing by repeated lavage; abundant phagocytosis of residual cocci following antiserum injections but a focus, especially over the anterior convexity, not appreciably reached by serum and thereby constituting a site for subsequent "reinfection" of other regions. Objectively a very decided benefit from treatment.

In view of the fact that it appeared quite possible to lavage certain regions of the central nervous system relatively free from organisms
and to reduce residual pneumococci by the phagocytosis resulting from the injection of immune serum, it was considered desirable to extend, if possible, the field of action of the serum to those portions of the brain not reached by lumbar or cisternal injections. It was determined to treat the convexity by subdural serum injections, after trephining over the frontal lobes. The question arose as to whether one trephine was sufficient, or whether the falx constituted such a complete barrier that bilateral trephining was necessary. To settle this point a single frontal trephine was made just above the frontal sinus in a normal dog under ether anesthesia and 2 cc. of methylene blue was injected. The animal later received likewise 2 cc. intracisternally. The methylene blue injected frontally passed rapidly to the cisterna and with the additional cisternal injection was almost immediately recovered from the lumbar subarachnoid space. The dog was etherized after 1 hour. Staining of the brain was, as far as the convexity was concerned, sharply limited to the hemisphere of the injected side (Fig. 3). This made it obvious that to treat the infected convexity bilateral trephine openings were necessary. The following protocol summarizes one of the early animals treated by quadruple puncture.

Dog 10.—Fox terrier, female; weight 7.5 kilos. March 18, 1927, 3.15 p.m., ether; cistern puncture; clear fluid. Injected 8,400,000 Type I pneumococci (6 hour dextrose broth culture; growth 1,400,000,000 per cc.). Good recovery.

21 hours—temperature 99.8°. Sluggish; refuses to stand; will not drink. Ether; lumbar puncture; cells 3-5 per o. i. f.; no cocci seen, but culture positive. Cistern puncture; cells 15-25 per o. i. f.; rare cocci; culture, marked growth but not confluent. Lavage lumbar to cistern with 15 cc. saline followed by 20 cc. antipneumococcus serum. Cistern needle withdrawn and left frontal trephine done. Injected very slowly 6 cc. serum. In view of the few organisms in the smear, it was felt that a second frontal trephine was not necessary.

45 hours—temperature 103.6°; unchanged clinically. No treatment.

66 hours—temperature 103.7°; clinically unchanged.

70 hours—diagnostic lumbar puncture; cells 3-5 per o. i. f.; cocci numerous.

72 hours—cistern and lumbar punctures; lavage of 15 cc. saline lumbar to cistern; followed by 15 cc. serum. First cisternal smear shows several hundred pneumococci per field; smear following treatment shows diminution and agglutination of cocci. Animal left head down for 30 minutes.

90 hours—temperature 102.7°; irritable but otherwise not notably worse. Cistern and lumbar tap. Cisternal fluid, yellow, purulent; contains about 150 cells per field; cocci markedly decreased and phagocyted. Culture: confluent growth. Lavage lumbar to cistern with 15 cc. serum. Rapid ether recovery.
114 hours—temperature 100.9°; weakness of hind legs; irritable; cistern and lumbar punctures; fluid nearly clear. Second trephine done.

Smears: lumbar—rare cells; no organisms seen. Cistern—rare cells; rare agglutinated pneumococci.

Cultures: fair growth.

Injected 2 cc. serum into each frontal trephine. 6 cc. lumbar subarachnoid space. 4 cc. cisterna.

Since sterilization was incomplete after several treatments, it was decided to test out the efficacy of ethylhydrocupreine hydrochloride (optochin) intrathecally. This effect is apparent in the continued protocol.

138 hours—temperature 99.4°. Unable to stand; lethargic. Lumbar and cistern punctures under ether. Cisternal smear: cells 5–10 per o. i. f.; cocci 15–20. Lavage with 15 cc., 0.0002 per cent ethylhydrocupreine hydrochloride in saline. Normal recovery.

168 hours—temperature 102°. Lumbar and cistern punctures under ether; cisternal fluid purulent; cocci very numerous. Injected 6 cc. 0.02 per cent optochin.

186 hours—usual double puncture; fluid contains hundreds of pus cells per field; cocci are diminished and markedly phagocytized. Injected 8 cc. of mixture of 15 cc. 0.2 per cent optochin and 5 cc. serum. Breathing ceased but was immediately renewed upon giving artificial respiration. Culture: rare colonies.

210 hours—dead. Autopsy: Moderate exudate over cord; brain congested; easily broken, fibrinous adhesions about cistern and over convexity; moderate seropurulent exudate at base and in both lateral ventricles; the latter are considerably dilated (Fig. 4). A small puncture wound in the floor of the fourth ventricle, just above calamus, with small hemorrhage extending into the central canal of the cord; this was the probable cause of death and is the inevitable result of frequent successive punctures into a dangerous region where working space is small. Smears from all regions show pneumococci—scanty in the cord, moderate in cistern and over convexity, fairly abundant at base and in lateral ventricles. The cellular reaction parallels the pneumococcus distribution in intensity. The principal feature in all smears is the extraordinary amount of phagocytosis and destruction of pneumococci. Many cells are loaded with bacterial débris (Fig. 5). Cultures: all regions positive; heart blood: no growth.

Summary: A mild initial infection; first treatment incomplete; considerable delay in instituting subsequent treatments; the latter usually not complete. Finally recourse to optochin in low concentration, resulting in exacerbation in growth of organisms. Gradual tendency toward sterilization with higher drug concentrations; course interrupted by traumatic death. A total of seven treatments, usually only partial in distribution.
As will be seen from the protocol, the initial growth of organisms in the spinal fluid was described as mild. We have made certain observations as to the rate of increase of these mild initial growths; for example, the fluid in one dog infected with 4 ½ million pneumococci is described as follows: 19 hours, 7 diplococci found in the entire smear; 23 hours, an average of 10 per oil immersion field; 42 hours, upwards of 100 cocci per field. The speed of progress of the infection becomes very evident and the significance of even a delay of a few hours in instituting treatment is obvious.

One dog afforded opportunity to compare the difference in numbers of pneumococci in washed and unwashed areas of cortex after a single large lavage, followed by optochin.

Dog 17.—Fox terrier, female; weight 5 kilos. March 30, 1927, 4.40 p.m., ether; cistern puncture; clear fluid. Injected 5,200,000 Type 1 pneumococci (6 hour dextrose broth culture; small transplant; growth 260 million per cc.). Good recovery.

24 hours—temperature 100.7°; in bad condition; irritable; unable to stand; convulsive. Morphine; ether; combined cistern and lumbar punctures; fluid cloudy; cells fairly numerous; hundreds of cocci per field. Lavaged from lumbar to cistern 50 cc. warm saline followed by 7 cc. 0.2 per cent optochin. On the 7th cc., respiration ceased, pulse continued strong, but short period of artificial respiration failed to revive; a drug death. Autopsy showed the cord, cistern, and cerebellum covered with a moderate film of exudate; a small triangular area of cortex just above the cerebellum was similar in appearance, but the base and balance of the cortex, where no lavage had penetrated, were overlaid with thick yellow pus and myriads of cocci. There was moderate bacteremia. Figs. 6 and 7 are offered for comparison of smears from washed and unwashed cortical areas. We are, of course, assuming that the initial distribution of cocci was the same.

This animal died from the effect of the drug on the respiratory center, and the manner of death typifies that which we have regularly seen with overdoses of drug. Respiration suddenly becomes shallow and slow, but pulse continues strong until some time after breathing has ceased. This effect on the respiratory center is transitory, and if artificial respiration is vigorously applied, with, if necessary, the addition of intracardiac adrenalin, it is almost always possible to revive the dog. When large concentrations of drug were used, respiratory difficulties were common, but deaths were few; artificial respiration was in two instances maintained for 20 minutes, during which time the
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pulse ceased, only to be renewed by adrenalin injections. Recovery ensued.

A word may be said about the drug concentration which has seemed safe. This has been found not to exceed that present in a mixture of 15 cc. of antiserum and 0.75 cc. of 1 per cent optochin. When no antiserum is used, this dose is too high; a safe maximum has not been determined for drug not diluted with serum. If much pus is present, an animal may withstand higher concentrations of drug than would otherwise be the case; if lavage is very effective and the return fluid practically clear, the drug concentration should never exceed that given above. Great caution should be observed if the fluid return through the cistern needle is blocked. With excellent drainage, the drug effect on the respiratory center appears a function of percentage concentration of optochin in the lavaging fluid, rather than one of total cc. of mixture lavaged through the meninges.

Having determined the dosages of optochin-serum mixture which were tolerated with little or no respiratory disturbance, systematic treatments were again undertaken. The results are evident from the following representative protocols. For convenience, we are illustrating the type of result obtained in mild initial infections and comparing these with that secured in severe initial infections.

Dog 25.—Female hound; weight 7½ kilos. April 25, 1927, 3.45 p.m., ether; cistern puncture; clear fluid. Injected 10,000,000 Type I pneumococci (6 hour dextrose broth culture; growth 800 million per cc.). Good recovery.

19 hours—temperature 103.4°. Sluggish; has eaten. Morphine; ether; lumbar and cistern punctures; fluid cloudy; contains 15-20 cells per o. i. f., and about 1 diplococcus to every 3 fields; lavage attempted, but fluid came through with great difficulty and was blood-tinged. Lavage consequently abandoned. After drainage a mixture of serum 10 cc. and 1 per cent optochin 0.5 cc. was distributed equally between cord and cistern. Double frontal trephines were done and 3 cc. of a similar mixture introduced on each side. Cistern culture, abundant growth.

42 hours—temperature 101.3°. Sluggish; lateral nystagmus (traumatic?). Morphine; ether; double puncture; yellow opalescent fluid; cells 15 per o. i. f.; only 1 coccus seen; culture grew but 2 colonies. Injected 10 cc. saline with poor recovery; followed by 5 cc. optochin-serum mixture in lumbar subarachnoid space, and 2 cc. in each frontal. Good recovery.

66 hours—temperature 99°. No treatment. Subsequent uneventful recovery.

Dog 26.—Female hound; weight 5½ kilos; a companion dog to the preceding. Infected with 8,000,000 pneumococci from the same culture.
20 hours—temperature 102.9°. Sluggish. Morphine; ether; quadruple puncture. Cisternal fluid cloudy; cells 15–30 per o. i. f.; cocci average 1 to every 2 fields. Saline lavage lumbar to cistern successful at first, but return then failed; repeated washing and draining via cisternal needle. Injection of mixture 15 cc. serum and 1.5 cc. 1 per cent optochin, divided 5 cc. cord, 5 cc. cistern, 2 cc. each frontal. Culture, abundant growth.

42 hours—temperature 102°. Clinically normal; usual double puncture, lumbar and cistern. Fluid clear; contains 2–3 cells per field, about half small lymphocytes. Introduced 6 cc. serum into lumbar subarachnoid space and placed head downward. No drug; no frontal treatment. Culture sterile.

66 hours—temperature 101°. No treatment. Remained unchanged until the 16th day. Found prostrate and convulsive; snapped and salivated and was thought to have rabies. Chloroformed. Typical pneumococcus meningitis, generalized over convexity and base; cord relatively free. Smears show large numbers of pus cells and diplococci; fairly good phagocytosis. Ventricles dilated; purulent ventriculitis. Type I pneumococci recovered from all regions including even the ethmoid cells.

Summary: Mild initial process; sterilization incomplete; reinfection delayed and from some focus so small that in the period between the first treatment and the cistern tap, 24 hours later, no reinfection of the cisternal fluid had occurred, thereby giving a false negative culture.

The subsequent protocol is illustrative of the importance of residual loci in reinfecting meninges not completely sterilized. Experiments such as this are the type which convince us of the inefficacy of partial lavages as practiced by Kolmer.

Dog 20.—Female airedale; weight 9½ kilos. Has canine distemper. April 6, 1927, morphine; ether; cisternal puncture; clear fluid; injected 10,500,000 Type I pneumococci (6 hour dextrose broth culture; growth 1,200,000,000 per cc.). Good recovery.

19 hours—temperature 102.8°; lethargic; morphine; ether; lumbar and cistern punctures; fluids purulent; contain several hundreds of cells and hundreds of pneumococci per o. i. f. (Fig. 8). Essentially no phagocytosis; a very severe infection. Lavage lumbar to cistern with 30 cc. saline followed by 11 cc. of mixture of serum 10 cc. and 1 per cent optochin 0.5 cc. Double frontal trephine with injection of 3 cc. of similar mixture on each side; 10 cc. of 800 unit antibody solution (Felton) intravenously; spinal fluid culture, confluent growth.

24 hours—temperature 100°. Ether; double puncture; lumbar and cistern; fluid clearer; cells 50 per o. i. f.; some contain phagocyted pneumococci (Fig. 9); practically no free organisms; culture, scattered colonies. Lavage lumbar to cistern with 10 cc. saline followed by 0.035 per cent optochin-serum 7 cc.; no frontal treatment.
43 hours—temperature 98.8°; sluggish but otherwise unchanged; morphine; ether; lumbar and cistern punctures; fluid yellowish, opalescent; cells 10–35 per o. i. f.; no organisms seen. Injected 6 cc. serum, lumbar subarachnoid space and placed head down. 75 cc. intraperitoneal saline. Culture yielded about 300 colonies from a 0.5 cc. planting.

65 hours—temperature 102.7°; quite ill; nose a mass of thick pus; cistern and lumbar punctures; fluid a culture of pneumococci, hundreds per field (Fig. 10); cells rare; no agglutination nor phagocytosis. Represents a massive reinfection of a nearly sterile region, following cessation of complete treatments. Injected 8 cc. 0.05 per cent optochin-serum mixture divided between cord and cistern and 3 cc. into each frontal.

90 hours—temperature 99.6°; weaker; able to stand; ataxic; no change in distemper; ether; cistern and lumbar punctures; no fluid obtained. Gave 5 cc. lumbar, 1 cc. cistern, 3 cc. each frontal of 0.1 per cent optochin-serum. Intraperitoneally 150 cc. 10 per cent glucose; milk by stomach tube.

114 hours—temperature below 94°; chloroformed. Autopsy: Material suggesting chicken fat clot in fourth ventricle, extending down over cervical cord. Explains the last "dry tap." Fibrin at base, about pituitary and optic chiasm. Cord elsewhere relatively clear; anterior convexity relatively clear; meninges over posterior convexity opaque; exudate purulent; some pus in lateral ventricles.

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<th>Region</th>
<th>Cells per o. i. f.</th>
<th>Cocci</th>
<th>Culture</th>
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<tr>
<td>Cord (lumbar)</td>
<td>5–10</td>
<td>Rare phagocyted; very rare free</td>
<td>0</td>
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<tr>
<td>Cistern</td>
<td>20–75</td>
<td>Moderate; free and phagocyted</td>
<td>+</td>
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<tr>
<td>Anterior convexity</td>
<td>5–25</td>
<td>Only 2 cocci in entire smear</td>
<td>0</td>
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<td>Posterior &quot;</td>
<td>75</td>
<td>Rare; phagocyted</td>
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<td>(Fig. 11)</td>
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<td>Base</td>
<td>25</td>
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<td>Lateral ventricle</td>
<td>75</td>
<td>Moderate, phagocyted; more free</td>
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A pneumonia practically lobar in distribution, entire left lung; culture, bronchisepticus. Negative heart's blood.

Summary: Initial massive infection; tendency to sterilization with treatment; "reinfection" with cessation of treatment; renewed tendency toward sterilization with resumption of treatment. Main focus of infection lateral ventricle; course of disease interrupted by fatal outcome of distemper.

As examples of severe infections sterilized slowly and progressively by successive complete treatments in one instance, seven in all, necessitating altogether ten etherizations, partially for treatment and partially for diagnosis, the following may be offered.
Dog 29.—Male hound; weight 7½ kilos. May 2, 1927, 4.00 p.m., morphine; ether; cistern puncture. Clear fluid. Injected 10,000,000 Type I pneumococci (6 hour dextrose broth culture; growth 680 million per cc.). Good recovery.

24 hours—temperature 102.2°. Sluggish; lumbar puncture; fluid under increased tension, opalescent, yellowish; cells 50 per o. i. f.; diplococci 5-12 per field. Not treated. Culture, confluent growth.

42 hours—temperature 103.7°. Sluggish but able to stand. Morphine; ether; quadruple puncture; lumbar fluid yellowish, opalescent; cells 8-10 per o. i. f.; diplococci about 100 per field; similar cisternal fluid (Fig. 13). Lavage attempted, but proceeded with difficulty and with poor return through cisternal needle; abandoned. Injected 7 cc. of mixture of serum 10 cc., optochin 1 per cent, 0.5 cc. cisterna, 3 cc. lumbar, and 2 cc. similar mixture in each frontal subarachnoid region. Cultures, confluent growth.

66 hours—temperature 102.9°. Clinically unchanged. Morphine; ether; quadruple puncture. Cisternal fluid clearing; cells rare; diplococci less than 20 per field. Lavaged lumbar to cistern with 10 cc. of saline; injected serum-optochin mixture as above. Gave 100 cc. intraperitoneal glucose. Culture, confluent growth.

90 hours—temperature 102.8°. Unchanged. Quadruple puncture; fluid more cloudy; cells 20-40 per field; cocci double in number. Last treatment repeated. No lavage. Culture, confluent growth.

96 hours—treatment repeated with exception of frontal injections. Cisternal fluid cloudier; cells increased but what few pneumococci are present are all phagocyted. Culture, few colonies.

114 hours—temperature 102.6°. Clinically unchanged. Morphine; ether; cisternal puncture; fluid scanty; 6-7 diplococci per field; culture, colonies increased. Complete treatment as above with exception of left frontal; no lavage.

138 hours—temperature 104°; unchanged; has eaten and drunk; stands and wags tail. Ether; quadruple puncture; lavage lumbar to cistern with 15 cc. serum-optochin mixture (serum 15 cc., optochin 0.75 cc. of 1 per cent solution); 1.5 cc. same mixture, both frontal trephines. Fluid clearing; cells 5-10 per field; no cocci seen. Culture, scanty growth.

162 hours—temperature 103.7°; canine distemper; less active; will not stand without assistance. Ether; lumbar and cistern punctures; cells 4-5 per field; no cocci; culture, sterile; lavage with 14 cc. serum-optochin mixture as above. Intraperitoneal glucose, 100 cc.

186 hours—temperature 102.7°; distemper worse; thick pus flowing from nostrils. No treatment.

210 hours—unchanged; diagnostic cistern puncture; rare cells; no cocci; injected 5 cc. of serum as prophylactic; culture, negative.

288 hours—very ill from distemper. Subnormal temperature. Chloroformed. Bilateral bronchopneumonia (B. bronchisepticus). Entire brain and cord grossly normal save for slight excess of small lymphocytes and endothelial leucocytes in
PNEUMOCOCCAL MENINGITIS. II

cisternal and ventricular fluids. Cultures taken from all regions, including the entire fluid contents of the lateral ventricles, failed to give growth.

Microscopically some slight generalized infiltration of the meninges by endothelial leucocytes, many fatty, a few with blood pigment; a typical late clearing up stage. In addition, collections of lymphocytes in the region just beneath the ependyma of ventricles. An organizing (purpuric?) hemorrhage of gray matter of cord.

_Dog 32._ Female hound; weight 5 kilos. May 4, 1927, 4.05 p.m., morphine; ether; cistern puncture; clear fluid. Injected 7,500,000 Type I pneumococci (6 hour dextrose broth culture; growth 750 million per cc.). Good recovery.

22 hours—temperature 104°; sluggish; irritable. Ether; lumbar puncture; fluid seropurulent; cells 15-20 per o. i. f.; cocci upwards of 150 per field (Fig. 14); cisternal fluid opalescent; cells rare; cocci 20-30 per field. Lavage, lumbar to cistern with 15 cc. saline. Frontal trephines; injection of mixture of 15 cc. antiserum and 0.75 cc. 1 per cent optochin as follows: lumbar subarachnoid space, 7 cc., cisterna magna, 3 cc., each frontal trephine, 2 cc. Culture, confluent growth.

42 hours—temperature 102.4°; stands weakly; attempts to walk; drinks. Ether; quadruple puncture; cisternal fluid clearing; cells 20-30 per field; no cocci seen; culture, colonies numerous but growth not confluent; injected serum-optochin mixture (as above) 7 cc. lumbar, 3 cc. cistern, 1 cc. each frontal.

66 hours—temperature 101°; marked loss of weight; weakness hind legs. Ether; quadruple puncture; fluid almost clear; cells 2-3 per field and cocci about 1 in every 2 or 3 fields; culture, colonies increased. Treatment as at 42 hours. Intraperitoneal glucose.

90 hours—temperature 98°; definite partial paraplegia hind legs; probably traumatic. No treatment.

114 hours—temperature 98.6°; unchanged; diagnostic cistern puncture; abundant crystal clear fluid; lymphocytes less than 1 per field; no organisms. Plated 2 cc. of fluid; no growth.

138 hours—temperature 99.3°; diagnostic puncture; negative culture. Subsequent course uneventful; persistent partial paraplegia.

The following may be offered as an example of a case where two complete treatments failed to sterilize the meninges appreciably. The condition was complicated by distemper.

_Dog 34._ Young female collie pup. May 10, 1927, 4.00 p.m., ether; cisternal puncture; clear fluid. Injected 9,000,000 Type I pneumococci (6 hour dextrose broth culture; growth 610 million per cc.). Good recovery.

19 hours—temperature 104°; sluggish; has developed severe distemper.

23 hours—ether; quadruple puncture; cistern and lumbar fluids seropurulent; cells 50-75 per field; diplococci very numerous. Lavage lumbar to cistern with
20 cc. saline, followed by lavage with serum-optochin mixture (serum 15 cc., 1 per cent optochin 0.75 cc.), lumbar to cistern 15 cc.; frontal trephines 2 cc. each of mixture of similar proportions. Intraperitoneal glucose, 100 cc. Culture, confluent growth.

42 hours—temperature 101.5°; unchanged. Quadruple puncture; complete treatment as above; 100 cc. glucose intraperitoneally. Smear: an 80 per cent reduction in organisms; cells average 12 per field. Culture, confluent growth.

66 hours—temperature below 94°. Chloroformed. In gross the brain and cord show only a trace of exudate; microscopically smears contain relatively few cells; diplococci present in large numbers and save in the cord region, where there is some phagocytosis, no evidence is seen of beneficial effect of treatment. Marked purulent bronchitis, but death is undoubtedly meningeal. Cultures all give confluent growth; heart’s blood, rare colonies. Histologically severe, diffuse leptomenigitis.

Comments.

Since optochin is somewhat under suspicion in view of its apparent tendency to produce transient amblyopia, we have endeavored to detect visual disturbances in recovered dogs. So far as was ascertained, none occurred; no ophthalmoscopic examinations were made. Some of the recovered dogs are deaf, but we know that in meningococcal meningitis permanent deafness may result.

A word may be said about lateral ventricular punctures. This has not been done in the dog; it is fairly certain that sterilization would be hastened and that more recoveries would have resulted had this been a routine procedure. Whereas the lateral ventricle of a dog, in the region where puncture is desirable, is a mere slit unless pathologic dilatation has occurred, it should offer no great problem in man. It is apparently the most difficult region to reach with serum by the methods applied in this study. In this respect the base in the region of the chiasm shares with the lateral ventricle and in case these methods should ever be employed in treating human disease, failure to sterilize by the quadruple puncture route should make one think seriously of ventricular punctures and punctures through to the base. The important thing in securing a cure is contact of all regions with the optochin-serum mixture—contact complete and frequently repeated. Undoubtedly lavage is a valuable adjuvant, but lavage alone cannot rid the meninges of organisms and if even a very small number remains, our experience shows that severe reinfection is almost inevitable. The object of treatment should be to wash out as many
organisms as possible and to control the residual bacteria by a pneumo-
coccidal drug and a phagocytosis-producing antiserum.

One of the most hopeful and surprising things observed in the entire
study was the manner in which lavage may be accomplished even in
the presence of a massive exudate, it being possible to lavage a cord in
an animal having almost frankly purulent cisternal and lumbar fluids,
until the return fluid is nearly crystal clear; full cognizance is, however,
taken of the fact that no matter how long one washes, within
reason, cells and pneumococci blocked in fibrin meshes, the former
certainly in large numbers, will remain. It has seemed that it is easier
to lavage out organisms than it is cells, since the latter are larger and
the network of fibrin offers more resistance to their free passage. As
judged by the pictures of phagocytosis in foci fairly distant from the
site of injection, the immune serum penetrates the exudate very satis-
factorily.

No statistics of cures are presented. In view of the prevalence of
traumatic deaths after frequently repeated cistern punctures, drug
deaths, when high optochin concentrations were used, and especially
mortality from epidemic canine distemper, such statistics would be
meaningless. The fact that progressive sterilization of the meninges
by methods employed in this study is possible has been established in
our opinion without question.

Pathology.

The microscopic pathology has been studied in all animals dying of
the disease, and in certain recovered dogs which succumbed to inter-
current disease (distemper) after becoming sterile. Sections show a
rapidly spreading, fibrinopurulent leptomenigitis, which as early as
23 hours after infection may involve all regions of the meninges (Fig.
15). In a control dog, or in a treated animal in which the disease
was not checked, the exudate reaches large proportions; the cord dura
is invaded; the spinal nerve roots are involved (Fig. 16); there is an
inflammatory process in the epidural fat (Fig. 17). Invasion and
destruction of the choroid plexuses, superficial encephalitis, spread
via the Virchow-Robin spaces are common (Figs. 18–20). An empy-
ema of the third or fourth ventricle with extension into the central
canal of the cord may occur (Fig. 21). Probably, too, the latter may
be reached from the ventral fissure. Once in the central canal the process may destroy the ependyma and invade the region of the commissures, and from thence the cornua by following a perivascular route, giving rise to a myelitis. Invasion, at least to any great extent, of the lateral ventricles occurs relatively late, probably because of the direction of flow of the spinal fluid; in other words, the lateral ventricles lavage themselves. With the development of a blocking exudate over the convexity or in the fourth ventricle or aqueduct, empyemas with dilatation of the lateral ventricles inevitably result. With the subsiding of the process, polymorphonuclears disappear and are replaced by fatty and phagocytic endothelial leucocytes and small lymphocytes, which cells apparently may persist for some time. Such was the picture observed in Dog 29, Fig. 22 (see protocol). Slight subependymal and peripheral cortical gliosis may be found.

*Reinfection Experiments.*

Five recovered dogs, together with a control, were reinfected to see if any immunity to meningeal infection existed. These animals (Dogs 1–5) were reinoculated 103, 79, 63, 47, and 42 days respectively after primary infection. Four ran an atypical course. The first two developed a very high grade, early leucocytosis; in one of these no organisms were observed in the 24 hour spinal fluid smear, and a culture gave no growth. Dog 1 died of severe purulent meningitis in 114 hours. Dog 2, whose culture was sterile 24 hours after infection, showed organisms at 66 hours; the number of cocci in the smears increased very slowly and at 7 days they were no more numerous than frequently seen after the 1st day in a primarily infected animal. As organisms increased, leucocytes diminished; phagocytosis was rarely seen and can scarcely account for the slow development of the disease. In none of the dogs were agglutinins demonstrable in the spinal fluids. Dog 2 died the 9th day. Dogs 3 and 5 gave negative cultures, and smears contained but 20–30 leucocytes per field 24 hours after infection; by 66 hours fluids were crystal clear and sterile. Dog 4 ran a typical course, but was sterilized by two complete optochin-serum treatments. This sterilization was unusually rapid. The control dog died typically in 48 hours. Hence a degree of increased resistance remained within the meninges.
RéSUMÉ AND CONCLUSIONS.

1. Extensive acute, fibrinopurulent meningitis may be produced in dogs by the intracisternal injection of virulent Type I pneumococci.

2. Given an equal number of virulent infecting organisms, the rate of establishment of infection depends upon the phase of growth and the quantitative growth per cc. of culture plus an uncontrollable individual factor in the animal.

3. The pathology of pneumococcal meningitis is discussed. It resembles very closely the similar disease in man.

4. Systematic lavage and treatment with optochin-serum mixtures by the method of quadruple puncture, as described above, have resulted in cures of Type I pneumococcal meningitis in dogs.

5. The important factor in obtaining cures is to bring all regions of the meninges into frequent contact with the therapeutic agent.

6. In the absence of such contact, incomplete sterilization results and "reinfection" is almost inevitable.

7. Protocols show the necessity of repeated negative cisternal fluids, both on smear and on culture, before sterilization can be assured.

8. Recovered dogs subjected to meningeal reinfection show some degree of resistance.

BIBLIOGRAPHY.


EXPLANATION OF PLATES.

PLATE 16.

**Fig. 1.** Control Dog 3. Massive basal exudate.

**Fig. 2.** Control Dog 7. Smear from convexity. × 1000.

**Fig. 3.** Distribution of dye introduced via left frontal trephine.

PLATE 17.

**Fig. 4.** Treated Dog 10. Dilated ventricles; purulent ventriculitis.

**Fig. 5.** Treated Dog 10. Ventricular smear showing extensive phagocytosis after ethylhydrocupreine hydrochloride treatment. × 1000.

**Fig. 6.** Treated Dog 17. Smear from posterior (lavaged) cortex. × 1000.
Plate 18.

Fig. 7. Treated Dog 17. Smear from anterior cortex unaffected by lavage and chemo-serotherapy. × 1000.

Fig. 8. Treated Dog 20. Initial 19 hour cisternal smear. × 1000.

Fig. 9. Same dog. 24 hour smear (after one treatment). × 1000.

Fig. 10. Same dog. 65 hour smear, showing result of cessation of treatment. × 1000.

Plate 19.

Fig. 11. Same dog. Smear from posterior convexity taken at 114 hours. Rare phagocyted cocci. × 1000.

Fig. 12. Same dog. Lateral ventricle smear. × 1000. Lateral ventricle a residual focus of infection.

Fig. 13. Recovered Dog 29. Cisternal smear before treatment. × 1000.

Fig. 14. Recovered Dog 32. Cisternal smear before treatment. × 1000.

Plate 20.

Fig. 15. Control dog, showing extent of exudate at 23 hours. × 50.

Fig. 16. Control dog. Massive exudate. Invasion of spinal nerve. × 50.

Fig. 17. Treated dog. Accidental death. Inflammatory exudate in epidural fat. × 50.

Fig. 18. Control dog. Purulent ventriculitis; superficial encephalitis; destruction of choroid plexus of lateral ventricle. × 50.

Plate 21.

Figs. 19 and 20. Control dog. Perivascular infiltration of cortex. × 50.

Fig. 21. Control dog. Empyema of central canal of cord; myelitis. × 50.

Fig. 22. Treated Dog 29. Sterile cultures; death from distemper; meninges essentially negative. × 50.
PLATE 18.

[Images of bacterial cultures or medical samples related to the study of pneumococcal meningitis.]
(Stewart: Pneumococcal meningitis. 11.)