

1920

Monographs of the RIMR. Vol. 12, 1920

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MONOGRAPHS OF THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH

No. 12

March 25, 1920

A STUDY OF EXPERIMENTAL MENINGITIS

A SERIES OF PAPERS FROM THE ARMY NEURO-SURGICAL LABORATORY

By

LEWIS H. WEED, M.D., PAUL WEGEFORTH, M.D., JAMES B.
AYER, M.D., AND LLOYD D. FELTON, M.D.



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Baltimore.)

PLATES 1 TO 17.

(Received for publication, May 15, 1919.)

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INTRODUCTION.

Among the major problems presented for investigation to the Army Neuro-Surgical Laboratory was the question of generalized infections of the central nervous system, in relation particularly to the possibility of successful therapy in the non-epidemic forms of purulent meningitis. Such cases were to be expected among the casualties of war in fairly large numbers, especially following open wounds of the head. These complicating infections of the meninges have not thus far proved amenable to surgical or other therapeutic measures; it was hoped that an experimental study of the basic factors concerned in these infections might ultimately prove of value. For the immediate purpose it was necessary to find microorganisms capable of producing in the common laboratory animals an acute and invariably fatal meningitis. The pathogenicity of the microorganisms should ideally be of such a standard that infections analogous to those of man would follow the subarachnoid introduction even of small numbers.

The search for microorganisms of suitable virulence has been quite successful; one strain of the *Bacillus mucosus capsulatus* group has produced acute and invariably fatal meningeal infections in the experimental animals. The maintenance of this high degree of infectivity in culture was soon found impossible and direct passage of the bacteria through the subarachnoid space was required. And in this phase of the work a relationship between the intrameningeal and the intraperitoneal pathogenicities was indicated.

But in larger part the activities of the Army Neuro-Surgical Laboratory were directed to the study of the hematogenous form of meningitis. An observation upon the production of meningitis by intravenous inoculation was followed by an extended investigation of certain of the factors which facilitate infection of the meninges. In these experiments the microorganisms which on subarachnoid inoculation proved to be of greatest virulence, also proved of greatest value in producing meningitis on intravenous injection; under these conditions, however, the process of meningeal infection required an addi-

tional procedure of physiological facilitation when the intravenous dosage was suitable. The investigation of these more fundamental aspects of the problem has seemed amply justified by the need for such data in the determination of the ultimate prophylaxis and therapy of the infections of the meninges.

There are gathered together in this volume the papers which represent the record of this study of experimental meningitis. The five chapters are in reality but separate phases of one investigation; the individual researches are credited to the proper members of the staff of the Army Neuro-Surgical Laboratory. The incorporation of the separate communications into this monograph has seemed desirable, as it presents under one cover the complete record of the experimental production of meningitis, both by subarachnoid and intravenous inoculation, of the maintenance and increase of the intrameningeal virulence of microorganisms, and of the pathological control of the experimental lesions.

Other papers from this laboratory dealing with the clinical aspects of the general problem and with the therapeutic measures employed in attempts to cure an otherwise fatal experimental meningitis have been arbitrarily excluded from this volume. Thus, the report of clinical observations, indicating a possible importance of certain phases of the experimental work, has already appeared in another journal (Wegeforth and Latham). The results of therapeutic irrigation of the meninges and of the effect of subarachnoid injection of antiseptics upon the central nervous system have likewise been published elsewhere (Weed and Wegeforth, Wegeforth and Essick).

I. THE PRODUCTION OF EXPERIMENTAL MENINGITIS BY DIRECT INOCULATION INTO THE SUBARACHNOID SPACE.

BY LLOYD D. FELTON, M.D., AND PAUL WEGEFORTH, M.D.

In the experimental study of the non-epidemic forms of purulent meningitis, it was soon found essential to employ microorganisms of marked virulence within the meninges of the animals chosen. Such microorganisms should be capable of producing infections within the central nervous system analogous to those seen in man; their pathogenicity should be uniform to the extent that after inoculation within the subarachnoid space a constant lesion could be predicted.

This requirement of relatively great intrameningeal virulence of the organism was largely based on the necessity for uniform controls when therapeutic procedures were instituted. But in a general consideration of the non-epidemic forms of meningitis the reactions of the experimental animal to the subarachnoid injection of many strains of microorganisms are also of importance. This chapter will deal with the results of our experiments in the search for microorganisms of great original pathogenicity within the meninges and will offer opportunity for comments on the relative virulence of the different strains of microorganisms tested. The data given here will, it is hoped, be of service to others in the study of the various problems of infections of the meninges.

The cat was selected for the major portion of the experiments, chiefly because the extensive employment of this animal in the study of the physiology of the nervous system made possible a broader use of the literature for the interpretation of many of the observations. Then, too, the animal is convenient in size and, even during war times, is available in sufficient numbers. Not much could be learned from the literature concerning the resistance of the meninges of this animal to infection. In consequence it was decided to conduct a series of routine experiments consisting of the direct inoculation of the subarach-

noid space with a large variety of organisms. The injections were made both through the occipito-atlantoid ligament (cf. Wegeforth, Ayer, and Essick) and the lumbosacral ligaments, although the former route proved more satisfactory. Since the relative pathogenicity of the organisms within the subarachnoid space was not known, an initial dosage of a cubic centimeter of a 24 hour broth culture was employed in most cases. If such an inoculation gave evidence that the animal was susceptible to infection with the organism, its virulence was titrated, and if the number necessary to produce a fatal infection was great, attempts were made to raise this virulence. Organisms which did not show on initial inoculation some indication of an original infectivity within the subarachnoid space were discarded.

The degree of the reaction caused by the activity of an organism in the meninges was determined by the clinical manifestations of the animal, the characteristics of the cerebrospinal fluid, and the pathological changes in the central nervous system seen at autopsy. Certain clinical signs, valuable in the diagnosis of acute infection of the meninges in man (variations in pulse rate and temperature) were found to be unreliable, as great variations occurred in normal animals. Examination of the eye-grounds was made in many instances but proved of no diagnostic importance. The general physical manifestations of animals suffering from meningitis are, however, definite enough to permit a diagnosis of the lesion in a large percentage of cases, so that a classification into various types of acute infection, as well as those of a chronic nature, could be made. The acute cases in these cats were classified as fulminating, ending in death within 24 to 48 hours, or protracted, ending sometimes in death after several days or going on to recovery. The clinical symptoms of the acute cases varied somewhat. The more severe were characterized by general prostration, convulsions, and extensor rigidities of the muscles, accompanied by frequent spontaneous cries. The more active manifestations, such as convulsions and outcries, appeared in paroxysms, so that, were the animal seen during a quiescent period, little could be noted aside from a slight lethargy. Under such conditions forcible retraction of the head was followed frequently by reactions which were characteristic of the individual cat at that time. A normal cat, unless wild, would allow its head to be retracted until the nose touched the

spine in the thoracic region without offering resistance; on releasing the head the original position would be resumed comparatively slowly and without ataxia or loss of balance. During such a manipulation in a cat suffering from acute meningitis, the neck was found to be stiff and a definite resistance to the procedure was encountered. When, after retraction, the head was released, it was thrown forward to its original position as if impelled by a spring. Immediately following this, the animal was seized with convulsions, the muscles of the entire body quivered and contracted, the limbs were held in an extensor rigidity, the tail was bushed, the eyes protruded, and the animal cried out. In other cases of the acute type these violent reactions were absent entirely and there was nothing in the behavior of the animal characteristic of the disease. Often there was only a moderate degree of lethargy and an apparent apathy to suggest the possible presence of some acute infection.

The chronic type of meningitis exhibited clinically signs, such as ataxia, variations in gait (spasticity), weakness, and paralysis of certain muscle groups, which were probably the result of permanent destructive lesions in the central nervous system. The diagnosis of these cases often depended largely on the findings in the cerebrospinal fluid and the postmortem study of the nervous tissues. A complete presentation of the spinal fluid changes occurring in acute and chronic experimental meningitis has been made by one of us elsewhere (Felton). Likewise, the pathology of these conditions is reported in the following chapter of this communication.

EXPERIMENTAL INJECTIONS.

In the subarachnoid space of the cat, large quantities of most of the ordinary pathogenic bacteria were destroyed and it was only by testing out a large variety of microorganisms that eventually a few were found which had some original virulence in the meninges of the animal. A classification of the organisms used in this work, 21 varieties and 102 strains, is given in Table I. Many of the specimens were obtained from recent autopsies, while others were taken from cultures kept in the laboratory for stock purposes.

TABLE I.

Microorganism.	No. of strains.	Comparative lethal dose.
Group I.		
<i>Meningococcus</i>	4	One-half blood agar slant did not produce fatal infection.
<i>M. flavus</i>	1	One-eighth.
<i>Streptococcus hæmolyticus</i>	36	4 strains, 1 cc. of broth culture; remainder avirulent in 1 cc. dose.
<i>Staphylococcus albus</i>	2	Avirulent.
<i>Staphylococcus aureus</i>	10	1 cc. of undiluted broth culture.
<i>Pneumococcus</i>	3	0.5 cc. of a broth culture.
<i>B. Influenzæ</i>	1	1 cc. of 1:10 blood broth culture.
Group II.		
<i>B. gallinarum</i>	1	One-fourth of an agar slant.
<i>B. subtilis</i>	1	One-twenty-fifth of an agar slant.
<i>B. smegmatis</i>	1	2 cc. of a broth culture.
<i>B. cereus</i>	1	Avirulent.
<i>B. proteus zoppii</i>	1	"
<i>B. proteus mirabilis</i>	1	1 cc. of a broth culture.
<i>B. anthracoides</i>	1	1 " " " "
<i>Spirillum metchnikovi</i>	1	1 " " " "
<i>B. dysenteriae</i>	6	One-fourth of an agar slant.
<i>B. typhosus</i>	1	One-half " " " "
Group III.		
<i>B. coli</i>	17	1 cc. of a 1:250 dilution of a broth culture was the greatest dilution. Few strains above 1 cc. of an undiluted culture.
<i>B. pyocyaneus</i>	2	0.5 cc. of a 1:10 dilution of broth culture. Virulence developed up to 1:10,000.
<i>B. paratyphosus</i>	2	0.1 cc. of a broth culture. Virulence increased to 1 cc. of a 1:10,000 dilution of a broth culture.
<i>B. mucosus capsulatus</i>	9	The greatest virulence, 1 cc. of a 1:10,000,000,000 dilution of a broth culture; the lowest dilution, 1:1,000.

Meningococcus.—The success that Flexner and others have had in infecting the meninges of monkeys with this organism naturally made it the subject of one of the earliest trials. It was not our primary object to work with sera, for our problem was ultimately to consider

means of treatment in those forms for which specific remedies were not applicable. If, however, the meningococcus had produced a purulent meningitis in the experimental animals selected, it would have been of the greatest possible service. Not much was anticipated from this organism, for even in monkeys Flexner, to produce meningeal lesions, had to use large doses, a factor that we desired to avoid if possible. A recently isolated strain of this organism (normal) was given to two cats in doses of one-half to one-third of an agar slant (24 hours) beneath the arachnoid. Neither animal developed an acute meningitis. In the rabbit this microorganism was found to possess an original virulence for the meninges; this original pathogenicity, however, did not hold for the cat.

Micrococcus flavus.—Although *Micrococcus flavus* was used only in two instances, it gave in each a certain amount of encouragement. The first animal died in 3 days of a meningitis following a subarachnoid injection of one-eighth of an agar slant, rubbed up in Locke's solution. The second case is of sufficient interest to be given in brief.

This cat (No. 138) was given on Jan. 7, 1918, directly into the subarachnoid space through the occipito-atlantoid ligament, an injection of one-eighth of an agar slant of *Micrococcus flavus* (H. L. H. strain). The following day the animal was bright and active. It then gradually began to show signs of weakness and ataxia in the hind legs, so that by Jan. 18 there was little doubt but that some pathological, probably infectious, process was present in the central nervous system. The cerebrospinal fluid on this date was cloudy (no red blood cells); the globulin content was 0.9 gm. per liter, and *Micrococcus flavus* was present on culture. The physical condition of the cat did not improve and in 3 days another specimen of spinal fluid showed the same pathological changes. On Jan. 24 (17 days after inoculation) the animal was found dead. Autopsy showed a slight subacute leptomeningitis.

This case is unusual in that the microorganism was present in the cerebrospinal fluid for so long a period after the inoculation. Ordinarily a cat, after subarachnoid injection of an organism, either succumbs in a few days or completely overcomes the infection, so that the specimens of spinal fluid obtained more than 4 to 6 days after inoculation seldom contain bacteria. The experiments indicate that the original virulence of *Micrococcus flavus* for the meninges of the cat is such as to be useful in the production of both acute and subacute

infections. Such infections with a Gram-negative diplococcus would be analogous in many respects to the infections produced in man by the Weichselbaum organism.

Streptococcus.—Various strains of the streptococcus, all of the hemolytic type, both of human and feline origin, were used. Injections of 25 recently isolated strains of human *Streptococcus hæmolyticus* obtained from the army camps were made into the subarachnoid space of cats. A dose of 1 cc. of the undiluted broth culture was given directly into the cisterna cerebello-medullaris of young cats with the result that only two of the animals developed any demonstrable inflammation of the central nervous system. The animals were usually unaffected and were kept under observation for periods varying from 3 to 17 days after the inoculation. Three additional strains of streptococcus, obtained from cases of human meningitic exudate, were tried. The cats inoculated with two of these failed to develop meningitis. The other strain, when given in large doses of the undiluted culture into the subarachnoid space of two cats, elicited a meningitis, killing in 4 and 7 hours, respectively. As soon, however, as the culture was diluted to 1:20 an acute transient meningitis was produced, followed by recovery. Little better success rewarded the effort made with strains of the organism obtained from the pus of spontaneous abscesses occurring among the stock animals. Five strains were tried. Three of these caused no reaction at all, even when 1 cc. of undiluted broth culture was injected. One, a long chain variety, killed an animal within 24 hours after the intraspinal injection of 1 cc. of the straight culture. Its virulence was greatly diminished when diluted 50 times, the animal so injected living 8 days. Some time later (one month) with storage in the ice box, the organism was again tried in full strength and its virulence was found to have disappeared entirely. Another strain (No. 209) caused a very promising reaction when it was first tried. The animal came down slowly and the pathological picture was that of a typical meningitis, but after being transferred on ascitic broth culture every other day for a short period of time, this strain also lost its virulence. This phenomenon of rapid loss of virulence is, of course, well known for the streptococcus. The following protocol shows the effect produced by one of these strains of streptococcus after injection into the subarachnoid space of a cat.

Cat 224.—Adult male.

Feb. 1, 1918, 12.10 p.m. Ether. Occipito-atlantoid puncture, clear cerebro-spinal fluid obtained. Subarachnoid injection of 1 cc. of a 24 hour broth culture of streptococcus. Strain obtained from abscess in Cat 209.

Feb. 2. Cat in fairly good condition, though weak in hind legs. Appears to be sick.

Feb. 3. Animal found dead in cage.

Pathological Diagnosis.—Acute purulent lepto- and pachymeningitis with exudate in the central canal of the spinal cord.

Three days later another cat injected in the same way with this organism developed a subacute infection and did not die until 15 days after the inoculation.

While the streptococcus was found to have a certain degree of virulence for the meninges of the cat, its effects were not uniform. Because of this characteristic variability in pathogenicity, the streptococcus does not lend itself for use in experiments in which the relative values of therapeutic measures are to be determined.

Staphylococcus.—Twelve strains of staphylococcus, including both *aureus* and *albus*, recently isolated from human and feline infections, were investigated. This series consisted of ten experiments, in which subarachnoid injections of 1 cc. of the undiluted culture of different strains were made. Three of the cats so inoculated died of a meningitis within 24 hours; the source of one of these strains was an abscess of a cat, while the other two were of human origin. Thirteen inoculations in which cultures of various dilutions (1:5 and 1:100) were injected intraspinaly failed to produce meningitis. It was thought that if the cultures were injected in such a way that the organism would be distributed over a large area of the central nervous system, a more marked effect might be obtained. Two experiments were therefore carried out with combined lumbar and occipito-atlantoid punctures. A syringe-full of the culture was then injected through the lumbar needle and the excess allowed to flow off through the occipital needle. In this way the entire cord was bathed with a culture of the organisms. In one case a dilution of 1:100 of the culture was used for this purpose, and in the other the undiluted broth culture was introduced. Neither animal showed subsequently evidences of infection.

Pus.—The uncertainty of meningeal infections from the staphylococcus and the streptococcus suggested subarachnoid injections of diluted pus, for it is well known that the virulence of an organism in such an exudate is maximal and that the presence of an aggressin facilitates infection. The pus was obtained from several sources (man and cat). The dosage used in this series varied from 1 cc. of a dilution of 1: 5 to 1 cc. of a dilution of 1: 150. Of the eight cats used, six developed an acute form of meningitis. The other two animals, receiving dilutions of 1: 50 and 1: 150 respectively (the highest used in the series), showed no evidences of infection. Attention was then directed to the combination of an organism grown *in vitro* with an aggressin obtained by filtering the exudate through a Mandel candle. A single experiment, in which a combined subarachnoid injection of such a filtrate with an avirulent staphylococcus, resulted in the production of an acute meningitis. The protocol of this experiment follows.

Cat 212.—Adult female.

Jan. 29, 1918, 11.10 a.m. Ether. Subarachnoid injection by occipito-atlantoid needle of one-twentieth of a 48 hour agar slant of *Staphylococcus* 68 plus 0.5 cc. sterile Berkefeld filtrate of pus obtained from an abscess in Cat 194.

Jan. 30. Animal is sick and lethargic but moves around fairly well.

Jan. 31. Cat's condition is worse. Drops heavily and prefers to remain quiet.

Feb. 1. Animal is down on its side, unable to do much more than move its legs. Extremely hypersensitive. Slight touching of the body produces crying and convulsive movements. Frequent spontaneous convulsions, characterized by beating motions of the legs. Death at 11.45 a.m.

Autopsy Diagnosis.—Acute exudative leptomeningitis.

The dosage of organisms in this case was small. This strain of staphylococcus in very much larger amounts had repeatedly failed to produce the slightest effect within the meninges. The method, therefore, promised to be useful and further experiments with other irritants were planned, but the finding of an organism with the desired subarachnoid virulence (*Bacillus mucosus capsulatus*) made such an indirect measure unnecessary.

Pneumococcus.—Three strains of pneumococcus, all of human origin, were given to four cats by subarachnoid injection. One strain

(Type IV) was from the stock culture in the laboratory. In one cat this was given intraspinally in 1 cc. dose of the undiluted broth culture; the animal developed a typical chronic meningitis, with organisms demonstrable in the cerebrospinal fluid for 3 days. The other animal received two subarachnoid injections of 0.4 and 1 cc., respectively, of the same strain; this cat also developed a chronic meningitis.

One of the other two strains was employed by direct subarachnoid injection of 3 cc. of turbid spinal fluid taken immediately at autopsy from a child dying of meningitis (*Pneumococcus* Type IV). This animal was definitely sick for 5 days, at the end of which time the cerebrospinal fluid showed 1,040 white cells and a colloidal gold reaction of 5555321000. The cat improved and became normal on the 17th day.

The third strain of pneumococcus belonged to Type III, and was obtained from the first culture of the spinal fluid of a patient dying of meningitis. A subarachnoid injection of 0.5 cc. of this first culture killed the animal in 36 hours with a typical meningitis.

These findings indicate a certain original virulence of the pneumococcus for the meninges of the cat. A similar observation has been made by Monti for the pneumococcus on subarachnoid injection in dogs. Bull's production of meningitis by large intravenous injections of pneumococci in dogs and the fact that Lamar was able to produce a typical meningitis in monkeys with 0.1 cc. of a 24 hour broth culture of pneumococci, also indicate an original intrameningeal pathogenicity of this organism.

Influenza.—The first experiment with the influenza bacillus consisted of a subarachnoid injection of 2 cc. of a culture diluted 1:10. This strain was obtained at autopsy from a case of human meningitis and it was used 18 hours after the spinal fluid was obtained. The injection was followed the next day by marked evidences of meningitis, and a specimen of spinal fluid taken at this time showed the presence of increased white cells and globulin, as well as typical bacilli. The animal died shortly after the specimen was secured and at autopsy an acute meningitis was diagnosed. Two other animals were given smaller doses (0.4 and 0.5 cc., respectively, of 1:5 dilution). Neither of them showed signs of meningitis, and after 2 weeks the fluid from one of them (No. 197) had the following analysis: globulin, 0.6 gm. per

liter; cells, 4; gold, 0111111000. These results were expected from a consideration of the fact that Wollstein, in monkeys, found only a few strains of sufficient virulence to produce a typical meningitis (two agar slants as dose).

Bacillus gallinarum.—Two subarachnoid injections of *Bacillus gallinarum* were made. The first cat received one-fourth of an agar slant, and, after a normal recovery from ether, became definitely sick and lethargic later in the day. The next morning the animal was dead. The other cat received 1 cc. of the same dilution intraspinally. For several days thereafter this animal appeared to be sick and toxic, but the manifestations of acute infection eventually disappeared. In the meantime there had developed a marked ataxia and uncertainty in movement, the neck was held in a position of torticollis, associated with a definite nystagmus. This picture of chronic meningitis persisted for very nearly 2 months, at the end of which time the cat died of a pulmonary infection. The spinal fluid 11 days after inoculation was positive culturally for *Bacillus gallinarum*, and another specimen 1 month after inoculation, contained 214 white cells per c.mm., but no organisms. Autopsy showed a subacute leptomeningitis and internal hydrocephalus.

Pathogenic Bacillus subtilis.—The experience with this organism in the first animals seemed highly promising, for in two experiments, using one-half and one-eighth of a 24 hour agar slant, respectively, death resulted in less than 24 hours. A third animal, receiving one-fifth of a 12 hour agar slant by subarachnoid injection, developed a typical meningitis with extreme extensor rigidity of the legs, retraction of the neck, and hypersensitiveness. Death occurred in 2 days. Pathological study of the central nervous system revealed an acute exudative leptomeningitis with involvement of the cord substance. Injection of one-twenty-fifth of a slant produced a subacute meningitis with death in 5 days, and still higher titration (1:500) resulted in an acute transient infection from which the animal made a complete recovery. The protocol of this last experiment follows.

Cat 176.—Adult female.

Jan. 17, 1918. Ether. Occipito-atlantoid puncture, cerebrospinal fluid clear. Subarachnoid injection of 1 cc. of a 24 hour culture of pathogenic *B. subtilis*, diluted 1:500.

Jan. 18. Animal shows no obvious signs of meningitis. Accurate in movements, and aside from being a little weak is apparently normal.

Jan. 21. Cat is practically normal and active. Spinal fluid, turbid; globulin, 0.5 gm. per liter; cells, 180; culture, positive for *B. subtilis*.

Jan. 28. Spinal fluid, turbid; globulin, 1.0 gm. per liter; cells, 180; gold, 1233210000; culture, negative. Animal normal.

Feb. 7. Condition continues normal. Fundus of eye examined, normal. Spinal fluid, turbid; globulin, 0.8 gm. per liter; cells, 90; gold, 0111000000.

Feb. 19. Spinal fluid clear.

Mar. 4. Spinal fluid clear. Animal normal.

Since a dose, even in concentrations of 1:500 directly into the subarachnoid space was followed by spontaneous recovery, it was not the type of bacterium desired for the work planned. Furthermore, the type of lesion produced, even in low titers, was too variable to permit of an accurate prognosis after inoculation.

Bacillus smegmatis, *Bacillus cereus*, *Bacillus proteus zoppii*, *Bacillus proteus mirabilis*, *Bacillus anthracoides*, and *Spirillum metchnikovi* were all used by subarachnoid injection in 1 and 2 cc. doses of the 24 hour broth culture without visible effect.

Bacillus dysenteriae.—Various strains (Flexner-Harris, Kruse, Hiss-Russell, and Duval) were inoculated intraspinaly without obtaining any uniformity of results. An injection of 0.75 cc. of a 24 hour broth culture of Hiss-Russell and one with 1 cc. (diluted 1:8) of the Kruse strains had no effect. The injection of 0.5 cc. of a 24 hour broth culture of the Duval strain killed a small cat in 3 days, the autopsy showed acute meningitis. Similarly the Flexner-Harris strain injected in a dose of 0.75 cc. into a small cat caused a meningitis with death in 4 days. Autopsy in this case also showed an acute meningitis, but another subarachnoid inoculation with the same organism in an adult cat produced a chronic meningitis.

Bacillus typhosus.—The experiments with *Bacillus typhosus* were quite similar to those with dysentery. One animal receiving one-half of an 18 hour agar slant, died in less than 24 hours. Another given one-eighth of the same slant appeared acutely sick for a few days, then developed a chronic meningitis and lived for 4 months. Seven days after the inoculation the spinal fluid of this cat contained *Bacillus typhosus*, and a culture of this strain was injected into the subarachnoid space of another cat. The second animal did not develop meningitis.

Bacillus paratyphosus.—The experiments with this organism afford an excellent example of the facility with which the intrameningeal virulence of a strain may be raised. Early experiments with one strain (B) were fruitless, but later attention was called to another recently isolated strain of *Bacillus paratyphosus* B (Autopsy 5,602). Subarachnoid injection of 0.8 cc. of a 24 hour broth culture of this organism killed a cat in less than 4 hours, during which time a marked exudate had formed in the meninges. A second experiment was therefore made, the animal being given this time 1 cc. of the culture diluted 1: 50. This, however, failed to cause a meningitis. Ordinarily this organism would have been abandoned, but the appearance of the exudate in the first experiment seemed to be so promising that an attempt to raise the virulence of the bacterium was made. Eventually, by passage through nine cats, such a marked pathogenicity for the meninges was attained that 1 cc. of a dilution of 1: 10,000 of the broth culture when introduced into the subarachnoid space killed the animal in 24 hours. The intravenous pathogenicity did not rise in proportion, however, so that the organism was subsequently used for precipitating meningeal infections from the blood stream. The results of these observations have been reported briefly elsewhere (Weed, Wegeforth, Ayer, and Felton), but are given in detail in the fourth chapter of this monograph. The strain had the disadvantage of losing its high virulence easily, unless it was continually passed through the meninges of cats. This procedure was unsatisfactory both on account of the number of animals required for the purpose and the amount of time necessary to keep such an artificially produced virulence in hand.

Colon Group.—It was soon found that some members of the colon group of bacteria possessed a feeble but definite natural virulence within the meninges. Strains of *Bacillus coli communior* especially were found to be capable of producing meningitis in dilution greater than that of any organisms previously used. Whether on account of extreme variations in individual resistance in the feline species toward this particular group of organisms, or whether due to difference in virulence of the organism itself, the results of such injections of the higher dilutions were far from uniform. The Behring strain of *coli communior* was the most promising of all the varieties in the early

experiments. One cubic centimeter of dilutions of 1:2 to 1:25 killed the animal within a day with the production of acute meningitis. As soon as the dilutions of this organism were increased, the natural resistance of the animals affected the constancy of the results, so that although a meningitis could be obtained even in dilutions as high as 1:500, the incidence and degree varied so much that no accurate prediction of the outcome could be made. For instance, on one day four animals were injected, each with 1 cc. of a dilution of 1:250. Three of these were dead by the following morning but one lived for 4 days, at the end of which time the spinal fluid was turbid and contained 700 cells. This, of course, was very satisfactory, so the following day the same dilution was again used, but the dosage was reduced to 0.5 cc. Of the three animals so inoculated, two died of a meningitis at the end of 4 and 6 days, respectively, but sections showed the occurrence of a secondary invasion with another organism (coccus). The remaining animal developed an acute meningitis but recovered and remained under observation for 46 days. A return to the original dosage of 1 cc. in the 1:250 dilution failed to give the uniform acute results noted in the first group. One of the three animals subsequently used died on the 4th day with an acute meningitis, and a second developed a subacute case and did not die until the 9th day. The third animal was under observation for 15 days, and repeated examination of the spinal fluid failed to reveal the existence of an infection in the meninges. Eleven experiments carried out with other strains of *Bacillus coli communior* gave similar results. A typical protocol of a cat developing subacute meningitis following subarachnoid inoculation with *Bacillus coli communior* follows.

Cat 255.—Adult male.

Feb. 11, 1918, 12.05 p.m. Ether. Occipito-atlantoid puncture; clear cerebrospinal fluid obtained. Subarachnoid injection of 1 cc. of a 24 hour broth culture of *B. coli communior* (Behring) diluted 1:250.

Feb. 12. Cat resents being moved. It is weak and lethargic. Fundus of eye normal.

Feb. 13. Spinal fluid very turbid, contains 2,400 cells per c.mm. and 5 gm. of globulin per liter; culture positive for *B. coli communior*; gold sol, 0001221000.

Feb. 14. Cat down on side. When it gets up it is very weak and ataxic. Clonus of head.

The animal's condition became progressively worse and on Feb. 19 it was found dead.

Autopsy Diagnosis.—Chronic generalized leptomeningitis.

Various strains of *Bacillus coli communis* were used in seven experiments. One cat after an inoculation of 1 cc. of undiluted culture died within 24 hours. The others receiving 1 cc. doses of dilutions varying between 1:10 and 1:500 remained under observation from 9 to 26 days without showing evidences of meningitis.

Bacillus pyocyaneus.—By using low dilutions (1:10) of *Bacillus pyocyaneus* a marked purulent meningitis was obtained in six out of eight of the experimental animals. In higher dilutions, however, the same uncertainty in results noted in other groups of organisms was found here. Of two animals receiving each 1 cc. of 1:50 dilution by subarachnoid injection, one died in 24 hours while the other after developing an acute meningitis, as shown by the spinal fluid analysis, went on to spontaneous recovery. Subsequently, the intrameningeal virulence of this organism was increased to the degree that 1 cc. of a 1:10,000 dilution of a 24 hour broth culture produced a fatal meningitis.

Spontaneous Meningitis.—During the course of the work in this laboratory, one of the stock animals developed a spontaneous meningitis. Theoretically, an organism which could cause a spontaneous meningitis in cats should be of value in experimental work, but that such a conclusion does not hold is illustrated by the following case.

Cat 359.—Adult female.

Mar. 5, 1918. Stock animal was found yesterday to have become sick spontaneously. Animal is crying with pain, has a lateral nystagmus, general rigidity of all limbs, and is unable to walk. Cerebrospinal fluid turbid, although containing few red blood cells. Cultures of the spinal fluid and blood show presence of a very small delicate Gram-negative bacillus.

Mar. 7. Animal shows clinically typical reactions of meningitis; retraction of head causes extension and spasticity of fore and hind legs. Has nystagmus and hypersensitiveness; cries out when disturbed. Spinal fluid turbid; contains 0.9 gm. globulin per liter and gives a colloidal gold reaction of 1234554310. Culture of fluid shows the same organism.

Mar. 8. Animal almost dead, down on side. Spinal fluid cloudy; contains 1,440 white cells per c.mm. Culture positive.

Mar. 10. Animal has continued in same moribund condition. Is still down on side breathing irregularly and intermittently. Killed with ether.

Autopsy Diagnosis.—Acute leptomeningitis.

The organism isolated from this animal was injected intravenously and also directly into the subarachnoid space of two animals in doses of 7 cc. and 1 cc., respectively. Neither of the animals was noticeably affected. The identity of the organism was not determined because of the inability to maintain its growth on subcultures.

Bacillus mucosus capsulatus.—The members of the group of *Bacillus mucosus capsulatus* have, in the experience of this laboratory, been the most satisfactory of all the organisms investigated. Nine different strains were employed, and while they all possessed original intrameningeal virulence, the one which proved itself of most value was *Bacillus lactis aerogenes*, a form very closely related to the bacillus of Friedländer. This organism fulfilled one of the first requirements for future work on the therapy of meningitis in that it was acutely and uniformly fatal. In this respect it corresponds very closely to the organisms (other than the meningococcus) causing purulent meningitis in man. Of the 59 injections made directly into the subarachnoid space with varying dilutions, only three failed to produce a fatal meningitis. One of these three was an animal which was given a lumbar injection of 1 cc. of a dilution of 1:10,000, and the other two were animals receiving the injection through the occipito-atlantoid ligament in dilutions of 1:100,000 and 1:1,000,000, respectively. Greater concentrations were not tried on these three animals, but a fourth which did not develop meningitis when given an injection of 1 cc. of a dilution of 1:50,000 came down later when the dilution was lowered to 1:100. Spontaneous recovery after a well recognized meningitis caused by this organism had established itself, took place only in one instance. The second quality which recommended this organism was the fact that it would produce a meningitis when used in high dilutions so that the number of bacteria necessary to cause infections in the meninges of animals corresponded perhaps rather closely to the number of organisms concerned in initiating a similar infection in man. Certainly the massive injections of small fractions of an ordinary culture, necessary to produce an experimental meningitis with many of the foregoing strains, do not represent an infection

analogous to those in man. The dilutions up to 1:2,500 were found to be extremely active, for none of the animals receiving injections of titrations lower than this lived longer than the day following the inoculation. With higher dilutions, some of the animals survived longer than 48 hours, but even among these, the animals succumbing within the first 24 hours were still greatly in excess. An idea of the relative lengths of survival with these titrations can be obtained from the following table.

Dilutions.	No. of experiments.	Died in 24 hrs.	Died in more than 24 hrs.	Did not come down.
Up to 1:2,500	21	21	0	0
Above 1:2,500	25	11	12	2

The smallest dosage employed at any time was 1 cc. of 1:10,000,000,000 dilution, which represents approximately 20 organisms; the estimation was reached by plating out in triplicate 1 cc. of the dilution injected. Two animals receiving this dose died of meningitis in less than 24 hours. A typical protocol, illustrating the great virulence of this organism within the meninges, follows.

Cat 313.—Adult male.

Feb. 25, 1918, 11.55 a.m. Ether. Occipito-atlantoid puncture; clear cerebrospinal fluid. Subarachnoid injection of 1 cc. of a 24 hour broth culture of *B. lactis aerogenes* (Perkins) diluted 1:100,000,000.

Feb. 26. Cat is very sick. Has frequent convulsions which develop into acute maniacal attacks, during which animal runs blindly about the cage. After such seizures it falls exhausted, with extreme retraction of the head and extensor rigidity of the legs. Spinal fluid, very turbid; contains 18,000 white blood cells per c.mm., and on stained film 4 to 5 bacilli can be seen to a field. Culture is positive for *B. lactis aerogenes*.

Feb. 27. Cat found dead in cage.

Anatomical Diagnosis.—Acute generalized exudative leptomeningitis with early encephalitis and myelitis.

A disadvantage of the great virulence possessed by this organism within the subarachnoid space was the short period of incubation and the rapid fulminating type of the meningitis produced, so that little time was afforded for the application of therapeutic measures. The effects of the injection could be delayed, it was found, by using lumbar

puncture instead of the cervical route. By inoculating in this region the same number of organisms in smaller volume of fluid (0.25 cc.), the animals did not succumb to the injection quite so rapidly as did those which had received the larger fluid amounts (1 cc.), at the occipito-atlantoid region. Such divergence in result was probably to be accounted for by the difference in the initial distributions of the infecting organisms.

DISCUSSION.

The foregoing types of microorganisms can be classified into three obvious groups; namely, the bacteria most frequently invading the meninges of man, a miscellaneous group consisting of Gram-negative and Gram-positive bacilli which are relatively of low virulence for the meninges of the cat, and a third group of very great virulence. In making this division it must be emphasized that the use of a comparatively small number of strains of the different types of microorganisms, some of which are virulent while others are avirulent, makes a more general statement impossible. However, study of these three groups discloses some rather interesting facts. The microorganisms that generally cause a fatal meningitis in man (*meningococcus*, *pneumococcus*, *streptococcus*, *staphylococcus*, *influenza*, etc.) were found to possess but slight pathogenicity for the meninges of a cat. This lack of intrameningeal virulence perhaps can be explained on the basis that the cat is comparatively insusceptible on intravenous inoculation to these same microorganisms, especially when of human origin. It is not unlikely that the factors of resistance to infection present in the blood stream or body tissues of an animal possessing a high degree of natural immunity, also maintain their activity to some degree within the subarachnoid space. That immune substances or specific antibodies are capable of passing through the meninges and of remaining in the cerebrospinal fluid, both of actively and passively immunized animals, has been demonstrated by Morax and Loiseau, Ransom, Kolmer and Sekiguchi, and others. The members of the second or miscellaneous group, as can be seen from Table I, possess about the same natural virulence for the meninges of the cat as those of the former group. However, in none of the strains was the pathogenicity uniform or of sufficient degree for the immediate purpose. Although

the majority of the organisms included in this group were stock cultures maintained on artificial media for months to years, they were still capable of producing a fatal meningitis on massive inoculation into the subarachnoid space. This suggests that an infection of the meninges of the cat can be brought about by direct subarachnoid injection of any of the known microorganisms, provided, of course, that the organism be of optimum virulence.

Of the four microorganisms of the last group (*Bacillus pyocyaneus*, *Bacillus coli*, *Bacillus paratyphosus* B, and the *mucosus capsulatus* group), a strain of *Bacillus lactis aerogenes* (Perkins' classification) of the *mucosus capsulatus* group possessed the greatest natural virulence for the meninges. This particular strain of *Bacillus lactis aerogenes* was isolated at autopsy both from the blood stream and the lungs of a patient dying from bronchopneumonia. Two months after its isolation, the cultures having been kept on plain agar at room temperature, the injection of 20 organisms in 1 cc. of Locke's solution produced in a cat a fatal meningitis of 24 hours duration. In comparing the natural pathogenicity of this microorganism with the others of this group, it is found that the virulence of *Bacillus lactis aerogenes* is 40,000,000 times as great as the most virulent strain of *Bacillus coli* (Behring); 1,000,000,000 times greater than a recently isolated *Bacillus paratyphosus* B, and the same number of times more virulent than a strain of *Bacillus pyocyaneus*. This computation, of course, assumes that the rate of multiplication and the number of organisms in a 24 hour meat infusion broth culture is the same with the different bacteria. Although for the purpose of this laboratory, the *Bacillus mucosus capsulatus* group has been the most useful, this organism has certain disadvantages in the investigation of other phases of the study of experimental meningitis. Its chief disadvantages relate to its extreme virulence, the difficulty experienced in producing an immune serum, and its relative infrequency as a meningeal invader in man. Other organisms, however, like *Micrococcus flavus*, although possessing only a slight natural pathogenicity for the meninges of the cat, seem more closely related to meningococcus infections in man,—a circumstance of possibly greater value in investigations of specific serum therapy.

The routine culturing at autopsy of the heart's blood of these animals revealed certain interesting facts in regard to the transfer of in-

fection between the meninges and the blood stream. In all acute fatal cases of meningitis caused by the injection of microorganisms into the subarachnoid space, every culture of heart's blood showed the presence of the same bacteria with which the animal had been inoculated intraspinally. However, when the number of bacteria initially injected into the subarachnoid space was not sufficient to produce a fatal meningitis in 24 to 48 hours, the animal would sometimes die in from 1 to 3 weeks. At autopsy in these cases the tissues of the central nervous system were practically normal, but on culturing the blood the same microorganism initially injected intraspinally was cultivated. This phenomenon was noticed with *Bacillus lactis aerogenes*, *Bacillus coli*, and *Bacillus pyocyaneus*. No doubt we were dealing here with a focus of an infection made by the lodging of bacteria in such a location or in such numbers that the offensive and defensive mechanisms of the host were unable to cope with them. As a result of this inability of the organism to kill, multiplication of the microorganism went on *in situ* until the defensive barriers were overcome; a septicemia causing the death of the animal was the final outcome. Two distinct methods of bacterial activity were observed, therefore, when the infecting agent was injected into the subarachnoid space: one, an acute infection with rapid destruction of the mesothelial walls between the leptomeninges and the blood stream with the production of a septicemia; the other, a chronic condition brought about by the survival and multiplication of a few bacteria in a local focus and, as in the other method of infection, a final septicemia.

From a consideration of the foregoing observations the question naturally arises as to whether the animals given injections of organisms into the subarachnoid space die as a result of an acute meningitis, or of an overwhelming septicemia, or of a combination of the two. The positive findings in the more chronic cases mentioned above—but little evidence of a meningitis, and a definite septicemia—argue in favor of the fatal issue being caused by a septicemia.

In the acute cases we are concerned both with a septicemia and a definite infection of the meninges. It is felt that the evidence favors the view that the death of the animal is caused by the two factors operating together. In our opinion the inoculation of a fatal dose of bacteria into the subarachnoid space may be likened to the injection

into the peritoneal cavity. In both cases we are dealing with an infection in a mesothelium-lined sac in which the defensive mechanism for certain organisms is apparently minimal. Multiplication of the bacteria there progresses until the restraining mechanisms are destroyed and the organisms invade the blood stream in numbers sufficient to cause a true septicemia. In one case a peritonitis occurs, in the other a meningitis, and a septicemia is present in both instances.

The explanation of the etiological mechanism of fatal infection by peritoneal injection is, perhaps, well recognized to be, with most bacteria, an overwhelming septicemia. Whether the analogy remains for the invasion of the blood stream from the subarachnoid space is left for future investigation. These questions are emphasized for the reason that it is generally held that meningococci may be demonstrated in the blood of only a small number of patients dying of epidemic meningitis. Does this intrameningeal localization of infection demonstrate a predilection of a microorganism for a specific tissue, or is the infection made possible by the low subarachnoid resistance to such an infection after the incident of inoculation? The occurrence of a septicemia at death of all the animals after subarachnoid inoculation, noted above, emphasizes a difference between the type of infection in the experimental animal and the analogous infection with the meningococcus in man. In one the meningitis is associated with an invariable septicemia, while in the other the meningitis at death seems the essential lesion, though improvement in technical methods may lead to the discovery of a positive meningococcus septicemia.

If the idea of a low subarachnoid resistance to most organisms is assumed, the direct injection of fewer bacteria in these spaces should lead to a more severe infection than after inoculation into the blood stream. This question of comparison of intrameningeal and blood stream virulence of different microorganisms will be taken up in the third chapter of this communication.

SUMMARY.

1. In a study of the natural intrameningeal virulence in the cat of 21 varieties of bacteria (102 strains), it was found that three arbitrary groups could be made.

- (a) The microorganisms most frequently isolated in human meningeal infections, possessed but slight natural virulence for the meninges.

(b) Miscellaneous groups of bacilli had very little or no natural virulence for the meninges.

(c) *Bacillus coli*, *Bacillus paratyphosus* B, *Bacillus pyocyaneus*, and *Bacillus mucosus capsulatus* possessed the greatest natural virulence for the meninges.

2. A strain of *Bacillus lactis aerogenes* (Perkins) of human origin was the most virulent for the meninges; the subarachnoid injection of as few as 20 organisms resulted in a fatal meningitis of 24 hours duration.

II. A PATHOLOGICAL STUDY OF EXPERIMENTAL MENINGITIS FROM SUBARACHNOID INOCULATION.

By JAMES B. AYER, M.D.

PLATES 1 TO 9.

This report represents a study of the meningeal reaction experimentally produced by subarachnoid inoculation of a large number of organisms, and it correlates from the pathological point of view the experiments described in the foregoing chapter. More than 100 of these animals have been examined both in gross and microscopically, and a larger number in gross alone.

Clinically the term meningitis may with reason be loosely applied to cover a variety of conditions in which meningeal symptoms occur but which are not all meningitis from a pathological standpoint. On a pathological basis we may divide meningitis (*i.e.*, leptomeningitis), into two general classes, aseptic and infectious. Into the first group are placed all types of meningeal reaction from the mildest reaction of the pia-arachnoid, as seen for example in connection with febrile states, to extensive exudation, as seen in subarachnoid injection of foreign substances such as sera and chemicals. Into the second group are placed all forms of meningitis in which living organisms are present. In this group, too, we find all degrees of intensity of meningeal reaction, closely simulating the types of aseptic meningitis, but showing one fundamental difference, the presence of living organisms, throughout the disease or at some stage.

In our experimental work, therefore, we must be constantly on our guard in the interpretation of types of meningitis resulting from inoculation, for it is to be expected that a reaction of the meninges will follow the injection into the subarachnoid space of almost any foreign substance. Significant factors in considering the virulence of a meningeal infection are the amount and character of the exudate, the viability or proliferating power of the organism in the subarachnoid space, its spread to the blood stream, and its invasion of the central nervous system.

We shall first consider the subject from the point of view of the infecting organisms, subsequently attempting to group the different pathological types, and finally commenting upon certain characteristics of the spread of infection in forms of acute meningitis. Examination of the tissues was for the most part made upon formalin-hardened material, subsequent to postmortem arterial injection with formalin; blocks embedded in paraffin and celloidin, and sections cut at 15 μ ; staining routinely with hematoxylin and eosin, and with toluidine blue. Examination of stained films, both wet and dried, cell counts, and dissection under the binocular microscope were employed in some cases.

EXPERIMENTS WITH DIFFERENT ORGANISMS.

Streptococcus.

In all, 36 strains of streptococcus were employed, the standard dose being 1 cc. of a 24 hour broth culture introduced by occipito-atlantoid puncture. Cats (half grown) and kittens were found for the most part insusceptible, most of them remaining normal and active. In such cases, if killed or if death occurred from other causes, the brain and cord appeared normal in gross, but microscopic examination frequently revealed chronic changes. The following case is of this character.

Experiment 237.—Cat. Subarachnoid inoculation by occipito-atlantoid puncture, 1 cc., 1:10 broth culture of streptococcus.

Cat developed an acute meningitis as shown by symptoms and by several examinations of the cerebrospinal fluid during the first week. In spite of improvement in symptoms and signs of meningitis, the cat became weaker and died on the 15th day after inoculation.

Gross Examination.—Brain and spinal cord, normal.

Microscopic Examination.—There is a moderate amount of exudate in the subarachnoid space over base of brain and in cervical cord, but very little in lumbar and thoracic cord and over cortex, and in the former situation only in discrete patches. The exudate consists principally of large mononuclear phagocytes, together with a lesser number of lymphocytes and polymorphonuclear leucocytes. Cocci in chains appear in small numbers and only where the exudate is in quantity. There is no abnormality of ventricles, canal, or central nervous system.

Diagnosis.—Focal subacute meningitis (Plate 7, Figs. 13 and 14).

With a few strains a moderately severe meningitis was brought about with death in 48 hours or less. In these cases organisms were few in number. The virulence of the streptococcus was found to be greatly increased by repeated subarachnoid inoculation, and one strain so treated produced a massive fulminating meningitis. The following experiment illustrates this.

Experiment 224.—Cat. Inoculation of subarachnoid space by cistern puncture with 1 cc. of a 24 hour broth culture of streptococcus from the spinal fluid of another cat. Became sick and weak in hind legs the next day and died about 42 hours after inoculation.

Gross Examination.—Vascular congestion throughout brain and cord, with hemorrhage in meninges of lower thoracic region. Pus in region of puncture.

Microscopic Examination.—In the subarachnoid space, at base of brain, and in cervical cord, there is a massive hemorrhagic exudate, composed chiefly of polymorphonuclear leucocytes and large mononuclear cells. Cocci appear frequently in masses, mostly free, but some in cells. The intensity of the meningitis progressively diminishes upward toward cortex or downward toward lumbar cord. Frank pus is likewise seen in the fourth and third ventricles and canal, but the lateral ventricles contain only a few cells and organisms.

The brain is invaded by polymorphonuclear cells about the ventricles and there are several small hemorrhages in the white matter of the cord, but organisms are not here present. Blood vessels are engorged, but no bacteria are seen.

Diagnosis.—Acute exudative meningitis.

The streptococcus produces great damage to the blood vessels. This was not especially noticeable in the above cases in which the organism was injected, but in several cases in which its toxin (method of Clark and Felton) alone was injected, lesions in the blood vessels were remarkable.

Experiment 1,348.—Cat. Subarachnoid injection of 3 cc. of streptococcus toxin. It became sick immediately and died the same night. Heart's blood gave negative culture at autopsy.

Microscopic Examination.—Throughout the subarachnoid space there is a moderate polymorphonuclear exudate. Cerebral cortex and spinal cord are densely infiltrated with polymorphonuclear leucocytes and the anterior horn cells show well marked chromatolytic changes. The most striking feature of the examination is the lesion produced in some of the blood vessels. The intima of arteries, large and small, is elevated and under it are seen accumulations of polymorphonuclear cells; so intense is this subintimal infiltration in the case of smaller vessels

that they appear to be almost obliterated (Plate 8, Figs. 15 and 16). No organisms are seen in the exudate, central nervous system, or in blood vessels.

Diagnosis.—Acute aseptic meningitis. Toxic endarteritis.

The streptococcus group must be considered as causing for most part a low grade of meningitis. Some strains, however, have been found to have a moderate original virulence, which on being artificially increased, produced a massive type of exudative meningitis, as in the second case. The last case shows the severe effect of the streptococcus toxin on the blood vessels.

Bacillus coli.

Members of the colon group generally produced a subacute or chronic type of meningitis, even when considerable numbers of organisms were employed. With larger doses, however, a fatal acute meningitis was at times produced. The following case is characteristic of the majority of infections with this organism.

Experiment 994.—Small cat. Subarachnoid injection by cistern puncture of 0.5 cc. of *B. coli communior*. Became promptly weak and sick. Next day meningeal symptoms developed and in a week the cat was unsteady, with abnormal gait and attitudes, which persisted until death in 14 days. Heart's blood culture, negative.

Gross Examination.—Brain and cord of normal color throughout. Membranes of brain thick, white, and opaque. Convolutions very slightly flattened. Cord normal.

Microscopic Examination.—(Plate 9, Fig. 17.) Throughout the subarachnoid space of brain and cord there is seen a small amount of exudate, in accumulations rather than generalized, especially in pockets and corners about medulla and cerebellum and about nerve roots where these join brain or cord or where they pierce the dura. The exudate consists exclusively of large mononuclear cells, lymphocytes, and plasma cells; no organisms are seen anywhere. The perivascular spaces contain a few cells of similar character. Ventricles and canal normal except for an occasional unidentified cell. The brain shows two small areas of degeneration, both subpial in location, one in medulla and one in cortex, in which the tissue is rarefied and in which large mononuclear cells are seen. Dura normal.

Diagnosis.—Subacute meningitis.

In this instance we are dealing with a subacute or chronic stage of what, judging by the symptoms, must have been an acute meningitis; unfortunately we have no examination of the spinal fluid during the

acute stage to prove this point, but in another cat, dying on the 8th day and showing a similar pathological picture, the fluid during the acute stage on the 2nd day showed 2,400 cells and a positive culture, although organisms must have been few in number as they were not seen in films.

With some strains and by inoculation of larger amounts of the culture, 1 cc. or more, an acute exudative meningitis was not infrequently obtained. The organisms are here seen in large numbers and are evidently proliferating, and death occurs in 1 or 2 days. However, this acute fatal meningitis is not the common form produced by the colon bacillus. While there is evidence that this organism possesses at times marked virulence in the meninges, it is more likely to die out rapidly leaving a residual chronic meningitis, with at times some destruction of nervous tissue.

Staphylococcus.

One would expect *a priori* that a generous inoculation of staphylococcus would produce massive meningitis, but this was rarely the case. Much more characteristic was a moderate subacute reaction, as in the following experiment.

Experiment 1,022.—Small cat. Subarachnoid inoculation (cistern puncture) of 1 cc. of a 24 hour broth culture of *Staphylococcus aureus* (human strain). Cat sick from time of inoculation; within 24 hours meningeal symptoms commenced and persisted for several days; later animal became spastic and very weak. Death on 6th day.

Gross Examination.—Normal brain and cord.

Microscopic Examination.—About cerebellum, base of brain, and cervical cord there is a moderate amount of exudate in subarachnoid space; all cells mononuclear, with a few cocci. Cortex and thoracic and lumbar cords show very little exudate of similar character. Perivascular spaces, ventricles, and substance of brain and cord appear unaffected.

Diagnosis.—Subacute meningitis.

The above is fairly characteristic of cases receiving staphylococcus. A low grade subacute meningitis was present, but the organism seemed to be dying out.

Bacillus pyocyaneus.

This organism frequently gave a massive purulent meningitis following the inoculation of rather small numbers of organisms, leading quickly to death. This result was not always obtained, however, and a similar injection frequently produced a non-lethal milder type of meningitis in which organisms rapidly disappeared.

The following cases briefly abstracted show the two types of reaction.

Experiment 1,039.—Cat. *B. pyocyaneus*, 1 cc., 1:10, by lumbar puncture. Death occurred over night. Heart's blood culture positive for *B. pyocyaneus*.

Microscopic Examination.—A marked purulent exudate is found with many bacilli throughout the subarachnoid space of the cord, being greatest in the lumbar sac. Base of brain shows rather less, and cortex is little affected.

Diagnosis.—Acute meningitis.

Experiment 956.—Cat. *B. pyocyaneus*, 1 cc., 1:10, by lumbar puncture. Became weak and remained so until death occurred during ether and cistern puncture 7 days after inoculation.

Gross Examination.—Normal.

Microscopic Examination.—(Plate 9, Fig. 18.) Scattered aggregations of large mononuclear cells, frequently with granular inclusions, and plasma cells are seen. No organisms. Spinal fluid and blood cultures negative.

Diagnosis.—Chronic meningitis.

Similar amounts of cultures of the same organism, though on different days, are here seen to have produced radically different results: in one an acute meningitis of considerable intensity with evident rapid growth of the organism; in the other an insignificant meningeal reaction with death of the organism, it being found neither in the spinal fluid nor in the blood.

In a number of cats dying of acute meningitis in more than 48 hours following somewhat larger doses, the gross appearance was striking. The central nervous system appeared throughout of a yellow-green color, and over the dura a considerable mucopurulent exudate was to be seen. Markings of brain and spinal cord were frequently obliterated by massive exudate, in which organisms were present in large numbers. Invasion of ventricles, of brain and cord, and of dura (Plate 3, Fig. 6) was extensive in these animals.

Bacillus paratyphosus B.

The following protocol shows an organism of considerable virulence.

Experiment 951.—Small cat. Cistern inoculation with 0.8 cc. of a broth culture of *B. paratyphosus B.* Death 4 hours later. Heart's blood gave negative culture.

Gross Examination.—Brain and cord of a dirty muddy color throughout, with marked congestion of cerebral vessels. Meninges transparent over cortex but opaque over base and throughout spinal cord. Convolutions greatly flattened.

Microscopic Examination.—In both cerebral and spinal subarachnoid space there is a moderate degree of exudate of the polymorphonuclear type, with considerable free blood and many bacilli. Ventricles and canal contain exudate and bacilli but the substance of the nervous system shows no invasion. Blood vessels engorged; no bacteria seen in them.

Diagnosis.—Early acute meningitis. Marked intracerebral pressure.

The cause of death in this cat may well have been due to the increased cerebral pressure, as evidenced by marked flattening of the convolutions; so it seems reasonable to attribute death directly to the meningitis.

The virulence of this organism was greatly increased by successive passage through cats until an acute meningitis, fatal in 24 hours, could be produced by inoculation with as little as 1 cc. of a broth culture, diluted 1:10,000.

Bacillus mucosus capsulatus.

By far the greatest success was obtained with an organism belonging to the group of *Bacillus mucosus capsulatus*. With this, *Bacillus lactis aerogenes*, a massive meningitis was regularly produced by the inoculation of a very small number of organisms. The meningitis was usually a fatal one within 48 hours; rarely did a cat live longer. As this bacillus proved so virulent and so constant in its effects, we shall consider the meningitis produced by it in greater detail, giving pathological descriptions of three cats, dying at different intervals.

Experiment 226.—Cat. Received 2 cc., 1:10 broth culture of *B. lactis aerogenes* by cistern puncture. Dead in 6 hours. Embalmed immediately.

Microscopic Examination.—Throughout the subarachnoid space of brain and spinal cord a large number of bacilli are found, doubtless more than were injected. Over the cortex there is considerable exudate, consisting mostly of fibrin and polymorphonuclear leucocytes; in the cord the exudate is less in amount. Perivascu-

lar spaces are prominent and for the most part well filled with exudate, both in brain and cord. The fourth and third ventricles, aqueduct, and central canal contain similar exudate with bacilli, but the lateral ventricles appear normal, as does the choroid plexus of all ventricles. There is no invasion of the central nervous tissue. The dura of the cervical cord shows areas of polymorphonuclear cells without organisms, but otherwise the exudate is confined to the subarachnoid, perivascular, and ventricular spaces (Plate 3, Fig. 5).

Diagnosis.—Early acute meningitis.

In this case we see after only 6 hours a well marked exudative meningitis with evidence of rapid growth of organisms, extending throughout the subarachnoid space and already invading the ventricles, though most conspicuous near the point of puncture.

Experiment 231.—Cat. Received 0.5 cc., 1:10 broth culture of *B. lactis aerogenes* by cistern puncture. Found dead in cage the next morning. Lived approximately 18 hours.

Microscopic Examination.—There is a fibrinopurulent exudate with a vast number of bacilli confined within the subarachnoid and perivascular spaces; bacilli (without exudate) are found in the ventricles. As in the previous case the exudate, while universal, is most noticeable there in cerebral and cerebellar sulci, near the point of puncture.

Bacilli are seen to be well confined within the boundaries of the arachnoid, neither spreading outward into the subdural space, nor spreading inward into the deeper layers of pia and nervous tissue itself. The inner surface of the arachnoid and trabeculae show evidence of cell proliferation, a condition frequently seen in meningeal infections. Large numbers of bacilli are seen in the blood vessels of this case.

Diagnosis.—Acute exudative meningitis (Plate 2, Fig. 3).

In this cat as in the previous one, the exudate is extensive, but is especially well marked in the cerebral and cerebellar meninges. In the short interval from the time of infection the exudate is seen to be well confined within the subarachnoid space. The large number of bacteria, both in the exudate (cf. Plate 2, Fig. 4) and in blood vessels, is of importance, although postmortem growth, in the latter instance probably accounts for part of the picture. Emphasis should be placed upon the importance of the arachnoid membrane. As seen in this case two functions should be attributed to it: first, limitation by it, with more or less efficiency, of the spread of exudate beyond the subarachnoid space; and secondly, formation, by the proliferation of its cells, of a considerable number of the large mononuclear elements to be seen early in meningitis in the subarachnoid space.

As a rule, with this organism animals die within 48 hours. The picture presented by cats dying after 2 days is a rather more intense generalized meningitis of similar character to the above cases, but one which tends to pass beyond the boundaries of the subarachnoid space. The following experiment, in which the therapeutic irrigation with saline solution may have helped to prolong life, is an example of this spread.

Experiment 746.—Cat. Lumbar subarachnoid inoculation of 0.25 cc. of a 1: 25,000 dilution of *B. lactis aerogenes*. Three hours later subarachnoid irrigation with 64 cc. of modified Ringer's solution from cisterna magna to lumbar cord (cf. Weed and Wegeforth). Death on 3rd day. Heart's blood culture positive for *B. lactis aerogenes*.

Gross Examination.—Markings of cord entirely obliterated by mottled pink and yellow exudate under dura; markings of base and cortex ill defined but visible. Evidence of increased pressure in brain and cord.

Microscopic Examination.—The same massive purulent exudate is seen in this as in the previous case, but rather more evenly distributed, although especially marked about the cervical cord. But there is here evidence of widespread extension beyond the subarachnoid space. Conspicuous is the infection of ventricles and canal, in which are seen exudate and bacilli. Conspicuous also is the involvement of the central nervous system in this case. About both lateral ventricles to a depth of about 2 mm. the nervous tissue has been infiltrated with pus and bacilli and the absence of any form of boundary to this exudate is evidence of progressively deeper extension of infection into surrounding brain tissue (Plate 6, Fig. 11). It is noticeable that the choroid plexus remains intact; although its stroma shows a polymorphonuclear infiltration, organisms are not present, and the ependyma is not broken, suggesting that the plexus is not involved in the infection which surrounds it. Another type of invasion is seen along the course of the velum interpositum, which is densely infiltrated with exudate; along its course the neighboring white matter of the brain shows irregular areas of invasion, obviously by direct extension. In similar manner the spinal cord at several levels is invaded from its infected canal with destruction of surrounding gray matter (cf. Plate 4, Fig. 8). Also the cervical cord presents a wedge-shaped area of invasion, apparently from direct extension from meninges (Plate 5, Figs. 9 and 10).

Diagnosis.—Massive acute exudative meningitis. Encephalitis and myelitis by extension.

In the few animals that lived longer there was progressively more frequent and more intense infection of ventricles and invasion of the nervous tissue.

In the three cases just described, well marked exudate occurred in all, but it increased with the greater period of time following infection until it became massive; in the first two cats the exudate was most noticeable in the neighborhood of puncture and inoculation, but in the last it was symmetrically distributed. As it spread throughout the subarachnoid space, so with the lapse of a longer time before death it spread beyond the confines of the subarachnoid space and invaded ventricles and the central nervous tissue. A significant fact is, that in all a large number of bacilli were noted, unmistakable evidence of active proliferation within the subarachnoid space (cf. Plate 6, Fig. 12).

Other Organisms.

The organisms already considered were the only ones in which a number of pathological examinations were made. A few examinations in isolated cases will be briefly mentioned.

Meningococcus.—Not until late in our work was there found a strain which in rabbits produced an acute purulent meningitis. These animals usually died within 24 hours and showed in the gross a generalized purulent meningitis. The exudate was of the polymorphonuclear type, was considerable, at times massive in amount, and contained numerous organisms, although not so many as in the other types of acute meningitis described.

Bacillus subtilis.—A cat inoculated with $\frac{1}{50}$ of a 12 hour agar slant of a pathogenic strain was killed because moribund in 48 hours. A summary of the examination of the spinal cord alone is as follows: acute exudative leptomeningitis, acute pachymeningitis, exudate in central canal. No organisms seen.

Absence of organisms at such a period suggests that in spite of the meningitis present their virulence had become spent. It was frequently found that this organism was not reliable in small dosage.

Bacillus gallinarum.—A cat received by cistern puncture $\frac{1}{4}$ of a 60 hour agar slant. At first sick, it later became unsteady in gait but subsequently improved. Death from distemper occurred in 6 weeks. Examination in this case shows clumps of mononuclear cells, some of which contain pigment granules, seen in cerebral sulci and in pockets formed by nerve roots with dura and nervous system. Ventricles are dilated but otherwise normal. No organisms seen.

ASEPTIC MENINGITIS.

As was indicated in the beginning of this paper, an aseptic meningitis is to be expected from introduction of almost any foreign substance into the subarachnoid space. In this laboratory a number of classes of substances have been so employed. Chemicals such as chloramine and flavine have been introduced and found to cause a profound reaction, exudative and destructive, both of meninges and central nervous system (cf. Wegeforth and Essick). Bacterial products, such as Berkefeld filtrates and dialysates, were found also to produce a severe type of acute meningitis, as also streptococcus toxin. A protocol illustrating the last named has already been given (page 28). Whole blood and laked blood have been employed; following injection of these a milder type of meningitis was obtained, involving especially the arachnoid membrane with proliferation of its cells, many of which were seen to become phagocytic for the blood corpuscular elements. A similar picture of arachnoid cell proliferation and phagocytosis was seen following the injection of inert particulate matter, such as carbon granules, India ink, and cinnabar, as described by Essick.

A number of experiments were carried out with blood sera in order to gain an idea of the type and duration of the meningitis following such injections. It was found that with serum, autogenous, homologous, or heterogenous, an acute meningitis resulted. Symptoms were frequently present and pathological changes well marked. Because of the striking similarity between aseptic meningitis produced by serum and some forms of infectious meningitis, two protocols are given, illustrating the acute and chronic stages of serum meningitis.

Experiment 1,265.—Cat.

Sept. 17, 10:45 a.m. Cistern puncture; fluid, clear; no cells. Injection of 3 cc. of blood serum obtained from a normal rabbit. 4.25 p.m. Cistern puncture; fluid, turbid; white cells, 22,080, mostly polymorphonuclears but also many mononuclears.

Sept. 18, 11:45 a.m. Normal, active animal. Cistern puncture; fluid, turbid; white cells, 7,880, mostly polymorphonuclears. Animal killed. Heart's blood culture, negative.

Pathological Examination.—In gross, central nervous system appears nearly normal, but microscopic examination shows a well marked exudate, composed almost entirely of polymorphonuclear leucocytes, universally distributed through-

out the subarachnoid space. The perivascular spaces are normal in appearance, as are the ventricles, canal, and substance of the nervous system; but the velum interpositum and choroidal stroma are infiltrated with polymorphonuclears. No organisms seen (cf. Plate 1, Figs. 1 and 2).

Diagnosis.—Acute exudative meningitis, aseptic.

The meningeal reaction in this case is promptly produced; in fact during the first few hours, as judged by spinal fluid findings, it is rather more marked than the acute reactions produced by virulent organisms. On the day following the injection, however, it is seen from the lowered cell count that the stimulus to exudation has already lessened. In a group of cases studied with this point in view it was found that following serum administration, white cell counts were always highest a few (4 to 6) hours after injection (usually about 7,000 per c.mm.). In 24 hours cells had diminished markedly in number, and from that time on progressively fewer cells were counted. This picture closely simulates that obtained from injections of weakly virulent organisms which produce at first acute meningitis, and then die, with resulting subacute or chronic meningitis.

The following protocol shows the late effect of serum in the meninges.

Experiment 1,470.—Cat.

Dec. 2. Cistern puncture; clear, colorless fluid. Subarachnoid injection of 2 cc. of normal horse serum. The animal, a little slow for a few hours following the operation, became normal and remained so until Dec. 17, when it was killed. Heart's blood culture, negative.

Gross Examination.—Brain and cord, normal.

Microscopic Examination.—Small accumulations of large mononuclear cells and lymphocytes in the cerebral sulci and about nerve roots are the only pathological findings. No organisms seen.

Diagnosis.—Focal subacute meningitis, aseptic.

This picture resembles closely that produced by weakly virulent organisms (some strains of *Bacillus coli* and streptococci).

PATHOLOGICAL TYPES OF INFECTION.

Analysis of the experiments dealing with the viability of the organisms in the subarachnoid space at once suggests two types: one in which the organism rapidly proliferates, the other in which it dies.

There are, however, many intermediate types of importance to be considered; moreover, the dosage, the age of the culture, the species of animal, and many other factors enter in. This subject has already been considered in the foregoing chapter.

Classification and Discussion of the Pathology.

The cases of experimentally produced meningitis here described may be grouped as follows: (1) focal subacute meningitis; (2) acute exudative meningitis of low grade type; (3) massive acute meningitis.

In the first group the brain and spinal cord are of normal gross appearance, but under the microscope are seen accumulations of lymphocytes, plasma cells, and frequently large mononuclear phagocytes. Rarely is an organism seen here. These foci of exudate are invariably in corners or pockets of the meninges, favorite loci being the sulci of cerebrum and cerebellum, and "root zones" of cranial and spinal nerves, at junction of nerve roots and dura, or nerve roots and brain or cord. Such cell groups are more frequently found in the deeper layers of the pia, close to the nervous tissue, than in the subarachnoid space. In this group, cultures both from the cerebrospinal fluid and blood are usually negative. Death, if it occurs, cannot be attributed directly to the meningitis.

This type of reaction was obtained with a number of strains of streptococcus, with staphylococcus, and with *Bacillus coli*; it is also similar to that seen late after serum injection.

In the second group the exudate varies in amount from little to much, but is never massive. In its character there is also variation; if seen early in the infection it is of the polymorphonuclear type; if later, it is of the lymphocytic formula. Organisms may be absent, but are more frequently few in number, never many. It must be assumed that in such cases the organism has exhibited a certain amount of subarachnoid virulence, but the smaller number of organisms is evidence of feeble viability. Death in such cases may be due in part to the meningitis but is more frequently a result of causes outside of the central nervous system.

Bacillus pyocyaneus and *Bacillus coli* gave excellent examples of this type of meningitis.

The third group is characterized by rapid proliferation of the organism within the meninges, even when the inoculation is minimal in amount. Exudate forms and promptly spreads throughout the subarachnoid space and beyond, causing death in a few hours or in one or two days, evidently for the most part as a direct result of the meningitis. It must be emphasized, however, that blood stream infection occurs promptly in these animals and that visceral lesions early show changes, which must be considered contributory to death.

This type of meningitis has been obtained with *Bacillus pyocyaneus*, *Bacillus coli*, *Bacillus paratyphosus*, meningococcus, and streptococcus, but not with constancy, and usually only after their virulence has been increased by repeated passage through animals. With *Bacillus lactis aerogenes* this type of fulminating meningitis has been obtained with very small numbers of organisms and with the regularity of prediction.

SPREAD OF INFECTION.

Of interest is the spread of infection from the point of inoculation in the subarachnoid space, based upon the study of the acute fulminating type of meningitis.

Subarachnoid Spread.—Whether the inoculation is made into the cisterna magna or into the lumbar sac, the organisms are disseminated very quickly throughout the subarachnoid space. Nevertheless, in animals dying or killed within 24 hours, it is generally possible to tell by the distribution of the exudate the seat of injection; after this time it can seldom be recognized.

Usually within the first 24 hours exudate is found for the most part well confined to the subarachnoid space (Plate 4, Fig. 7). The arachnoid membrane is here seen to serve an important function in limiting the spread both outward into the subdural space and inward into the deeper layers of the pia and the substance of the nervous system.

There is anatomical and physiological evidence to show that there exists free communication between perivascular and subarachnoid spaces (Weed). Certain it is that perivascular spaces promptly become infiltrated with exudate, apparently synchronously with the spread of exudate in the subarachnoid space. As a rule, however, it is the cortical vessels in the brain and the central vessels in the spinal

cord which show the earliest and most marked perivascular infiltration. Another point of interest is that usually few organisms are seen in the perivascular exudate at a distance from the meninges, even though many exudative cells are present. This, however, is by no means constant.

Spread to Ventricles and Spinal Canal.—Very early, in fact in a few hours, organisms are seen with great regularity in the fourth ventricle and canal, and somewhat later in the third and lateral ventricles; and shortly afterwards exudate makes its appearance also. The appearance of exudate in the ventricles seems to be associated with a similar infiltration of the velum interpositum and velum medullare, such infiltration preceding ventricular invasion. To show how early involvement of the ventricles occurs, of 39 cases analyzed, 95 per cent of the animals dying or killed within 24 hours showed invasion of ventricles or canal with organisms or exudate.

Invasion of Central Nervous System.—Somewhat later than the ventricles, but still early, infection of the parenchyma is seen. This occurred in 48 per cent of animals living less than 24 hours, and in 54 per cent of the total 39 cases analyzed. Usually invasion is by direct extension from the lateral ventricles and spinal canal; it may be in the nature of a rapid infiltration with organisms and polymorphonuclear leucocytes, or it may proceed more slowly, leaving complete destruction of nervous tissue in its path (Plate 4, Fig. 8 and Plate 6, Fig. 11).

Marginal infection is seen less frequently than the periventricular type. In this form invasion of the cord or cortex occurs by direct invasion from the meninges (Plate 5, Fig. 10). In these instances the infiltration is usually not so deep and not so rapid as in the previous type of invasion.

The formation of small foci of infection in brain and cord are occasionally seen, presumably secondary to infected blood vessels or perivascular spaces, but this type of brain and cord invasion is more rarely seen than either of the other types.

Spread Outside of the Subarachnoid Space.—As has been stated, the arachnoid membrane forms an efficient boundary to the spread of infection outward. In severe infections the arachnoid becomes in places loosely adherent to the dura, as can be demonstrated by dissection under the microscope, and the dura at this point frequently

shows a focal cellular reaction in which organisms may at times be observed. More commonly the exudate is seen leaving the subarachnoid space along with spinal nerves and attaining an epidural position about nerve roots and root ganglia. This is so conspicuous in some of the cases with *Bacillus pyocyaneus* that the dura becomes covered to a large extent by a greenish yellow exudate. At these "root zones" the dura is almost always infiltrated with exudate.

Blood Infection.—Animals dying of meningitis seldom fail to show organisms in the blood vessels, either microscopically or culturally. In very few animals, killed after a few hours, blood infection may be lacking, but after 18 hours it is almost invariably present. This is important, as this septicemia probably plays an important part in the death of the animal in meningitis.

The above considerations are based on the findings of acute fulminating meningitis alone. The cases of a subacute and chronic nature included in Groups I and II show certain of the above characteristics of spread only. In some there is evidence of generalized subarachnoid spread, but frequently the chronic type of exudate is found confined to certain areas of brain or cord alone; in these it is difficult to say whether the exudate has ever been generalized. There is reason to believe that invasion of ventricles and substance of the nervous system has never taken place in many of these milder types of meningitis, but has remained confined to the subarachnoid space. Blood stream infection frequently occurs and is doubtless the primary cause of death in many of these less acute forms of meningitis.

SUMMARY AND CONCLUSIONS.

Meningitis by subarachnoid inoculation was produced experimentally by a number of different organisms. The dosage of injection employed was never large (seldom over 1 cc. of a 24 hour broth culture), and was frequently small, in some cases infinitesimal (20 organisms with *Bacillus lactis aerogenes*).

The resulting meningitis may be grouped in three pathological types: (1) Focal subacute meningitis, in which small accumulations of exudate are found in isolated foci, especially the deeper layers of the pia. Organisms are usually absent from meninges and blood, both culturally and on microscopic examination. (2) Acute exuda-

tive meningitis of low grade type. Here the exudate may be scanty or considerable in amount, of polymorphonuclear or lymphocytic formula, but organisms, if present, are few in number and are considered to possess only mild subarachnoid virulence. (3) Massive acute meningitis, in which there is evidence of extreme virulence and proliferation of the organism. In this type alone is death considered to be due primarily to the meningitis.

In acute meningitis the exudate soon passes beyond the subarachnoid space into the ventricles, and a little more tardily invades the brain and spinal cord. In 24 hours approximately one-half of the cases show such involvement of the central nervous system; this involvement usually occurs by direct extension from ventricles and canal. The exudate also spreads outward with nerve roots and a patchy or diffuse epidural infection then results. The dura itself becomes infiltrated at areas of root perforation. The blood vessels early become infected in such acute meningitis, as seen in section and on culture, and it is likely that this septicemia plays an important part in the death of the animal.

In the less acute forms of meningitis the arachnoid membrane more or less effectively limits the spread of infection from without and from within. Moreover, in proliferation and budding off of its cells is seen the origin of at least some of the mononuclear phagocytes of the subarachnoid space.

EXPLANATION OF PLATES.

PLATE 1.

FIG. 1. Cat. Subarachnoid injection of heterologous serum. Killed in 24 hours. Pia of spinal cord shows marked sterile polymorphonuclear meningitis. $\times 720$.

FIG. 2. Same case as in Fig. 1. Aseptic meningitis of cortex. $\times 107$.

PLATE 2.

FIG. 3. Cat. Subarachnoid inoculation with *B. lactis aerogenes*; broth culture, 0.5 cc., 1:10. Symptoms of meningitis. Death in about 18 hours. Pia-arachnoid shows much exudate with very large number of bacilli. $\times 1,080$.

FIG. 4. Cat. Subarachnoid inoculation with *B. lactis aerogenes*; broth culture, 1 cc., 1:500. Death in about 18 hours. Film of meningeal exudate showing polymorphonuclear leucocytes and a large number of encapsulated bacilli. $\times 1,750$.

PLATE 3.

FIG. 5. Cat. Subarachnoid inoculation with *B. lactis aerogenes*; broth culture, 2 cc., 1:10. Death in 6 hours. Shows well marked meningitis with a large number of bacilli. Deeper layer of pia immediately overlying spinal cord free from organisms. $\times 720$.

FIG. 6. Cat. Subarachnoid inoculation with *B. pyocyaneus*; broth culture, 1 cc., 1:10. Killed when moribund in 48 hours. Shows circumscribed area of exudate in spinal dura caused by direct extension of infection from subarachnoid space. Arachnoid is seen adhering to inner surface of dura except over its infiltrated portion where the arachnoid is involved in the inflammatory process. $\times 107$.

PLATE 4.

FIG. 7. Cat. Subarachnoid inoculation of *B. lactis aerogenes*; broth culture, 1 cc., 1:400. Meningeal symptoms. Death in 30 hours. Cervical cord shows moderate amount of exudate. The pia mater and arachnoid membrane confine the exudate within the subarachnoid space. $\times 15$.

FIG. 8. Cat. Subarachnoid inoculation with *B. lactis aerogenes*; broth culture, 0.5 cc., 1:1,500. Meningeal symptoms. Death in 3 days. Spinal cord shows remnant of ependymal ring surrounding the central canal, which is bulging with exudate. Infection is seen spreading laterally in central gray and posteriorly into the white matter. This is a common type of invasion of the central nervous system occurring frequently after infections of over 48 hours. $\times 60$.

PLATE 5.

FIG. 9. Cat. Subarachnoid inoculation with *B. lactis aerogenes*; broth culture, 0.25 cc., 1:25,000. Meningeal symptoms. Death in 3 days. Shows massive exudate in cervical cord. Exudate at this time not confined to subarachnoid space, but invades canal, subdural space and dura, and substance of spinal cord. $\times 15$.

FIG. 10. Same section as Fig. 9. To show wedge-shaped area of invasion of spinal cord from meninges. $\times 60$.

PLATE 6.

FIG. 11. Same case as shown in Figs. 9 and 10. Shows invasion of brain tissue presumably by direct extension from lateral ventricle which contains exudate. Choroid plexus intact except for infiltration of polymorphonuclear leucocytes in stroma. $\times 60$.

FIG. 12. Same case as Fig. 8. Film preparation of meningeal exudate. Shows polymorphonuclear and large mononuclear cells, both types being phagocytic for bacilli. $\times 1,750$.

PLATE 7.

FIG. 13. Cat. Subarachnoid inoculation of streptococcus; 24 hour broth culture, 1 cc., 1: 10. Symptoms of meningitis with leucocytosis of cerebrospinal fluid, but few bacteria next day. Recovered from acute meningitis, but never became well. Death with abscess of neck 15 days after inoculation. Shows subacute meningitis of cortex. $\times 60$.

FIG. 14. Same section as in Fig. 13. Shows type of subacute meningitis, chiefly large mononuclear cells, some phagocytic, lymphocytes, and plasma cells. No organisms. $\times 720$.

PLATE 8.

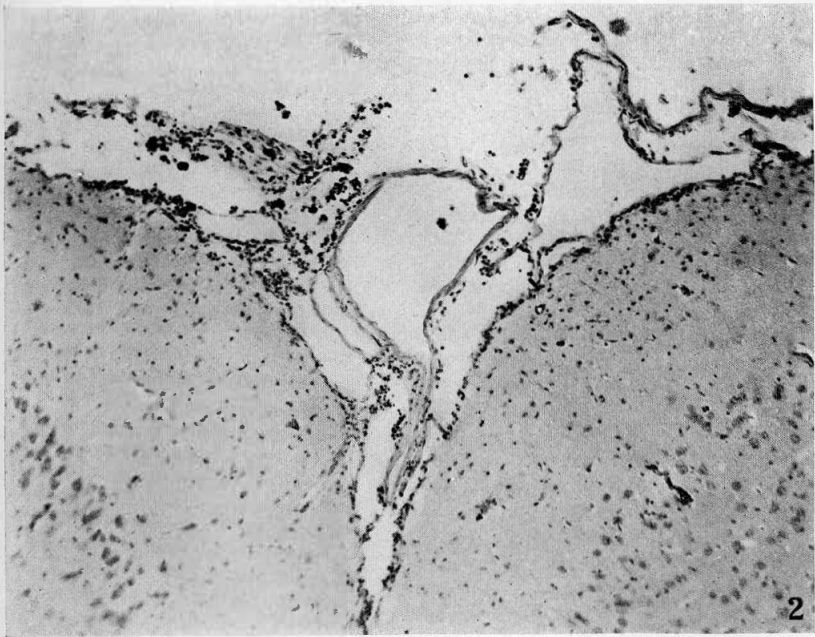
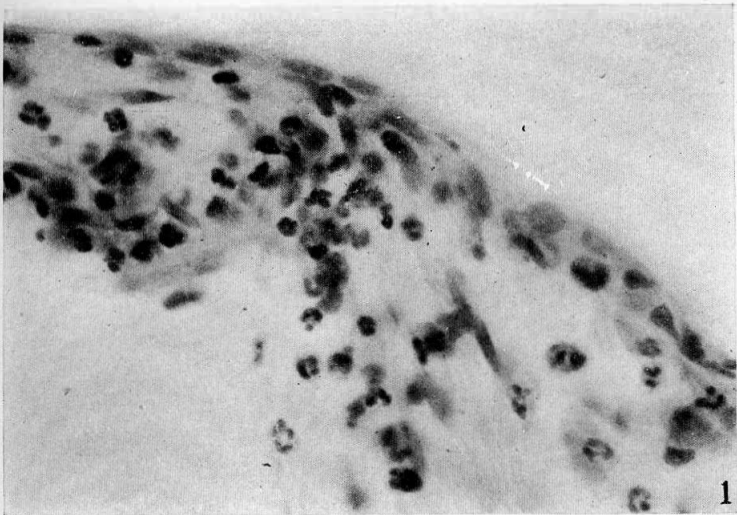
FIG. 15. Cat. Subarachnoid injection of streptococcus toxin, 3 cc. Meningeal symptoms. Death in about 18 hours. Shows artery in brain with marked subintimal exudation, and perivascular exudate. $\times 330$.

FIG. 16. Same section as in Fig. 15. Shows better the elevation of intima and polymorphonuclear leucocytes under it. $\times 540$.

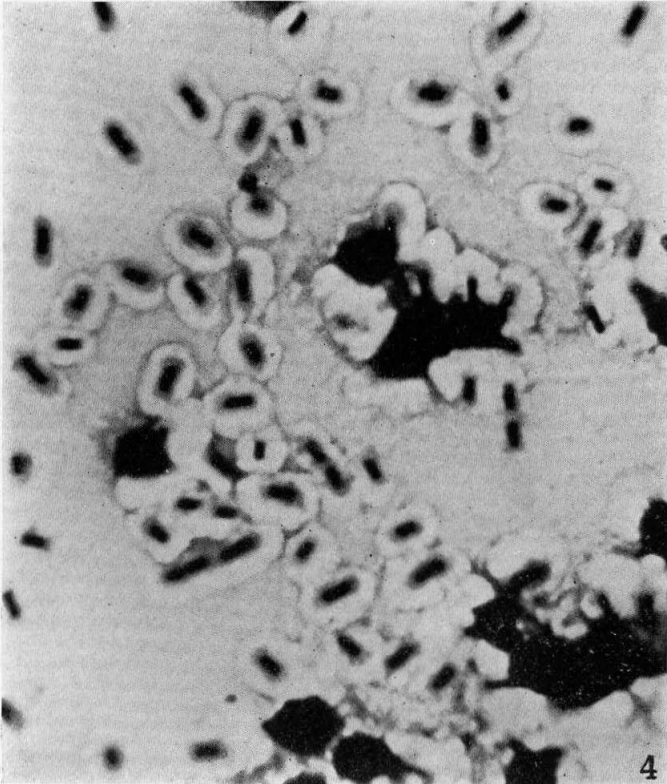
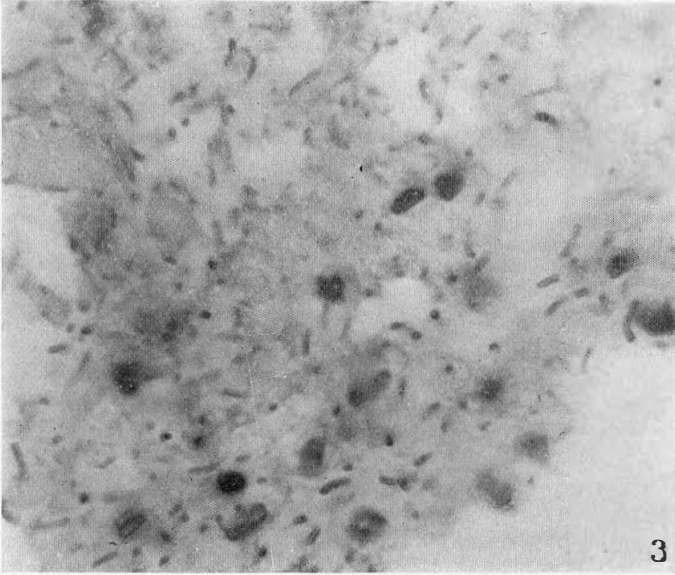
PLATE 9.

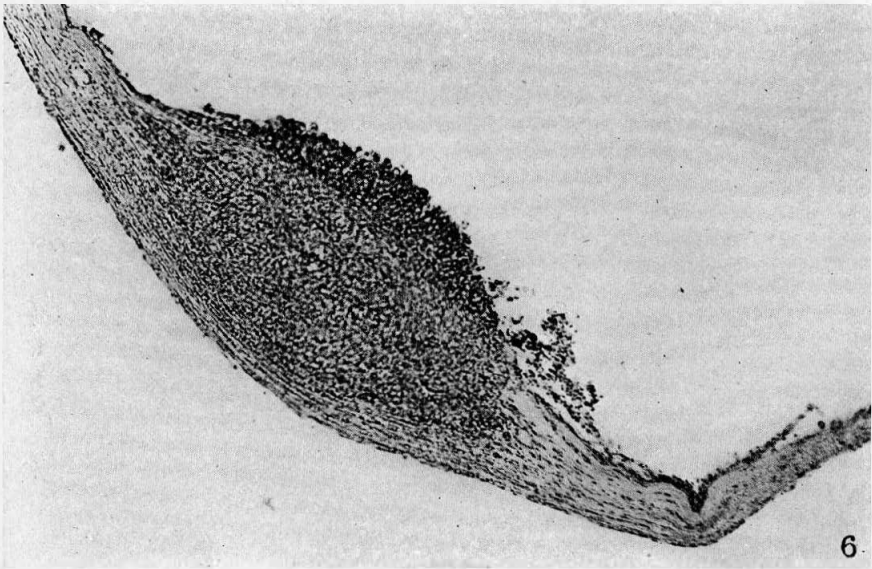
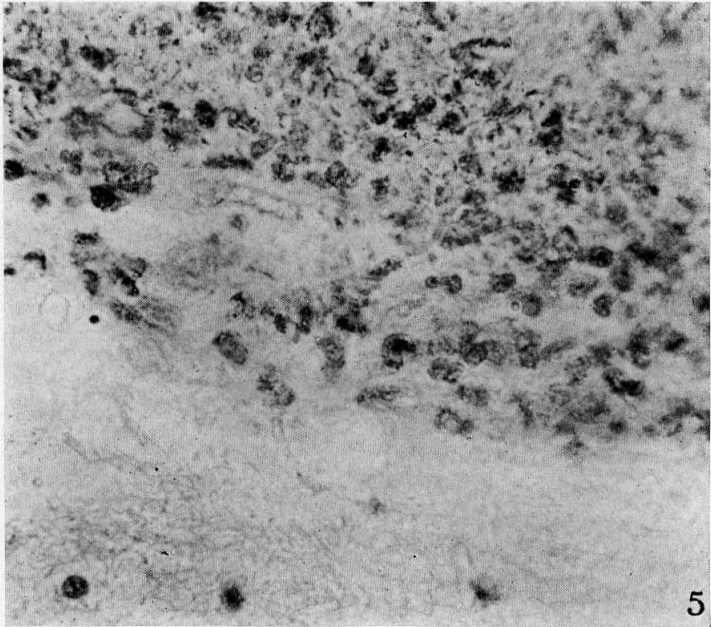
FIG. 17. Cat. Subarachnoid inoculation with *B. coli communior*; 24 hour broth culture, 0.5 cc. Symptoms of acute meningitis early, followed by symptoms of chronic meningitis. Death in 2 weeks. Shows localized subacute meningitis of cortex. $\times 150$.

FIG. 18. Cat. Subarachnoid inoculation with *B. pyocyaneus*; broth culture, 1 cc., 1: 10. Sick from onset, but no definite symptoms of meningitis. Killed after 7 days. Shows localized subacute meningitis in deep layer of pia mater of spinal cord close to an emergent root. Subarachnoid space free. $\times 107$.

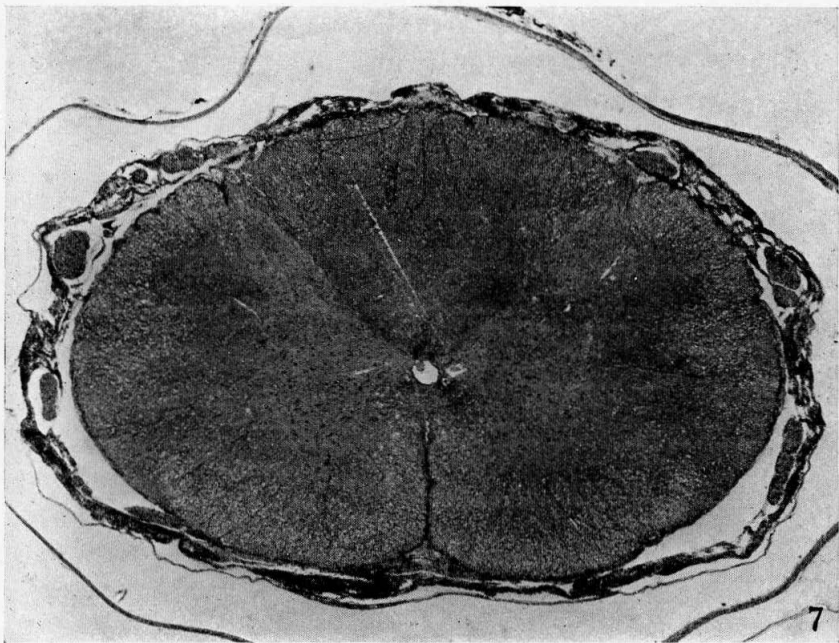


(Ayer: Pathological study of experimental meningitis.)



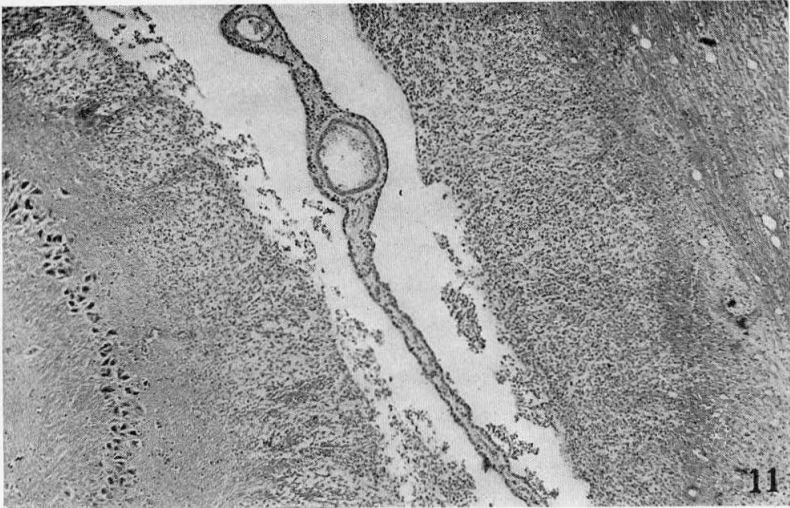


(Ayer: Pathological study of experimental meningitis.)

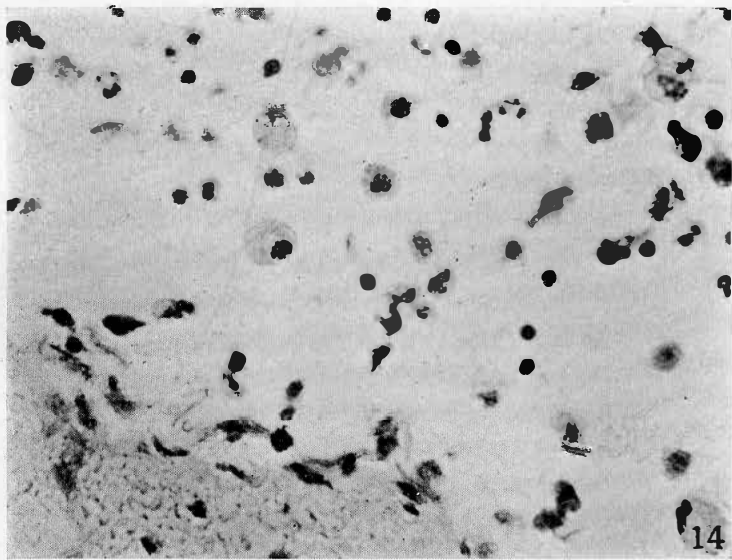
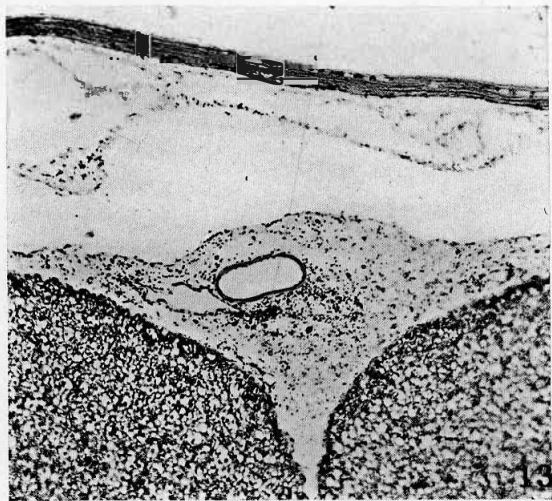


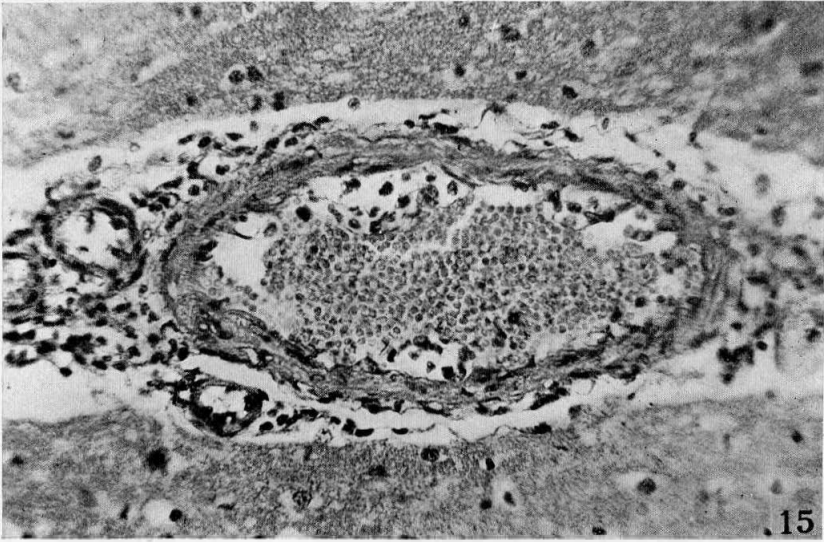
(Ayer: Pathological study of experimental meningitis.)



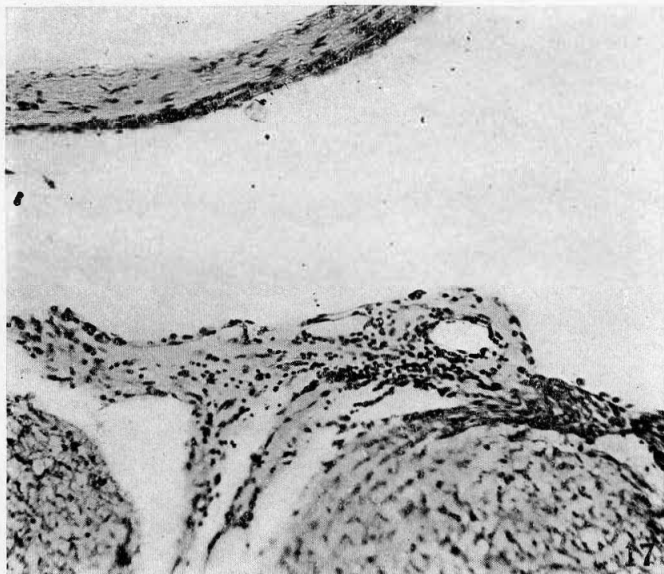


(Ayer: Pathological study of experimental meningitis.)





(Ayer: Pathological study of experimental meningitis.)



(Ayer: Pathological study of experimental meningitis.)

III. THE INTRAMENINGEAL VIRULENCE OF MICROORGANISMS.

By LLOYD D. FELTON, M.D.

In determining the original virulence of 102 strains of 21 groups of microorganisms, it was found that certain bacteria or groups of bacteria were more capable than others of producing a fatal infection when injected into the meningeal spaces. The term "original" virulence is understood to mean the pathogenicity of recently isolated strains of microorganisms, before increase in virulence by animal passage. As reported in the first chapter of this monograph the groups of bacteria which have been most commonly found to produce an acute meningitis in man, possessed a relatively low original virulence for the meninges of a cat, but the members of the *Bacillus mucosus capsulatus* group were demonstrated to possess a special pathogenicity or original virulence, not discovered in any of the other microorganisms studied. It is our purpose to present here a method for enhancing the original intrameningeal virulence of strains of *Bacillus mucosus capsulatus*, hemolytic streptococcus, meningococcus, and *Bacillus paratyphosus*, and to compare, as far as possible, this with other methods for increasing the invasive power of microorganisms. This work was undertaken for a two-fold reason; to maintain the virulence of microorganisms so that the production of an experimental meningitis could be standardized, and also to make possible the extension of the investigation of the factors favoring infection of the meninges from the blood stream. The biological principle that the withdrawal of cerebrospinal fluid from an animal during an experimental bacteremia with an organism possessing original virulence within the meninges, results in the development of an acute meningitis, required for its establishment organisms of uniform marked virulence. This latter phase of the work is taken up in the following chapter of this publication.

EXPERIMENTAL DATA.

Bacillus lactis aerogenes.—During the course of the investigation of experimental meningitis, it was found that a certain strain of the *Bacillus mucosus capsulatus* group, *Bacillus lactis aerogenes* (Perkins' classification), was an ideal microorganism for producing, in the common laboratory animals, a uniformly fatal acute meningitis with a relatively small number of bacteria. Three months after isolation of the strain, with storage during this period on ordinary agar at room temperature, one-billionth of a cubic centimeter of a 24 hour meat infusion dextrose broth on subarachnoid injection caused the death of the cat over night. The maintenance of virulence by this microorganism over this long period without special precaution, led to daily transfers of the culture on either a brain broth medium (macerated cat's brain in 150 cc. of meat infusion broth) or a cat's blood broth (10 per cent blood in meat infusion broth). On Feb. 26, 1918, 5 months after isolation of the strain, one-billionth of a cubic centimeter of the broth culture still produced, on subarachnoid injection, an acute meningitis with death of the animal (Cat 320) in 24 hours. But on July 16, 1918, 1,000 times the above dose, (1 cc. of 1:1,000,000 into subarachnoid space) had no noticeable effect on an animal (Cat 946), although the microorganism was still highly pathogenic as compared with any of the other bacteria tested. Even at this time its intrameningeal virulence was at least 1,000 times greater than that of other organisms. As such great decreases in invasive power vitiated many of our experiments, attempts were accordingly made to restore the lost activity by "animal passage."

The method first employed consisted of the intravenous injection into a cat of a supposedly lethal dose of the bacteria. About 1 hour after death of the animal, approximately 1 cc. of heart's blood was transferred into a culture tube, containing about 9 cc. of meat infusion broth. This inoculated medium was incubated for 18 hours and a smaller dose than initially used was injected into the vein of another cat. By this usual routine intravenous method, *Bacillus lactis aerogenes* was passed through six animals. The virulence increased to the degree that 2 cc. of an 18 hour meat infusion broth culture produced a fatal septicemia in 18 hours; 5 cc. of a culture of the same age

had been required to kill the first cat in this series. However, the intrameningeal virulence remained practically unchanged, 0.000,001 cc., when injected into the subarachnoid space, being necessary to cause the death of an animal.

The failure to bring about a marked difference in the pathogenicity of this culture either for the meninges or in the blood stream by this method compelled us to try animal passage by means of inoculation directly into the meninges, puncture being made through the occipito-atlantoid ligament into the cisterna magna (cf. Wegeforth, Ayer, and Essick). Martinotti and Tedeschi had observed in their work on anthrax that microorganisms cultivated from the brain after subdural injections were more virulent by intravenous route than the organisms isolated from the blood stream or spleen. Although their work has not been precisely confirmed, the major part of the contention was substantiated by our experiments.

By making use of the occipito-atlantoid puncture the technique of subarachnoid injection of microorganisms and of postmortem culture of the cerebrospinal fluid is very simple and most satisfactory. The routine procedure consisted in shaving the hair from the occipital region and painting the exposed area of skin with tincture of iodine. The head of the puncture needle (No. 18G) was covered with a small fold of sterile cotton, and the puncture was made through the sterile field. On withdrawal of the stylet a few drops of cerebrospinal fluid were allowed to escape, and from a syringe the desired number of microorganisms diluted to 1 cc. with Locke's solution was slowly injected. Locke's solution was used as a diluent in preference to normal salt to avoid any toxic effect of the pure sodium salt within the meninges (Weed and Wegeforth). As far as possible, within 2 hours after the death of the animal, occipito-atlantoid puncture was made again, with the same aseptic precautions. Often under these conditions enough cerebrospinal fluid will flow out of the puncture needle to inoculate a culture tube. But generally, slight suction must be applied with a tight fitting syringe to obtain the fluid. At no time was there failure to obtain sufficient fluid for the purpose of inoculation. As in the intravenous method the culture was incubated 18 hours and a portion of this was injected into the subarachnoid space of another animal. Table I demonstrates the value of this method.

In Table I the data included under the heading "Intravenous" refer only to the virulence of the culture of *Bacillus lactis aerogenes* when injected into the blood stream. The phenomenal augmentation of bacterial pathogenicity within the meninges is made manifest by the comparison of the number of organisms required to produce a fatal infection in Animals 21F and 28F. Assuming that the intradural injection of a smaller dose than 0.0005 cc. of an 18 hour culture (4,000,000 organisms) would not cause at that time the death of a cat,

TABLE I.
Virulence of Bacillus lactis aerogenes in Cats.
Intrameningeal and Intravenous.

Intrameningeal.				Intravenous.		
No. of animal.	Dilution.	No. of organisms.	Killing time.	No. of animal.	Dose.	Killing time.
			hrs.		cc.	hrs.
17F	0.1	2 billion.	12	1,253	2.5	Lived.
18F	0.02		15			
19F	0.01		8			
20F	0.002		15			
21F	0.0005	4 million. 870,000	15	1,173	1	Lived.
22F	0.00001		15			
23F	0.000001		15			
24F	0.0000001		36			
25F	0.00000001	210	48			
26F	0.000000001		24			
27F	0.0000000001		48			
28F	0.00000000001		24	1,245	1	Lived.
29F	0.000000000001	None.	Lived.			
				1,427	0.75	48

50,000,000 times more were injected into Animal 21F than into 28F. Or if we judge from the number of bacteria in each dilution, as determined by the plating method, the invasive property of each microorganism was increased 200,000-fold. The intravenous virulence did not increase in any such ratio. In an experiment 2 days before this series was begun, 5 cc. of an undiluted 18 hour broth culture of the organism killed an adult cat in 24 hours. On the day of the first subarachnoid injection in this series, 2.5 cc. of the undiluted culture had no noticeable effect upon an adult animal; the lethal dose was

between 5 cc. and 2.5 cc. of the straight culture. At the end of this experiment 0.75 cc. of a broth culture was required to kill a small cat (No. 1,427). However, 2 days previously, 1 cc. produced no appreciable reaction on an adult animal. At the most, the increase of intravenous virulence did not exceed 5 times, in contradistinction to the 50,000,000 increase in the subarachnoid pathogenicity. The subarachnoid pathogenicity of this microorganism was by this procedure increased 10,000,000 times more than the intravenous pathogenicity.

TABLE II.

<i>Streptococcus</i> (cats).				<i>Meningococcus</i> (rabbits).				<i>B. paratyphosus</i> B (cats).			
No. of animal.	Weight.	Dose.	Killing time.	No. of animal.	Weight.	Dose.	Killing time.	No. of animal.	Weight.	Dose.	Killing time.
	gm.	cc.	hrs.		gm.	cc.	hrs.		gm.	cc.	hrs.
8F	370	0.5	14	408	1,200	1.5	48	30F	500	0.5	7
9F	350	0.2	48	440	1,100	0.5	15	31F	640	0.2	10
10F	360	0.1	10	463	1,200	0.25	15	32F	500	0.1	Lived.
11F	340	0.05	15	475	1,100	0.1	18	33F	370	0.15	24
12F	800	0.01	72	499	800	0.1	15	34F	380	0.05	10
13F	900	0.01	15	516	1,150	0.05	20	35F	360	0.02	8
14F	700	0.005	12	523	1,100	0.05	15	36F	800	0.01	10
15F	900	0.001	12	526	1,100	0.025	24	37F	525	0.0001	24
16F	1,100	0.0001	Lived.	529	1,000	0.025	15	38F	400	0.00001	15
				537	1,000	0.025	15	Culture kept at room temperature for 12 days.			
				538	9,000	0.025	15	42F	500	0.01	Lived.
				539	1,150	0.001	17				

Bacillus paratyphosus B (Cats).—Table II represents the results of the application of the direct method of developing intrameningeal virulence of a strain of *Bacillus paratyphosus*, meningococcus, and hemolytic streptococcus. A culture of *Bacillus paratyphosus* B was chosen for this experiment for the reason that it possessed a certain degree of natural virulence on injection into the meninges of a cat. Animals 30F, 31F, and 32F show the record of a titration of the pathogenicity of this strain on the same day, the lethal dose at the beginning of this series lying, therefore, between 0.2 and 0.1 cc. of the undiluted 18 hour culture. The rapidity with which the intrameningeal infec-

tivity was augmented demonstrates characteristics similar to those shown by the strain of *Bacillus lactis aerogenes*; the intrameningeal virulence of the paratyphosus increased 1,000 times on passage through the meninges of six animals. There is a marked difference in reaction from the *Bacillus lactis aerogenes*, however, in that it is found after five trials that 0.0001 cc. of an 18 hour broth culture represented the smallest number of organisms that would produce a fatal meningeal infection. A titration of the intravenous infectivity at the time when the culture was of the greatest virulence showed that 0.25 cc. of a broth culture failed to kill, while 0.5 cc. of the same killed in 120 hours, and 1 cc. killed in 15 hours. The results indicated that the ratio of intrameningeal and intravenous lethal doses of this strain of *Bacillus paratyphosus* B was as 1:5,000.

Meningococcus (Rabbits).—The demonstration that the meningococcus was avirulent for the meninges of a cat, as detailed in the first chapter of this monograph, led to the choice of the rabbit for the possible development of the infectivity of this microorganism, by means of passage through the meninges. Many of the investigators have been unable to produce a meningitis in animals by direct inoculation of the meningococcus intraspinally; the successful experiments of Flexner were accomplished only by injection of fairly large doses of the organism, (2 loops being the smallest amount producing a fatal meningitis in a monkey, and the average dose required being three-fourths of a dextrose sheep serum slant). It was felt that any method facilitating the production of an experimental meningococcus meningitis would be of definite value ultimately in the field of therapeutics. The results with this organism (normal strain) would indicate that the development of a marked increase in its virulence was, by this method, more difficult than in the case of the former organisms cited (Table II). The medium used for the growth of the microorganism from the cerebrospinal fluid was unheated defibrinated rabbit blood, diluted 1 part to 3 parts with Locke's solution. The inoculated medium was incubated for 18 to 24 hours. A comparison of the subarachnoid dose given to Rabbits 408 and 539 reveals an increase of the invasive power of the meningococcus of 1,500 times. The intravenous lethal dose was not determined in this instance. At the time when the lethal intrameningeal dose was 0.025 cc. of the blood

media culture, an intravenous injection of 1 cc. of the same culture failed to kill. This demonstrated that at least 40 times more organisms were required to produce a fatal infection on intravenous injection of the meningococcus than after subarachnoid. Judging by the data obtained with other microorganisms, the ratio of the virulence of this strain of meningococci in the two modes of infection at the time of its highest activity would undoubtedly be 1,000 to 1. These results are interesting in the light of the failure of von Lingelsheim and Leuchs to increase the virulence of any of 10 different strains of meningococcus by passage through white mice (intraperitoneal injection). They were not only unable to increase specific bacterial pathogenicity, but state that the cultures became more avirulent after passage through as few as four or five animals. Albrecht and Ghon had a similar experience working with guinea pigs.

Streptococcus (Cats).—The marked insusceptibility of the cat to intravenous injection of a virulent strain of hemolytic streptococci, and the similar insusceptibility when the organism was injected into the subarachnoid space led us to attempt the development of its virulence for this animal by inoculation through the subarachnoid route. The culture of hemolytic streptococcus selected had, on the day used, a lethal dose of 0.0001 cc. for a rabbit on intraperitoneal injection. As can be seen by the weights of the animals (Table II), small cats were used, not by choice but because of the scarcity of adults. The rates at which its pathogenicity was increased were surprising. Although in Animals 9F and 12F the number of bacteria were not sufficient to cause death in 24 hours, a repetition of the same dose in the next cat (No. 13F) showed by the rate of death of the animals, that the pathogenicity had increased somewhat. It was found in these experiments that the success in this method, as well as in any other, can best be obtained when the dose is so regulated that the animal dies promptly within 24 hours. It is better to inject too many organisms and secure prompt death of the animals than to make use of insufficient numbers with consequent prolongation of killing time. The repetition of the same dose until the animal succumbs in the desired time gives the best results. In this instance the increase in intrameningeal virulence was 5,000 times. The intravenous injection of 2 cc. of an undiluted 18 hour serum broth culture had no effect on a small cat. Thus, a

lethal dose for the blood stream was at least 2,000 times greater than the dose necessary to kill when injected directly into the meninges.

Hemolytic Streptococcus (Rabbits).—In the above experiments the possibility of increasing the virulence of four varieties of microorganisms by means of subarachnoid inoculations has been demonstrated. It is our purpose now to give results of a comparison of this method with the other two that are used continually in bacteriological laboratories, intravenous and intraperitoneal injections. The usual technique was employed throughout; the heart's blood of rabbits receiving the intravenous injection was cultured in acetic broth for 18 hours and with that culture another rabbit was inoculated. The peritoneal cavity of rabbits dying of an infection following the intraperitoneal injection was washed out not later than 2 hours after death with 3 cc. of Locke's solution, this suspension being used for the desired dilutions for the next rabbit. An army strain of hemolytic streptococcus (Beebe) was employed after it had been stored in semisolid medium for about 2 months. Table III gives the details of this experiment. There was a more marked difference in the increase of virulence by the intravenous method than by the other two. Although in this series of ten animals the virulence of the streptococcus culture did not develop beyond 2 cc. of an undiluted culture on intravenous injection, subsequently, after passage through many more animals, 1 cc. of the undiluted culture produced a fatal infection.

A remarkable similarity in the rate and degree of virulence attained by the intraperitoneal and intrameningeal injections is at once apparent. Undoubtedly the decrease in the number of organisms used might have been the same in both instances. For, as can be seen from Table III, with one exception in the first ten, all intraperitoneal animals died within the 24 hours, while, in the intrameningeal series, three out of the ten lived over 24 hours, and one survived for a period of 96 hours; these results indicate that a smaller number of organisms would have produced a fatal infection. However, approximately the same degree of virulence was developed by both the intraperitoneal and intrameningeal methods. Although the time allotment for the accomplishment of the work did not permit of a careful comparison of the degree of virulence developed, an error made during the course of the experiments throws some light on this question. Rabbit 171 of the intra-

meningeal series received the dilution meant for Rabbit 5 of the intraperitoneal. Both animals died early, and for this reason the cultures were not returned to their original series. That the two cultures were of the same virulence and continued to increase in pathogenicity in the different locations would demonstrate, as far as this one strain of hemolytic streptococcus is concerned, that the increased virulence in the different sites is an indicant of the location of inoculation and

TABLE III.
Virulence of Streptococcus (Rabbits).

Intraperitoneal.				Intravenous.				Intrameningeal.			
No. of animal.	Weight.	Dose.	Killing time.	No. of animal.	Weight.	Dose.	Killing time.	No. of animal.	Weight.	Dose.	Killing time.
	gm.	cc.	hrs.		gm.	cc.	hrs.		gm.	cc.	hrs.
100	700	5	24	53	900	7	24	297	1,500	2	15
204	500	4	18	238	1,100	6	24	476	900	1	18
499	500	3	18	351	600	5	8	116	1,400	0.8	18
416	400	2.5	18	471	750	4	36	360	1,100	0.4	20
489	300	2	14	355	800	2	18	126	400	0.1	7
314	500	1	15	485	300	2.5	20	132	980	0.05	12
225	450	0.1	15	463	600	2	192	150	100	0.005	96
227	450	0.02	18	80	600	2	72	171	1,050	0.005	15
3	900	0.01	20	144	900	2	24	188	1,000	0.001	32
5	700	0.005	30	413	800	1	Lived.	193	1,000	0.0005	36
11	800	0.003	24								
13	1,200	0.002	15								
14	1,250	0.001	24								
21	1,300	0.0005	18								
19	1,200	0.00025	36								

bears no relationship to predilection for specific tissue. That both Rabbits 21 and 193 on intravenous injection of 0.0005 cc. of the culture with subsequent release of cerebrospinal fluid, died and were found at autopsy to have definite meningitis, with organisms in the cerebrospinal fluid (as determined by culturing), also argues that the virulence of this strain of streptococcus is not a phenomenon of predilection for specific tissue. For it will be shown in the following chapter that the localization of infection within the meninges from the blood stream is dependent upon altered pressure conditions within the

cranium, and the biological principle involved has been established by the successful employment of five different organisms.

A titration of the intravenous virulence at the end of the experiments showed that the strain of organisms in the intraperitoneal series, possessing an intraperitoneal virulence of 0.00025 cc., revealed a pathogenicity of 0.1 cc. on intravenous injection. The same titration with the intrameningeal strain, 0.0005 cc. being the lethal dose in the subarachnoid space, required the intravenous injection of 0.2 cc. to kill. Thus, the intraperitoneal virulence in comparison with the intravenous was as 400 : 1, and the intrameningeal as 200 : 1, while in comparison with the virulence as developed by means of intravenous method, the ratios would be 4,000 : 1 and 2,000 : 1, respectively. The method, therefore, can probably be used with advantage in developing the intravenous pathogenicity of other microorganisms.

DISCUSSION.

From the positive reports in the literature one must conclude that the infection of the meninges of laboratory animals is a difficult undertaking. Councilman, Mallory, and Wright were unable to produce meningococcic meningitis in rabbits, guinea pigs, or cats, but were successful in a single instance. These writers caused the death of a goat by the intradural injection of 1 cc. of a broth culture of this organism, and an acute meningitis was revealed by postmortem examination. The inconstant results of Weichselbaum, Bettencourt and França, Albrecht and Ghon, von Lingelsheim and Leuchs, and others in their attempts to produce an infection of the meninges of laboratory animals with recently isolated strains of meningococci are surprising. The satisfactory results of Flexner were in part due to the fact that he made use in his experiments of a more susceptible animal (the monkey) than those used by the other investigators. Inasmuch as practically all the work was done with recently isolated strains of the microorganisms, the age of the culture of the experiments in the literature may be considered a fairly constant factor. The only variable factor was the different species of animal. Wollstein in her work on influenza meningitis selected virulent strains of organisms as tested on other animals and then was compelled to make use of as much as two blood agar slants to produce a fatal meningitis in monkeys. On the other hand, Lamar was able to produce a fatal infection of the meninges of a monkey with pneumococcus by using a highly virulent strain of this organism. It is interesting to note that he used mice to maintain the virulence of the organism and consequently showed that a strain of pneumococcus virulent within the peritoneal cavity of a mouse is also virulent within the meninges of the monkey. Thus was demonstrated the possibility of using the intraperitoneal injection to increase the pathogenicity of the microorganism for the purpose of producing meningitis (with, at least, the pneumococcus).

From the consideration of the data given above, it may be assumed that the former work on the production of experimental meningitis by direct subarachnoid inoculation has been largely unsuccessful. Since it has been shown to be comparatively easy to increase the intrameningeal virulence of four microorganisms as in the above experiments, so far that a uniformly fatal meningitis follows the subarachnoid injection of a virulent strain, it is possible that this pathogenicity for any specific animal species is the essential potential activity for the infection of the meninges of that animal. That is, a strain of meningococcus of human origin may be capable of infecting human meninges but have little or no pathogenicity for the meninges of another species. However, after animal passage by means of subarachnoid injections, the microorganism may become virulent for the meninges of the experimental animal of another species and consequently the inoculation of the meninges may be followed by a typical fatal meningitis. It is a problem how far this species specificity operates in the reproduction of infectious diseases in experimental animals.

It has been demonstrated that the fatal dosage of bacteria virulent for the meninges and for the peritoneal cavity is much smaller when inoculated into these spaces than when given in the blood stream. The explanation of this phenomenon undoubtedly lies in the fact that the defensive mechanism of the animal is largely contained within the circulating blood. The rapidity with which a sublethal dose of microorganisms disappears from the blood stream after their injection is well known. According to Ransom, Morax and Loiseau, Takano, and others, there are immune bodies in the cerebrospinal fluid of immunized animals. But as shown by the present work, either the natural protective mechanism (natural immunity) is present in a very small degree within the meninges, or the central nervous system is extremely sensitive to bacterial toxins; such a deduction infers a tremendous difference between the intravenous and the intrameningeal virulence of microorganisms. The fact that the same numbers of virulent streptococci on injection both into the subarachnoid space and into the peritoneal cavity result in a fatal infection indicates that the sensitivity of the meninges was not alone the determining factor in the reaction. It seems most likely that in both cases we are dealing

with a mesothelium-lined sac serving as a good incubator, with enough nutriment for the development of the bacteria injected and with very slight inhibitory power of the body elements. As a result, the few organisms injected multiply and cause the death of the animal just as quickly as when a far larger dose is injected intravenously. The fact that bacteria injected intrameningeally, if in sufficient number to cause death, invade the blood stream, seems to warrant the conclusion that the relatively small number of bacteria injected either intraperitoneally or intrameningeally is equivalent to the massive dose injected intravenously. That is, the threshold of infection in the meninges being lower than in the circulating blood, the bacteria multiply until sufficient infectivity is developed to cause an invasion of the blood stream.

SUMMARY.

The method of intrameningeal injection has proved to be of value as a means of increasing the virulence of microorganisms within the subarachnoid space.

It was found possible by this method to increase the virulence of four different strains of microorganisms, representing as many groups to the degree indicated: *Bacillus lactis aerogenes*, 0.000,000,000,01 cc. of a 24 hour broth culture, killing in 24 hours (cats); *Bacillus paratyphosus* B, 0.0001 cc. (cats); hemolytic streptococcus, (cats) 0.001 cc. and (rabbits) 0.0005 cc.; meningococcus, (rabbits) 0.001 cc.

By the intraperitoneal and intrameningeal methods, approximately the same degree of virulence was developed with streptococcus in rabbits. The intrameningeal virulence became at least 500 times greater than the intravenous..

The ratio of the intrameningeal and intravenous pathogenicity of *Bacillus lactis aerogenes*, of *Bacillus paratyphosus* B, of streptococcus on cats and on rabbits, and of meningococcus was respectively 10,000,000 : 1; 1,000 : 1; 1,000 : 1 at least; 500 : 1.

IV. THE INFLUENCE OF CERTAIN EXPERIMENTAL PROCEDURES UPON THE PRODUCTION OF MENINGITIS BY INTRAVENOUS INOCULATION.

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PLATES 10 AND 11.

The first chapter of this publication recorded the experimental injections of living cultures of bacteria within the subarachnoid space of laboratory animals (cat, rabbit). Of all the various groups of organisms tested, a certain strain of *Bacillus mucosus capsulatus* was found to be of the greatest virulence; a typical acute and fatal meningitis has been produced by this organism when given in such dilutions that not over twenty bacteria, as determined by plating, were introduced into the cerebrospinal spaces. This organism, as differentiated in the classification of Perkins, should be grouped as *Bacillus lactis aerogenes*. Many other organisms were tested for virulence within the meninges but none was as effective as this particular *Bacillus mucosus capsulatus*. Strains of streptococci, after repeated passage through subarachnoid spaces attained a marked degree of virulence; likewise certain cultures of the paratyphoid and colon groups were capable of producing an acute and fatal meningitis. *Bacillus pyocyaneus* also was found to be an organism possessing powers of bringing about an acute meningitis in the cat. But the *Bacillus lactis aerogenes* must be considered as being the only organism so far worked with capable of producing an infection of the meninges in concentrations analogous to those causing similar infections in man.

When injected directly into the blood stream *Bacillus lactis aerogenes* was fairly quickly killed, except in those cases in which very large doses were administered. This intravascular virulence was relatively very low as compared with the intraspinal, and in consequence it was early felt that it might be possible to produce in the blood stream a fairly efficient antiserum. In the course of one of

these experiments dealing with the intravenous injection of living cultures of the organism into a cat, cerebrospinal fluid was withdrawn by occipito-atlantoid puncture. This fluid was clear and normal in every way. Within 24 hours this animal showed clinical signs of meningitis; the cerebrospinal fluid at this time was definitely turbid and the animal died shortly after of the meningitis. This observation was repeated and a definite relationship between the removal of cerebrospinal fluid during an experimental bacteremia and the subsequent development of a meningitis was thereby indicated.

As soon as this process favoring lodgment of circulating organisms within the meninges seemed established, further experiments dealing with other favoring factors were carried out. Thus the proper development of this work necessitated investigation of the effect of congestion of the cerebral veins during the height of such an experimental bacteremia and of the effect of temporary stoppage of the heart. Likewise, the reduction of the pressure of the cerebrospinal fluid by intravenous injection of concentrated solutions of salts was related to the production of a meningitis by organisms circulating within the blood stream. Also of importance in the serum therapy of the meninges was the investigation of the part played by an existent sterile meningitis upon the subsequent localization of an intravenous infection within the meninges. It is with the invasion of the meninges from the blood stream under the influence of these various factors that this chapter will deal.

METHODS OF INVESTIGATION.

The major portion of this work was done on cats. The selection of this animal was due to its availability in large numbers and to the fact that it has been the subject upon which much of the recent neurological work has been performed. The cat has proved to be of great value in this experimental study of meningitis, for it has shown susceptibility to certain of the organisms which are virulent for the other laboratory mammals and for man. In addition to the general employment of cats for this investigation, other laboratory mammals—monkey, rabbit, guinea pig, and white rat—have been

subjected to many of the same procedures in order that wider application might be given to the various biological principles involved.

The important observations were always made in series. A number of normal healthy adult cats would be selected and the experimental procedures carried out on several with the same or varying doses of the organism. One or more control animals were always included in every series; these were routinely given double the intravenous dose of bacteria as those subjected to the procedure of localization. These intravenous control animals were customarily allowed to live for 1 month; they were then killed and pathological examinations of the meninges made. Others were killed when the experimental animals of the series died, to afford necessary histological controls.

The variable procedure in these experiments naturally concerned the production of conditions which favored lodgment of the intravenous organisms within the meninges. The first of these facilitating processes was the release of cerebrospinal fluid; the removal of this fluid was brought about by either lumbar or occipito-atlantoid puncture. These punctures were performed before, synchronously with, or immediately after the intravenous inoculation, or at some later period. Routinely, the release of cerebrospinal fluid was accomplished 2 minutes after the injection of organisms into the vein—at the height of the experimental bacteremia. Such practice resulted inevitably in the production of a fatal meningitis if certain other conditions were met. In general, cerebral vascular congestion during a bacteremia was accomplished by pressure upon the jugular veins in the neck; temporary stoppage of the heart was brought about by excessive concentrations of ether by cone, with resumption of function after cardiac massage. To reduce the pressure of the cerebrospinal fluid without puncture of the meninges, intravenous injections of hypertonic solutions were given a few minutes before or at the same time as the intravenous inoculation. And as a means of altering the protective mechanisms of the meninges by sterile inflammatory processes, various sera were introduced into the subarachnoid space at intervals of from 5 to 120 hours before the intravenous inoculation.

Routinely, cultures of 24 hours growth were used for the intravenous injections. In a few of the experiments 18 hour cultures were injected; the longest period of growth was 72 hours. For *Bacillus lactis aerogenes* a meat infusion broth was found to be as satisfactory as any; but a brain (cat) infusion broth was for a time employed. For the other organisms the meat infusion medium was generally preferred.

The virulence of the culture at the time of experimental inoculation was soon found to be of the greatest importance. It was impossible to maintain this virulence within the meninges on any of the ordinary media and it became the customary practice to raise the virulence by frequent passage through animals. The intrameningeal virulence was not heightened by intravenous passage through cats; direct subarachnoid inoculations were essential in the rapid raising of the intraspinal virulence. With *Bacillus lactis aerogenes* it was found that a fairly constant and marked virulence within the meninges could be maintained by passage through cats in this manner once a week. With the other organisms it was advisable to perform the critical experiments shortly after the virulence within the subarachnoid space was at its maximum. Too great emphasis cannot be placed upon the importance of maintaining the high intrameningeal virulence of the organisms at the time of intravenous injection.

To control properly this important factor of the virulence of the particular culture used in any one series, one or more animals were injected with doses considered lethal under the conditions of the observation. In the earlier experiments dealing with intravenous inoculation and release of cerebrospinal fluid, the virulence was established for the culture by direct subarachnoid injection. After the demonstration that the withdrawal of spinal fluid during an experimental bacteremia is an inevitable means of producing a meningitis, this method was used as the means of control for all subsequent procedures. The death of such control animals within 24 to 48 hours was considered the necessary verification of the virulence of the organism.

Bacteriological studies of many phases of this work were carried out. Thus many cultures of the cerebrospinal fluid withdrawn were

made as well as of the heart's blood, in animals dying of meningeal infections or killed as controls. The results of these cultures will be given later.

The methods of investigation outlined above have been followed in general, though many alterations in the various experiments have been made as required by the investigation of particular angles of the problem. The essential controls for the different observations have been those concerned with the intravenous injection alone, and with the virulence of the particular culture as determined by the development of a fatal meningitis.

THE PRODUCTION OF MENINGITIS BY RELEASE OF CEREBROSPINAL FLUID DURING AN EXPERIMENTAL BACTEREMIA.

As soon as a possible relationship between the withdrawal of the cerebrospinal fluid and the subsequent development of a meningitis was indicated in the earliest observations with the intravenous inoculation of *Bacillus lactis aerogenes*, other experiments were devised to test out this hypothesis. The series were ordinarily so planned that the intravenous injection could be controlled by one or two animals receiving at least double the unit dose. It was early ascertained that a considerable margin between the lethal intravenous inoculation and the proper experimental dose existed. This permitted extensive rearrangements in the series so that various aspects of the problem could be investigated. In general, the control animal received an intravenous injection of 0.5 cc. of a 24 hour meat infusion broth culture of *Bacillus lactis aerogenes*, while the other animals in which withdrawal of cerebrospinal fluid was accomplished were given half this amount, 0.25 cc. Variations from this dosage were frequently made, being determined by the virulence of the particular culture.

Reactions of the Experimental Animals.—In the typical series of experiments, the control cat which had been given the intravenous injection of 0.5 cc. of the 24 hour culture of *Bacillus lactis aerogenes* customarily exhibited no abnormality in reaction. If no ether was administered, as in a rather small percentage of the cases, the animals could hardly at any time be distinguished from the normal. Those receiving the injection under anesthesia made rapid recoveries from the ether and became, in an hour or less, normal and active.

In 5 or 6 hours, these cats seemed frequently to be a little slow and cautious in movement, though for the most part they were as active as any of the stock animals. By the next morning it was quite unusual to find one of these control cats at all slow or weak; customarily they were very active and vigorous. Heart's blood cultures taken 24 hours after the initial injection have proved uniformly negative. Cerebrospinal fluid obtained at the same time has not shown any real increase in cells (below 10 per c.mm.) and has yielded negative cultures. These animals have remained normal until killed (usually at the end of 1 month) for histological control.

Although these intravenous control animals usually showed no real abnormality in reaction following the injection, there was during the first 24 hours apparently some disturbance of the animal's well-being. This was shown by the frequent observation of slowness and lack of desire for movement on the part of the animals 5 or 6 hours after the injection. For it must be assumed that during the destruction of the organisms intravenously there is a certain amount of fever and some malaise. This passed off ordinarily in a few hours, so that the morning following the injection the animal was normal and active. Furthermore such a febrile (?) reaction would account for a very slight increase in the mononuclear elements of the cerebrospinal fluid. Many observations of the temperature of these animals were made during these experiments; the variations in the temperature under the conditions of the laboratory were so extreme that no reliance could be placed on the findings. But on recovery from this short period during which the bacteria in the blood were being destroyed, the animals became and remained wholly normal. In pregnant animals abortion usually occurred during the first night but recovery was prompt and uninterrupted.

Contrasted strongly with these control cats which received double the unit number of organisms intravenously, were the other animals which, in addition to the unit intravenous injection of *Bacillus lactis aerogenes*, were subjected to withdrawal of cerebrospinal fluid. In the routine experiment the puncture into the subarachnoid space was done at the height of the experimental bacteremia—usually two minutes after the intravenous injection. The amount of clear cerebrospinal fluid removed was usually from 1 to 1.5 cc., the quan-

tity which would easily escape from a short lumbar puncture needle (18 G). For 5 or 6 hours afterward, no essential difference between the intravenous control and the punctured animal could be made out. During this time both behaved normally, but the punctured cat soon began to be slow and cautious in movement. This initial slowness was, in some cases, postponed for as long as 48 hours, but in the great majority of animals it was noted before 24 hours had passed. Added to this initial slowness and caution in movement was the frequent phenomenon of weakness in the posterior half of the body, exhibited as a definite drag and ataxia of the hind legs. Such a weakness could be demonstrated easily in a cat by dropping the animal from the height of a few inches. Instead of landing lightly and gracefully like the normal cat, the weakened cat would fall more heavily, rather clumsily, and would stagger slightly before recovery.

After the initial stage of slowness the animal receiving the intravenous inoculation with release of cerebrospinal fluid usually exhibited the various signs of meningeal (cortical) irritation. This stage was often wholly lacking and the cat might show nothing more than the weakness and slowness, with death occurring in the same length of time. In the cat meningeal irritation gave rise to many and varied signs and it was quite unusual for one animal to exhibit all the phases. Hypersensitiveness was only fairly common; a mild degree might, however, often be overlooked. This hyperesthesia has been so pronounced, that mere stroking of the animal's fur elicited crying and other signs of acute discomfort. On the motor side the more common findings were those related to the rigidities, though it may probably well be argued that these were the result of the removal of customary inhibition. In the milder cases the animals showed such extensor tendencies only when roused from the position voluntarily selected; thus an animal might appear on superficial inspection to be fairly normal when undisturbed, but if the head were retracted a more or less prolonged spasm might be elicited. Many cats suffering from such acute meningitis never showed spontaneous spasms or rigidities; others with no apparent external excitation passed through prolonged and repeated convulsions in which the extensor outthrust was typical. Still others re-

acted, during the stage of excitability, to loud noises, handclapping, etc., giving the motor response which characterized the animal at the time of excitation. The percentage of such infected animals which went through a phase of acute mania (running madly about the cages, climbing about in apparently sightless ferocity, and so forth) was not small; fortunately, however, most of the animals did not exhibit these abnormalities.

The clinical picture of the rigidities was quite typical and was wholly analogous to that occurring in man. In the common seizure the animal lay on its side with all four legs outthrust in an extensor rigidity, while the head was usually retracted into the position of opisthotonos. This extensor stiffness occasionally endured for long periods but usually passed off soon after the removal of the external stimulus. The effect of these prolonged extensor spasms seemed very exhausting to the infected animal. In the very severe cases these extensor rigidities were alternated with more or less prolonged movements of progression; the latter usually occurred in the moribund or unconscious animal. The development of such progressive movements seemed to have no other significance than to indicate a very severe infection with more or less loss of cortical function.

Cats, then, given intravenous injections of *Bacillus lactis aerogenes* with associated release of cerebrospinal fluid, exhibited within a few hours phenomena of meningeal infection. The earliest sign of such involvement was a slight weakness and slowness; a stage of excitement might, however, supersede all other indications. The animal rapidly became worse and gave the typical clinical signs of an acute meningitis. Most of the animals went through a stage of meningeal irritation, though a small proportion showed no abnormality except a slight weakness and an excessive caution in movement. The power of orientation was rarely badly impaired. Death ensued with regularity, in animals infected with *Bacillus lactis aerogenes*, in from 16 to 120 hours. The majority of the cats receiving the inoculation and puncture died in the period of from 24 to 48 hours. Even with signs of an overwhelming infection some of these animals have lived for 5 days. Others seemed to be rapidly overcome by the toxic products of the organism and succumbed readily before much exudation had occurred in the meninges. The cultures of the heart's blood in all

these cats at death have been positive for *Bacillus lactis aerogenes*. The production of a fatal meningitis in these animals by the release of the cerebrospinal fluid, following a suitable intravenous inoculation was practically invariable if the organism at the time of experimentation possessed virulence within the meninges and was given in proper dosage. Practically no cat in a very large series was found to be non-susceptible to this meningitis caused by *Bacillus lactis aerogenes*; when the virulence of the culture was for a time relatively low a more chronic form ensued. The relationship of the withdrawal of the cerebrospinal fluid during an artificial bacteremia to the later production of an acute meningitis seems therefore indicated.

Two condensed protocols from a typical series of experiments to show the consequences of the withdrawal of cerebrospinal fluid during an artificial bacteremia are given below in parallel columns. On the left appears the record of the control animal; on the right is given that of the cat punctured 2 minutes after the intravenous injection of the unit dose of the same culture (Series V, page 66).

The protocols given illustrate in a graphic way the essential difference in reaction of the cat receiving only the intravenous inoculation and that suffering withdrawal of the spinal fluid immediately after the inoculation. In the control animal some degree of reaction may have occurred following the injection, but the animal gave no evidence of sickness and remained normal and active. On the other hand, the punctured cat in 9 hours was definitely weak and sick; the next morning it exhibited the typical signs of an acute meningitis; in 24 hours from the time of experimentation it was dead. The photomicrographs of sections from similar areas of the cerebral cortex of the two cats show in the control normal meninges in every way. In the experimental animal, however, a typical leptomeningitis is shown, with distention of the subarachnoid space by exudative cells, bacteria, and fibrin.

Thus far emphasis has been laid upon the withdrawal of cerebrospinal fluid as an essential factor in the facilitation of the infection of the meninges from the blood stream. Such a view-point is substantiated by the comparative findings after the fluid is released by either occipito-atlantoid or lumbar puncture. Both punctures give identical pathological pictures of meningeal involvement; the resultant infection of these membranes from the blood stream is as invariable

Series V.

Cat 463.	Cat 467.
<p>Apr. 2, 1918, 10.30 a.m. Ether. Intravenous injection of 0.5 cc. of a 24 hour broth culture of <i>B. lactis aerogenes</i>.</p> <p>5.00 p.m. Normal and active.</p> <p>Apr. 3, 9.00 a.m. Normal, active animal.</p> <p>Animal remained normal and active; subsequently it developed a <i>bronchi-septicus</i> infection from which it recovered.</p> <p>May 6. Still normal and active. Ether. Heart's blood culture negative. Occipito-atlantoid puncture yielded 1.5 cc. clear cerebrospinal fluid, giving a negative culture.</p> <p>May 8. Still normal and active. Killed for control.</p> <p><i>Gross Pathological Diagnosis</i>.—Normal brain and spinal cord.</p> <p><i>Microscopic Examination</i>.—Normal central nervous system throughout (Plate 10, Fig. 1).</p>	<p>Apr. 2, 1918, 11.00 a.m. Ether. Intravenous injection of 0.25 cc. of the same broth culture of <i>B. lactis aerogenes</i>. 2 minutes later, occipito-atlantoid puncture with release of 2 cc. of clear cerebrospinal fluid, giving negative culture.</p> <p>5.00 p.m. Practically normal; not sick.</p> <p>7.45 p.m. Cat is definitely sick and dislikes to move. Weak in hind legs. Drops in sprawled-out position.</p> <p>Apr. 3, 9.00 a.m. Cat crouches in cage with head lowered. Very rapid respirations. Retraction of neck causes marked extensor rigidity as soon as head is released. Shows extensor outthrust and rigidities when excited. Sick and lethargic.</p> <p>11.30 a.m. Animal dead in cage. Culture of heart's blood gave positive <i>B. lactis aerogenes</i>.</p> <p><i>Gross Pathological Diagnosis</i>.—Acute leptomeningitis chiefly of brain.</p> <p><i>Microscopic Examination</i>.—Acute cerebrospinal meningitis; greatest amount of exudate in brain (Plate 10, Fig. 2).</p>

and certain when the fluid is removed by one route as by the other. In animals, puncture through the occipito-atlantoid ligament, as practiced by Dixon and Halliburton, offers a splendid method for the obtaining of cerebrospinal fluid, unmixed by blood contaminations. Lumbar puncture can also be done on cats and rabbits with ease after sufficient skill is acquired.

Below are given condensed protocols showing the production of meningitis by removal of spinal fluid by lumbar puncture during an intravenous inoculation and the typical reactions of the animal and its control (Series AU).

Series AU.

Cat 964.	Cat 971.
<p>July 22, 1918, 12 noon. Intravenous injection of 0.5 cc. of a 24 hour broth culture of <i>B. lactis aerogenes</i>.</p>	<p>July 22, 1918, 12.28 p.m. Ether. Lumbar puncture yielded 1 cc. of clear cerebrospinal fluid. While fluid was flowing from the needle, intravenous injection of 0.25 cc. of same broth culture of <i>B. lactis aerogenes</i>. Needle withdrawn and anesthesia stopped.</p>
<p>5.00 p.m. Normal, active animal.</p>	<p>5.00 p.m. Normal, active animal.</p>
<p>July 23, 9.00 a.m. Normal and active.</p>	<p>July 23, 9.00 a.m. Animal is quite slow in movement. Seems a little weak. Retraction of head causes no real reaction.</p>
<p>5.00 p.m. Normal and active. Animal remained normal and active until Aug. 20, when it was killed.</p>	<p>2.00 p.m. Dead in cage. Culture of heart's blood gave positive <i>B. lactis aerogenes</i>.</p>
<p><i>Gross Pathological Diagnosis.</i>—Brain and cord normal throughout.</p>	<p><i>Gross Pathological Diagnosis.</i>—Acute cerebrospinal meningitis with increased cerebral and spinal pressure, symmetrical.</p>
<p><i>Microscopic Examination.</i>—Normal meninges and central nervous system (Plate 10, Fig. 3).</p>	<p><i>Microscopic Examination.</i>—Acute exudative (mostly polymorphonuclear) leptomeningitis. Exudate greatest over cerebral cortex and in lumbar region of cord; slight in thoracic and cervical cord. Free bacilli in subarachnoid space. Exudate in cerebral ventricles, most in fourth. Bacilemia. (Plate 10, Fig. 4.)</p>

That infection of the meninges from the blood stream occurs in cats after release of cerebrospinal fluid by lumbar puncture as well as by occipito-atlantoid is amply demonstrated by the foregoing protocols. Likewise, the distribution of the exudate in the two cases (Cats 467 and 971) was not dissimilar; in both the greater involvement of the cortical meninges was recorded. Further note of the significance of this cerebral distribution will be made in a future paragraph of this communication. But of considerable importance in this study is the time relation of the withdrawal of the spinal fluid to the intravenous inoculation. In this experiment the lumbar puncture was first performed and while the fluid was still flowing from the puncture needle the intravenous injection of organisms was given. Further experiments along this line were carried out to determine with accuracy the time relations of the various procedures.

Application of the Same Procedures to Other Animals.—The invariable production of a meningitis by the release of the spinal fluid in an experimental bacteremia with *Bacillus lactis aerogenes* in cats naturally suggested that the phenomenon was due to a biological facilitation, and that it would occur equally well in other animals. Tests were immediately made on the other laboratory mammals and the inevitable occurrence of the meningitis under these conditions gave support to the hypothesis. The observations were carried out on monkeys, rabbits, guinea pigs, and white rats.

Two white rats were given the same intravenous injection of 0.2 cc. (1:100) of a 24 hour broth culture of *Bacillus lactis aerogenes*. 2 minutes later three drops of clear cerebrospinal fluid were obtained from one of the rats (No. 407) by occipito-atlantoid puncture, while the other (No. 406) was used as a control. The next day the control animal was normal while the punctured rat was very sick, remained lying on its side, and was just able to move. The condition of this punctured animal grew worse; on the 2nd day it had a persisting intense tremor and lay on its side with legs outstretched and tail retracted. On the morning of the 3rd day this rat was found dead in the cage, while the control animal remained active and well. Microscopically the punctured rat showed an extreme exudative leptomeningitis with involvement of the dura and of the substance of the nervous system. The control was killed on the 13th day; its nervous system was normal in every respect.

The experiments with rabbits were of the same positive character. The control animal (No. 402) was given an intravenous injection of 0.5 cc. (1:100) of a 24 hour broth culture of *Bacillus lactis aerogenes*. The other animal (No. 403) received one-half this amount (0.25 cc.) of the same culture, and 2 minutes later 1 cc. of cerebrospinal fluid was withdrawn by occipito-atlantoid puncture. The next morning the punctured rabbit was definitely sick and weak. It could not move about, constantly falling when progression was attempted. In the evening the animal was down on its side in the position of opisthotonos, and occasional spontaneous convulsions were noted. The next morning the animal was dead in the cage; an acute diffuse leptomeningitis was found on gross and microscopic examination. The control rabbit remained normal and active; it was killed 2 weeks later and at autopsy no abnormality of the nervous system was present. Microscopically a slight increase in the mononuclear cells in the leptomeninges was recorded—a normal postfebrile reaction.

Quite similar is the result of the application of the experimental procedure to guinea pigs. Two of these animals were given equal doses (0.3 cc., 1:100) of a 24 hour broth culture of *Bacillus lactis aerogenes*. The control animal (No. 404) was not affected by the injection but remained normal and active. It was killed on the 13th day for tissue control; the nervous system was normal on macroscopic and microscopic examination. The other guinea pig (No. 405) was subjected to occipito-atlantoid puncture 2 minutes after the intravenous inoculation; one drop of clear cerebrospinal fluid yielding a negative culture was withdrawn. The next morning at 9 a.m., this punctured guinea pig was definitely sick. It was undergoing spontaneous convulsions during which all four legs were rigidly outstretched and the neck was retracted. It could not walk or move around, due to a gross clonus and ataxia. At 10.45 a.m. that morning, the animal died. Microscopically a typical leptomeningitis of both brain and cord was found.

With the two monkeys at the disposal of this laboratory, similar experiments were performed. The control monkey (No. 411) was given intravenously 0.75 cc. (1:100) of a 60 hour agar slant of *Bacillus lactis aerogenes*. The other monkey (No. 412) was given a similar intravenous injection of the same culture, and 2 minutes later occipito-

atlantoid puncture was performed, with release of 2 cc. of cerebrospinal fluid. This fluid gave a negative culture, contained 0.3 gm. of globulin per liter, and the gold sol was recorded as 0011100000. The next day the control monkey was normal and in excellent condition, while the punctured animal was noted as being quite sick and weak. It was able to climb around the cage but was easily fatigued. The gait was somewhat ataxic in the hind legs. On the 2nd morning the control animal was in the same excellent condition; it was very pugnacious and lively. Contrasted with the reaction of this control monkey was the condition of the punctured animal. The following note regarding the latter was made:

"Animal quite sick. If left undisturbed it crouches on floor of cage with head down. Cries out frequently and respirations are rapid. As soon as animal is touched it cries, especially when the back and neck regions are handled. This handling brings out a coarse tremor with convulsive movements of all the limbs; retraction of the head cannot be tolerated. If placed on side on floor animal will maintain the abnormal position in preference to moving. Passive extension of limbs is resented."

With this difference in the clinical picture between the two animals 2 cc. of cerebrospinal fluid were obtained from both by occipito-atlantoid puncture at this time (43 hours after the initial injection). The fluid of the control monkey (No. 411) was clear, contained 20 red blood cells but no white cells to the c.mm., gave a negative culture, yielded 0.3 gm. of globulin per liter, and resulted in the following gold sol, 0011100000. From the punctured animal (No. 412) there was obtained a very turbid fluid, containing 14,000 white blood cells and giving a positive culture of *Bacillus lactis aerogenes*. The globulin content of this turbid fluid was 6 gm. per liter and the gold sol was recorded as 0012234444. In the film from this fluid, there were numerous white blood cells (mostly polymorphonuclears) and many encapsulated bacilli, both free and intracellular.

The punctured animal continued to be hypersensitive and to show extreme extensor tendencies. It died at 8.30 p.m. on the 2nd day, 53 hours after the intravenous injection and withdrawal of cerebrospinal fluid. At autopsy a widespread acute hemorrhagic purulent leptomeningitis was found; the spleen and the liver were somewhat swollen but no abscesses were found. The lungs were clear through-

out. The control monkey (No. 411), which had received only the intravenous dose, continued to be active and normal for 6 months; it then developed an acute tuberculosis from which it died 7 months after the original injection. At autopsy the central nervous system and meninges were normal.

The production of meningitis in these other laboratory animals (white rats, rabbits, monkeys, and guinea pigs) by intravenous injection of suitable doses of *Bacillus lactis aerogenes* followed by release of cerebrospinal fluid, indicates clearly that the withdrawal of this fluid causes within the meninges of such mammals a condition which favors the lodgment of organisms there. As a biological factor in the facilitation of the infection of the meninges from the blood stream, this withdrawal of cerebrospinal fluid seems indicated.

Withdrawal of Spinal Fluid during Bacteremias with Other Organisms.—The production of meningitis by the withdrawal of cerebrospinal fluid during an artificial bacteremia with *Bacillus lactis aerogenes* immediately suggested the employment of other organisms for the purpose. It was early learned that the occurrence of the meningitis under the experimental conditions depended largely upon the virulence of the organism within the meninges, and upon the number of organisms circulating within the blood stream at the time of subarachnoid puncture. The second condition was a subject of easy control; the first was limited by the efficacy of the method available for the raising of the intrameningeal virulence of the organisms.

It was further necessary not only that the intrameningeal virulence should be great but that the intravenous virulence should be fairly low. For the ordinary laboratory mammals *Bacillus lactis aerogenes* met these requirements ideally; even fairly large numbers of these organisms were quickly destroyed in the blood stream, while in the subarachnoid space small numbers sufficed to inaugurate an overwhelming infection. Several other organisms, notably *Bacillus pyocyaneus*, *Bacillus paratyphosus*, a strain of streptococci, and another of meningococci, were selected for trial. One of the earliest cases (Cat 475) when given *Bacillus pyocyaneus* intravenously with release of cerebrospinal fluid 2 minutes later developed a "mild generalized cerebrospinal leptomeningitis," as determined pathologically.

The great difficulty with the experiments on cats with *Bacillus pyocyaneus* was related to the intravenous virulence of this organism. This was usually quite high as compared with its subarachnoid virulence. Many experiments were carried out, but the results were of little value because the organism continued to grow within the blood stream, finally killing the animal. It was only when the subarachnoid virulence was raised by passage through animals that the experimental evidence could be relied upon. This heightening of the subarachnoid virulence was accomplished by lethal injection directly into the subarachnoid space, withdrawal of the infected spinal fluid from the animals at death, and injection of this fluid into another animal. Thus the intraspinal virulence was raised without relative increase in the intravascular. With the intraspinal virulence of the organism raised by this method, a typical leptomeningitis could be produced in cats by intravenous inoculation followed in 2 minutes by withdrawal of cerebrospinal fluid by occipito-atlantoid puncture.

The results of one series of cats injected with *Bacillus pyocyaneus* will be recorded here. The control animal (No. 626) was given an intravenous injection of 0.5 cc. of a 24 hour broth culture of *Bacillus pyocyaneus*; it showed no abnormality and remained active. The other animal (No. 627) was given the same dose (0.5 cc.) of the culture intravenously, with release of cerebrospinal fluid by occipito-atlantoid puncture 2 minutes later. Culture of this evacuated fluid was negative. On the 2nd morning this cat showed definite signs of weakness, but no other abnormality in reaction was noted before death occurred on the 5th morning. The culture of the heart's blood at death was positive for *Bacillus pyocyaneus*. The gross pathological diagnosis was recorded as "intracranial pressure; meningitis, especially cerebral." Microscopically, a mild mononuclear infiltration of both spinal and cerebral subarachnoid spaces, with a few pus cells and free organisms in one isolated area, permitted the diagnosis of "mild cerebrospinal leptomeningitis."

The same method of raising virulence was employed with a strain of *Bacillus paratyphosus* B. When the subarachnoid virulence had become as great as possible, the series of experiments was undertaken. The control animals received usually 2 cc. of a 24 hour meat infu-

sion broth culture intravenously and practically all remained normal. The other animals of the series were given 1.5 cc. of the same culture with release of cerebrospinal fluid in 2 minutes. In most instances the punctured animal showed more or less weakness with death in 5 to 10 days. At autopsy a mild leptomeningitis could be made out. In a few instances the control animal died from the septicemia, but without meningeal involvement. The meningitis produced with this organism in cats was not of the fulminating variety, but was subacute and chronic in nature and not necessarily fatal. The interpretation of these findings is more difficult than with organisms like *Bacillus lactis aerogenes*, but a definite facilitation of the infection of the meninges from the blood stream seems indicated.

By repeated passage through the meninges of rabbits, a strain of streptococcus was raised to the necessary degree in intraspinal virulence. Several series of experiments were carried out on these animals. In the larger series three rabbits (Nos. 1,403, 1,404, and 1,405) were given intravenous injections of an 18 hour culture of streptococcus, 1 cc. each of a 1:1,000, 1:2,000, and 1:5,000 dilution, respectively. All three of these animals remained normal and active, being killed on the 25th day. Cultures of the heart's blood of all these rabbits at death were negative; the nervous system and meninges of all were without abnormality. Three other rabbits of the same weight (Nos. 1,412, 1,413, and 1,414) were given intravenous injections of similar dosage and dilution of the same culture; cerebrospinal fluid was withdrawn from each by occipito-atlantoid puncture 2 minutes after the inoculation. Two of these animals (No. 1,412, receiving the 1:1,000 dilution, and No. 1,414, the 1:5,000 dilution) were noted as normal 5 hours later but were found dead in the cage the next morning. Heart's blood from both yielded, on culture at death, streptococcus, and at autopsy an acute early cerebrospinal leptomeningitis was found. Microscopically, a typical purulent leptomeningitis was diagnosed. The third rabbit (No. 1,413, receiving the 1:2,000 dilution) was somewhat sick and slow for 36 hours and then developed typical signs of an acute meningitis. It was hypersensitive, weak, and spastic in gait, and retraction of the head caused a convulsive reaction. For several days it remained in this critical

condition with a complete paraplegia developing; then it improved slightly and showed signs of a chronic meningitis in its spastic gait and general condition. It exhibited signs of this chronic lesion for 10 days, dying finally on the 17th day. Heart's blood culture at death was positive for streptococcus; the pathological diagnosis (gross and microscopic) was "mild subacute cerebrospinal meningitis."

After repeated passage through the meninges of rabbits the intraspinal virulence of a strain of meningococci from Camp Jackson was much heightened. Experiments were then carried out in series, the intravenous injection being usually 1 cc. of a 24 hour broth culture. In one series the control animal (No. 1,523 A) receiving the intravenous inoculation remained normal for a month before being transferred. On the other hand, the rabbit receiving the same intravenous injection of the culture but with release of clear cerebrospinal fluid 2 minutes later, developed a typical meningitis and died in 42 hours. Thirty minutes after death, turbid cerebrospinal fluid, containing 200 red and 200 white blood cells per c.mm., was obtained. The gross pathological diagnosis was "acute generalized leptomeningitis," while microscopically an acute exudative and hemorrhagic meningitis was found. In the film preparation from this fluid a few organisms were identified.

The production of meningitis by release of the cerebrospinal fluid during bacteremias with other organisms seems to establish the conception of the facilitation of the infection by the procedure on a fairly firm basis. With cats, *Bacillus pyocyaneus* and *Bacillus paratyphosus* B have been successfully employed; with rabbits the streptococcus and also the meningococcus have given positive results. The success of the method seems to depend solely upon the raising of the virulence of the organism within the meninges to the proper point and upon the injection of the suitable concentration. This contention is amply illustrated by the experiments with streptococcus detailed above. The strain of organisms was obtained by throat culture in an Army camp. It was passed through the meninges of seventeen rabbits before being tried out intravenously; by such repeated direct inoculations into the subarachnoid space its virulence for the meninges was markedly accentuated. The dilutions of the culture for the intravenous injections were as great as 1:5,000; the animal re-

ceiving this injection with release of the cerebrospinal fluid died over night. This dilution is higher and the intravenous dosage smaller than has been employed with *Bacillus lactis aerogenes* on cats. It is most likely, however, that with *Bacillus lactis aerogenes* the procedure could be successfully carried out with far greater dilutions than those employed, if the virulence was increased by the same method. This passage through animals was not necessary in the case of this organism, for the intravascular virulence was always very low as compared with the intraspinal. It seems fair to assume that in rabbits almost any of the common pyogenic organisms could be sufficiently increased in intrameningeal virulence to produce meningitis by intravenous inoculation with release of the cerebrospinal fluid. In cats the process would be more difficult owing to the relatively low susceptibility of the cat's tissues to the organisms common to man.

Time Relations of Meningeal Infection and Withdrawal of Fluid.—It has been shown in detail that if the cerebrospinal fluid is withdrawn during the height of a suitable bacteremia, infection of the meninges will invariably occur. This release of fluid was routinely accomplished by puncture 2 minutes after the intravenous inoculation; *i.e.*, at the height of the artificial bacteremia. Likewise, in a foregoing protocol (Series AU), the fluid was still flowing from the needle when the injection of organisms into the blood stream was given; meningitis resulted as in the other cases, demonstrating that the distribution of organisms was rapidly accomplished or that the effects of the withdrawal of fluid were not immediately overcome. Both these factors are of importance, but the latter is seemingly of greater significance.

To determine, then, the time limits at which release of the cerebrospinal fluid might be accomplished before the intravenous inoculation, a series of experiments was undertaken in which the fluid was withdrawn by occipito-atlantoid puncture before the intravenous inoculation with *Bacillus lactis aerogenes*. The data obtained are of significance in the correct understanding of the factors favoring the intrameningeal lodgment of organisms from the blood stream. In Cats 437 and 438 occipito-atlantoid puncture yielded 1.5 and 3 cc. of clear cerebrospinal fluid respectively; $4\frac{1}{2}$ hours later suitable intravenous injections of *Bacillus lactis aerogenes* were given. Both

animals remained normal and active, showing no signs of meningitis. Again, in Cats 458 and 474 withdrawal of 1.5 to 2 cc. of clear spinal fluid 2 hours before the intravenous inoculation gave no resulting meningitis. Similarly, in Cat 466 the puncture (2 cc. of clear fluid) was done 1 hour before the inoculations; the animal showed no signs clinically of meningeal infection. In Cat 456 release of cerebrospinal fluid was done 30 minutes before the intravenous injection of organisms; no meningitis followed. When, however, the interval was decreased below a half hour the occurrence of meningitis became more certain; if the puncture is done only a few minutes before the inoculation, a typical leptomeningitis will invariably result. Thus, in Cat 374 occipito-atlantoid puncture yielding 1 cc. of clear cerebrospinal fluid was performed 4 minutes before the intravenous injection of *Bacillus lactis aerogenes*; the animal showed typical signs of meningitis and died in 23 hours. At autopsy an acute exudative leptomeningitis (chiefly in the brain) was found; the control intravenous cat had a normal central nervous system. In general, therefore, it may be assumed that release of cerebrospinal fluid may be accomplished a half hour or more before the intravenous inoculation without the development of an acute leptomeningitis. Compensation for the loss of the fluid must be fairly efficient in the half hour interval.

Only one exception to this margin of 30 minutes has been found in this fairly large series. This was Cat 457, in which 2 cc. of clear cerebrospinal fluid were withdrawn 1 hour before the intravenous injection of *Bacillus lactis aerogenes*. The animal developed a subcutaneous abscess at the site of injection and was killed because of this on the 3rd day. Except for the local infection the animal was clinically normal in every way. The mild acute leptomeningitis found at autopsy was probably accounted for by the persisting infection of the blood stream from the abscess, the gradual breaking down of the animal's resistance, and the final involvement of all the tissues.

Studies have been made to determine how long after intravenous inoculation with *Bacillus lactis aerogenes* the withdrawal of cerebrospinal fluid may be accomplished and meningitis develop. It has been found that the length of this period varies with the virulence

and dosage of the organism, and with the resistance of the individual animal. Experiments to test out this time relationship were performed with routine intravenous injections of *Bacillus lactis aerogenes*. In one series (V) the control animal was given 0.5 cc. of a 24 hour broth culture of this organism; it remained normal and active until killed 5 weeks later. The other animals were given half this dose (0.25 cc.) of the same culture. Withdrawal of cerebrospinal fluid was carried out at varying intervals thereafter; those punctured up to and including 5 hours after the injection developed meningitis, while the two punctured at 7 and 9 hours, respectively, after the intravenous inoculation did not develop meningitis. The cerebrospinal fluid removed from all these animals by initial puncture gave a negative culture.

The interpretation of these findings does not seem difficult. For the limited dosage of organisms used, the animal possesses adequate protective mechanisms within the blood stream, as is shown by the fact that the control animals remain normal and give negative cultures in the heart's blood within 24 hours. On this basis the number of organisms within the blood stream constantly diminishes after the injection, and the number available for the infection of the meninges is, in consequence, constantly being reduced. The delay in the withdrawal of the spinal fluid therefore renders the infection of the meninges from the blood stream less likely, as the number of organisms available for this purpose is constantly becoming smaller. This hypothesis has been further substantiated by experiments in which the size of intravenous injection was decreased. A small enough number of organisms can be injected intravenously, so that no infection of the meninges results even if the withdrawal of fluid is accomplished at the height of the bacteremia. With virulent cultures, animals have many times been given meningitis by this procedure even when the injection was only 1 cc. of a 1:100 dilution of *Bacillus lactis aerogenes*. Routinely, however, 0.25 cc. of the undiluted culture has been injected; this yields a wholly sufficient number of organisms and is well below the lethal intravenous dosage. With streptococci in rabbits, the intravenous dosage has been as small as 1 cc. of a 1:5,000 dilution.

Observations have been made to determine how soon the infection of the meninges from the blood stream occurs after the release of the spinal fluid. The animals were all given routine intravenous injections of 0.25 cc. of a 24 hour broth culture of *Bacillus lactis aerogenes*, and 1 to 2 cc. of cerebrospinal fluid were withdrawn in 2 minutes. Cultures of this fluid were negative in all the animals in the series. The animals were killed after 1 hour, 2 hours, 4½ hours, and 6 hours. Films were then taken from the subarachnoid space in different situations and from the cerebral ventricles.

In the 1 hour animal, the brain and cord with meninges appeared normal in gross. From the subarachnoid space over the cerebral cortex, a few mononuclear cells (arachnoidal) were seen, together with four free bacilli. From the thoracic and lumbar spaces, plaques of arachnoidal cells were found; in these plaques a few bacilli were identified. In the 2 hour animal, no abnormality in the cord could be made out macroscopically in the fresh specimen, but the cerebral gyri seemed yellowish and somewhat flattened. Films from all portions of the subarachnoid spaces showed bacilli in small numbers and a few mononuclear (arachnoidal) cells. No polymorphonuclear cells were identified. In microscopic sections from these tissues no inflammatory reaction could be made out, but a single bacillus free in the cortical subarachnoid space was identified.

Evidences of exudation were present in the nervous system of the cat killed 4 hours after the puncture of the subarachnoid space. In the fresh state the spinal meninges and cord were not abnormal on gross examination. Over the cerebral hemispheres the surface patterns were obscured, particularly in the anterior region. The left frontal lobe was covered by a yellowish exudate, surrounded by a zone of hyperemia and petechial hemorrhages. Films from the cerebral cortex showed an exudate rich in cells, with a relatively great increase in polymorphonuclear leucocytes.

In the last animal in this series, a second occipito-atlantoid puncture was done 6 hours after the initial inoculation and puncture; the cerebrospinal fluid obtained by this second puncture was definitely turbid, contained 8,000 cells per c.mm., and gave a positive culture of *Bacillus lactis aerogenes*. The animal was then killed; cultures of the heart's blood immediately thereafter were positive

for the same organism. The gross pathological diagnosis of the central nervous system was "slight leptomeningitis, possibly more severe over cerebral cortex than in spinal region." Microscopic examination of sections from fixed material resulted in the following summary: "Acute exudative leptomeningitis, universal but with cerebral involvement greater than spinal; polymorphonuclear in type; rare bacillus free in subarachnoid space."

From the observations, then, on the time relations between the release of cerebrospinal fluid and the intravenous inoculation, it becomes evident that localization of the infection in the meninges occurs quickly after the fluid is withdrawn, provided that the organisms are virulent within the meninges and are circulating in sufficient numbers. Withdrawal of the cerebrospinal fluid more than 30 minutes before the intravenous inoculation has not facilitated the lodgment of bacteria within the meninges. Delay in the release of spinal fluid after the intravenous injection of virulent organisms decreases the likelihood of meningeal infection and a point is reached when the number of circulating organisms becomes too small to cause infection, so that the withdrawal of fluid does not result in meningitis. Exudative cells could not be demonstrated in the meninges until 4 hours after the essential experimental procedures were carried out; infection as demonstrated by the finding of bacilli free within the subarachnoid spaces occurred very quickly after the release of the fluid.

Determination of the Mode of Meningeal Infection from the Blood Stream.—The invariable occurrence of a meningitis after the withdrawal of cerebrospinal fluid during an experimental bacteremia suggested many hypotheses to account for the phenomenon. Naturally, one of the most prominent ideas dealt with the infection of the subarachnoid space as the result of a spread of the infection from the blood stream along the pathway of the needle; for the entrance of the needle in puncture through the dura makes a hole which is relatively enormous when compared with the red blood cell and the ordinary bacillus. Such a puncture, whether in the lumbar or occipital region, breaks its way through the various tissues, rupturing a number of capillaries and other smaller vessels. When a large number of organisms are circulating in the blood stream, infection

of the meningeal spaces could easily be conceived to occur along the pathway of the needle. Especially was this possible in the infections with *Bacillus lactis aerogenes*, of which the intrameningeal virulence was extreme.

Experiments were devised to test out the hypothesis that the infection of the meninges under these experimental conditions resulted from a local spread from the track of the needle. Absolute proof was not obtained by the data given below; the proper interpretation of the evidence, however, inclines strongly to the view that the infection is the result of a period, more or less short, of low pressure of the cerebrospinal fluid, altering in some way the normal defenses of the nervous system against organisms circulating within the blood stream. The evidence collected follows.

(a) *Cultures*.—Many cultures of the cerebrospinal fluid withdrawn at the height of the artificial bacteremia have been taken. These have proved negative in a great majority of the cases, showing that the infection of the spinal fluid is not immediate and that the passage of the needle through tissues supplied by blood, containing organisms, has not resulted in initial infection of the fluid. The character of the cerebrospinal fluid obtained determined the results of the cultures; these have proved negative in all specimens except those markedly contaminated with blood. Usually for these bacteriological controls the whole amount of evacuated cerebrospinal fluid (1 to 2 cc.) has been cultured, a procedure which should give a positive growth even if the number of organisms is small. In this respect it is fortunate that *Bacillus lactis aerogenes* yields abundant growths on the ordinary laboratory media.

(b) *Replacements*.—With the idea that after the withdrawal of the needle blood might escape through the puncture hole into the subarachnoid space (where the pressure was markedly reduced), the cerebrospinal fluid in certain experiments was replaced with normal saline or Ringer's solution. In Cats 476 and 477 occipito-atlantoid puncture was first done and 1 to 2 cc. of clear cerebrospinal fluid were allowed to escape. This fluid was in both cases replaced immediately by injection through the needle of 2.5 cc. of Ringer's solution. Two minutes after this procedure, an intravenous injection of 0.25 cc. of a 24 hour broth culture of *Bacillus lactis aerogenes* was given.

Neither animal developed meningitis, the experiments demonstrating apparently some protection.

Another procedure was then carried out with the animal receiving a primary intravenous injection, followed by occipito-atlantoid puncture. As soon as the initial flow of cerebrospinal fluid stopped, the stylet was replaced in the needle, and the closed needle left undisturbed for 2 minutes. The stylet was again withdrawn and from 2 to 2.5 cc. of Ringer's solution were injected into the subarachnoid space to replace the cerebrospinal fluid. Such procedure should determine whether the temporary decrease in the subarachnoid tension would be sufficient to facilitate the lodgment of organisms within the meninges. It is surprising that such a replacement of the fluid during the height of the artificial bacteremia invariably caused a typical leptomeningitis. The protocols of such a test observation are included below (Series Z, page 82).

These observations indicate that if the replacement of the evacuated cerebrospinal fluid is done before the intravenous injection is given, no meningitis will develop. If, on the other hand, the fluid is withdrawn during the bacteremia, the pressure maintained at a low value for 2 minutes, and replacement with Ringer's solution then carried out, meningitis will develop as in the routine experiment. The findings suggest that even a very short period of low tension of the cerebrospinal fluid is sufficient for the facilitation of the infection of the meninges. Support is also added by these replacements to the idea that infection of the meninges from the blood stream is in no way dependent upon the leakage from the track of the needle, but follows upon even temporary reductions of the pressure of the cerebrospinal fluid.

Other experiments, however, indicate that too much reliance cannot be placed upon the results of such replacements of the cerebrospinal fluid. In certain animals the routine injection of organisms was given intravenously and after 2 minutes puncture into the subarachnoid space through the occipito-atlantoid ligament was made; the needle was then withdrawn without allowing any fluid to escape to the exterior. Likewise, under similar conditions, puncture of the ligament was done with the stylet of the needle alone. Animals after both of these experimental procedures developed meningitis.

Series Z.

Cat 500.	Cat 501.
Apr. 16, 1918, 10.45 a.m. Ether. Intravenous injection of 0.5 cc. of a 24 hour broth culture of <i>B. lactis aerogenes</i> .	Apr. 16, 1918, 10.50 a.m. Ether. Intravenous injection of 0.25 cc. of same broth culture of <i>B. lactis aerogenes</i> . 2 minutes later occipito-atlantoid puncture. Clear fluid, 1.5 cc. Culture negative. The needle was kept in place; the stylet inserted and the closed needle left undisturbed for 2 minutes. Stylet again removed and subarachnoid injection of 2 cc. of Ringer's solution made, to replace cerebrospinal fluid.
5.00 p.m. Normal, active animal.	5.00 p.m. Normal, active animal.
Apr. 17, 9.00 a.m. Normal and active.	Apr. 17, 9.00 a.m. Cat is very weak and lethargic. It falls heavily and moves slowly and gingerly. Retraction of the head causes no reaction.
5.00 p.m. Normal and active.	5.00 p.m. Cat is not very lively and is weak in hind legs. Not so lethargic as in morning.
Apr. 18, 9.00 a.m. Normal and active.	Apr. 18, 9.00 a.m. Found dead in cage. Heart's blood culture positive for <i>B. lactis aerogenes</i> .
Animal remained normal and active.	
May 23, under ether, occipito-atlantoid puncture yielded 1 cc. clear cerebrospinal fluid, containing 10 white blood cells and no red blood cells, culture negative. Globulin 0.5 gm. per liter. Heart's blood culture negative. Remained normal and active until May 27, when killed.	
<i>Gross Pathological Diagnosis</i> .—Normal brain and cord.	<i>Gross Pathological Diagnosis</i> .—Acute exudative leptomeningitis of brain and cervical cord.
<i>Microscopic Examination</i> .—Normal meninges and central nervous system (Plate 11, Fig. 5).	<i>Microscopic Examination</i> .—Extensive acute leptomeningitis, universal, but massive only in cerebral and cervical portions. Early slight pachymeningitis. (Plate 11, Fig. 6.)

These observations suggested immediately that the puncture hole through the dura permitted escape of the spinal fluid into the looser tissues outside the dura, and thereby effected a reduction of the intracerebral pressure sufficient to facilitate the lodgment of organisms within the meninges. To verify this view, cerebrospinal fluid was withdrawn in another cat by occipito-atlantoid puncture and an equal quantity of India ink injected into the subarachnoid space for replacement. Subsequently these carbon granules were found to have spread extensively throughout the softer tissues of the neck, demonstrating the final distribution of the fluid of replacement. These latter findings, then, strongly indicate that mere opening of the dura and of the arachnoid allows an escape of fluid into the softer tissues of the region, thus reducing the intracranial pressure to a degree sufficient to permit invasion of the meninges from the blood stream.

(c) *Distribution of Meningeal Exudate.*—It is not proposed, in the present communication, to discuss at length the pathology of the meningitis caused by intravenous inoculation and release of cerebrospinal fluid. However, a certain gross aspect of the distribution of exudate in these cases has a distinct bearing on the problem of the method of infection.

In all the animals in which meningitis was produced by direct inoculation of virulent organisms into the subarachnoid space, the distribution of the exudate in the early cases indicated a direct spread from the local point of injection. The exudative process was most extreme in the region immediately adjacent to this point of puncture and gradually became less marked as one progressed from this point. This finding held, of course, merely for the earlier cases, for the specimens of several days duration of infection showed a generalized and equal exudation throughout the whole subarachnoid space.

Quite different from this type of infection resulting from the spread from the local point was the character of the meningitis brought about by the intravenous inoculation and release of cerebrospinal fluid. In this, as evidenced in the protocols given, the infective process was largely cerebral and the spinal cord became only secondarily affected. This marked involvement of the cerebral meninges was found to hold in about 90 per cent of the animals dying or

killed under 24 hours. In a very small percentage of cases the spinal exudate was greater than that of the brain. Reduced to simplest form, the distribution of the exudate in the meninges following intravenous inoculation with release of the cerebrospinal fluid suggests strongly that the infection was not from a local point of puncture through the dura mater. The usual cortical distribution of exudate when the release of fluid was brought about by lumbar puncture argues strongly against the idea of a spread from a local point.

The evidence presented above is not in any way absolute, but it indicates quite strongly that the facilitation of the involvement of the meninges from the blood stream after removal of the cerebrospinal fluid is dependent upon the reduction of the pressure of the fluid or other general intracranial reaction, and is not related to the opening of the meninges by the needle except as this procedure permits escape of the fluid. In the following sections, further experiments dealing with this hypothesis of the mode of infection will be given.

CEREBRAL CONGESTION AS A FACTOR IN PRODUCING MENINGITIS.

The many and varied experiments dealing with the release of cerebrospinal fluid during an experimental bacteremia led to the idea that the essential factor in the invasion of the meninges from the blood stream was the lowered pressure of the cerebrospinal fluid or other intracranial readjustment. Numerous observations contributed to the support of this hypothesis,—the pathological lesion, the result of replacements of fluid, and the general consideration of the whole process. But it was impossible to exclude absolutely the interpretation that the invasion of the meninges was the result of the spread from the local puncture hole of the needle through the dura and arachnoid. Consequently other experiments were required to give further data upon which to base the essential hypothesis of the mechanism of meningeal lodgment of circulating organisms.

Consideration was immediately given to the intracranial readjustments which necessarily ensued upon the withdrawal of the cerebrospinal fluid. It was assumed that the cranial cavity was of relatively fixed capacity (within certain limits) and was completely filled

by three elements—brain, blood, and cerebrospinal fluid. Alterations in the volume of any one of these were compensated for, to a greater or lesser degree, by change in one or both of the other elements. Thus an increase in the bulk of the brain may be considered to cause in the initial stages a compensatory dislocation of the cerebrospinal fluid, followed later by alteration in the blood volume of the cranium. A fairly efficient mechanism of readjustment seems to exist within the cranial cavity. Removal of a certain amount of cerebrospinal fluid is followed by a period of low intracerebral pressure, apparently due to the fact that the compensation for the loss of this fluid while adequate, is not immediate. But certain vascular adjustments, occurring quickly upon withdrawal of the cerebrospinal fluid, must necessarily fill a large part of the space occupied by the evacuated fluid. The chief vascular alteration seems to be a more or less extensive venous engorgement, with associated slowing of the blood stream, sufficient to account for at least a portion of the space occupied by the fluid withdrawn.

It seemed wholly possible to subject to experimental proof, this hypothesis of a partial vascular compensation for the cerebrospinal fluid withdrawn. With the lowered pressure of the fluid resulting in venous engorgement and slowing of the blood stream, bacteria might well be assumed to find that the ordinary protective agencies in the meninges were altered or destroyed, so that invasion of the subarachnoid space was possible. Likewise, the period of low intracerebral pressure might possibly cause physiological alterations in the cellular boundaries of the meningeal spaces.

Hence, it was proposed to bring about, in animals, subjected shortly before to intravenous inoculation with an organism virulent within the meninges, a venous congestion within the cranium. The experimental congestion produced really amounted to an incomplete, temporary stasis, for the pressure applied by the fingers over the jugular vein was unquestionably referred to all the deeper structures of the neck. This digital compression was maintained in the routine experiment for 2 minutes and the animals were then allowed to recover from the ether. All the observations were carried out on cats with intravenous injections of broth cultures of *Bacillus mucosus capsulatus*. The intravenous controls, with the same or double the

inoculation, were included in every group of experiments. Likewise, the virulence of the culture was established by the further inclusion in every series of a cat, given the same intravenous injection, but from which cerebrospinal fluid was withdrawn at the height of the experimental bacteremia.

About one-half of the animals subjected to this jugular compression at the height of the bacteremia developed a typical leptomeningitis. Certainly the experimental procedure of digital compression was variable enough to account for the failure of the other animals to become infected. But the positive cases are of importance as they indicated quite strongly that the brief cerebral venous engorgement was sufficient to facilitate the infection of the meninges. The animals subjected to this compression of the neck usually did not show signs of meningeal involvement until the 2nd or even the 3rd day; the controls receiving the same dose but with release of cerebrospinal fluid usually died within 48 hours. It must be assumed, then, that the number of organisms lodging within the meninges after congestion is smaller than after escape of spinal fluid; this infection of the meninges with the smaller number of bacteria necessitates a longer period of growth before the process becomes widespread enough to cause outspoken signs. The meningitis produced is wholly similar to that recorded in foregoing sections.

The protocols of a control cat and one subjected to the routine compression of the jugular veins and adjacent tissues are included below (Series AV, page 87).

The fact that a typical leptomeningitis could be produced in about 50 per cent of the cases by this variable, brief digital compression of the neck offers support to the view that the infection of the meninges from the blood stream, in the animals from which cerebrospinal fluid was withdrawn by puncture, was not the result of a spread from a local point. It must be assumed that this crude method of compression produced a temporary, yet marked venous engorgement. The first result of this engorgement was probably the expulsion of as much cerebrospinal fluid as possible; this expulsion of fluid was reduced to a minimum by the rise in venous pressure and the slowing of the venous stream. Following the compression, however, the vascular adjustments were rapid and the

Series AV.

Cat 984.	Cat 987.
<p>July 24, 1918, 11.13 a.m. Intravenous injection of 0.5 cc. of a 24 hour broth culture of <i>B. lactis aerogenes</i>.</p>	<p>July 24, 1918, 11.37 a.m. Ether. Intravenous injection of 0.5 cc. of same broth culture of <i>B. lactis aerogenes</i>. 2 minutes later both jugular veins and adjacent tissues of neck were compressed for 2 minutes. During this compression the heart rate did not vary.</p>
<p>5.00 p.m. Normal, active animal.</p>	<p>5.00 p.m. Normal, active animal.</p>
<p>July 25. Normal and active. Aborted.</p>	<p>July 25. Normal, active animal.</p>
<p>July 26. Very good condition. Normal.</p>	<p>July 26. A little slow and weak but otherwise normal. Retraction of head causes no reaction.</p>
<p>July 27. Very good condition. Normal.</p>	<p>July 27, 9.00 a.m. Weak and somewhat slow. Retraction of head causes profound and prolonged extensor spasm of all four legs with bushing of tail. Sprawls out on floor following spasms.</p>
<p>July 28, 9.00 a.m. Normal, active animal. Animal remained normal and active for a month when killed for tissue control on Aug. 23, 1918. Heart's blood immediately after death negative.</p>	<p>4.30 p.m. No change in condition.</p>
<p><i>Gross Pathological Diagnosis.</i>—Normal meninges and central nervous system.</p>	<p>July 28, 9.00 a.m. Dead in cage. Culture of heart's blood positive for <i>B. lactis aerogenes</i>.</p>
<p><i>Microscopic Examination.</i>—Normal central nervous system and meninges (Plate 11, Fig. 7).</p>	<p><i>Gross Pathological Diagnosis.</i>—Cerebral leptomeningitis. Spinal cord shows edema and perhaps early meningitis.</p> <p><i>Microscopic Examination.</i>—Acute leptomeningitis with cerebral involvement greater than that of cord (Plate 11, Fig. 8).</p>

return to normal was accomplished quickly. It seems likely that a period of low pressure of the cerebrospinal fluid should follow the compression; this lowering of the pressure could be accounted for by the variable amount of the initially expelled fluid. Observations

were made to test out this possible lowering of the fluid pressure, but no positive data were obtained.

It seems best to explain this production of meningitis by cerebral venous congestion during an experimental bacteremia on the basis that the organisms were able to overcome the normal protective agencies of the central nervous system during the period of slowed blood flow through the cranium, with or without alterations in the cerebrospinal fluid.

STOPPAGE OF THE CIRCULATION AND THE LODGMENT OF BACTERIA WITHIN THE MENINGES.

The influence of the slowing of the cerebral venous flow on the production of meningitis by intravenous inoculation led naturally to the idea that similar facilitation of the infection would result from temporary cessation of the circulation. It was felt that with virulent bacteria in the blood stream, the complete stasis of the blood, even for a very limited time, would allow organisms to invade the tissues otherwise invulnerable, and under the experimental conditions the involvement of the meninges might be predicted. Any radical variation between the pressure of the cerebrospinal fluid and that of the vascular system would be avoided by this complete arterial and venous stasis.

Stoppage of the circulation within the cranium alone was the logical procedure, but this was impracticable experimentally, even with the application of a tourniquet to the neck, for the spinal arteries afford an intracranial blood supply not affected by compression of the neck. In consequence recourse was had to the stopping of the heart by increasing the concentration of the anesthetic. Under these conditions the series of observations were so planned that a control animal was given the same or double the intravenous dose, while the virulence of the particular culture was established by the production of meningitis in another animal by similar intravenous inoculation and release of the cerebrospinal fluid. The experiments were all done on cats and the organism used was *Bacillus lactis aerogenes*.

The stopping of the heart was accomplished without difficulty by pushing the ether to maximum concentration in the inspired air. The heart beat was followed with a stethoscope, and when total cessation was noted a period of 30 seconds was allowed to pass. Car-

diac massage was then begun and artificial respiration with a bellows applied. By this means usually three out of every four animals could be revived; these animals recovered from the anesthesia and the manipulation normally. For the most part they were recorded as normal animals 6 hours later. Of the animals subjected to and surviving this experimental procedure, approximately one-half developed a fatal meningitis. The remainder of the animals under observation showed no abnormality.

The protocols of a control and experimental animal follow.

Series BC.

Cat 1,053.	Cat 1,049.
<p>Aug. 12, 1918, 11.00 a.m. Ether. Intravenous injection of 0.75 cc. of a 24 hour broth culture of <i>B. lactis aerogenes</i>.</p>	<p>Aug. 12, 1918, 12.10 p.m. Ether. Intravenous injection of 0.75 cc. of same broth culture of <i>B. lactis aerogenes</i>. Immediately after this injection ether was pushed to limit, stopping respiration and heart at 12.16. Heart was stopped for 30 seconds. Resumption of respiration and heart beat after cardiac massage and use of artificial respiration.</p>
<p>4.30 p.m. Normal and active.</p>	<p>4.30 p.m. Lying down in a corner of the cage. Somewhat weak and cautious.</p>
<p>Aug. 13, 9.00 a.m. Normal, active animal. Cat remained normal and active until killed 1 month later (Sept. 12). Culture of heart's blood negative.</p>	<p>Aug. 13, 9.00 a.m. Dead in cage. Culture of heart's blood positive for <i>B. lactis aerogenes</i>. Films made in fresh state from subarachnoid space showed free bacilli and a few mononuclear cells.</p>
<p><i>Gross Pathological Diagnosis.</i>—Normal central nervous system and meninges.</p>	<p><i>Gross Pathological Diagnosis.</i>—Intracerebral and intraspinal pressure (edema?). Slight symmetrical meningitis of brain and spinal cord.</p>
<p><i>Microscopic Examination.</i>—Normal central nervous system and meninges.</p>	<p><i>Microscopic Examination.</i>—Free bacilli in cerebral subarachnoid space, with excess of mononuclear cells. Dilated perivascular spaces, containing free bacilli. Bacillema. Spinal cord relatively normal.</p>

The meningitis produced by this temporary cardiac stasis during an artificial bacteremia was in general of a fulminating variety with often very little exudation but with enormous numbers of organisms. The occurrence of these large numbers of organisms was noted not only in the cases found dead over night (in which a post-mortem multiplication of organisms could not be excluded), but also in those injected with formalin immediately after death. Others of the infected animals not only showed typical meningitis clinically, but pathologically the customary polymorphonuclear and mononuclear exudation was present.

The entrance of virulent organisms from the blood stream into the meninges was apparently facilitated by the temporary cessation of the circulation. This facilitation occurred in about 50 per cent of the animals used. It cannot in any way be looked upon as an invariable method of producing meningitis. It has interest in this discussion only as it affords further supporting evidence to the view that infection of the meninges is rendered less difficult if the blood flow through the cranium is impeded. That this facilitation of infection occurs after primary alterations in the cerebral blood flow as well as after secondary changes due to readjustments for lowered pressure of the cerebrospinal fluid is seemingly of some importance. The character of the meningitis in most of the cases succumbing to this experimental procedure indicates that when the facilitation due to cardiac stasis is at all efficient, an overwhelming type of infection occurs.

FACILITATION OF INFECTION BY HYPERTONIC SALTS.

It has been pointed out in the foregoing sections of this communication that infection of the meninges from the blood stream is facilitated by lowering of the pressure of the cerebrospinal fluid by puncture, or by causing congestion or stasis in the cerebral circulation. The lowering of the fluid pressure by either occipito-atlanto or lumbar puncture was most efficacious in producing such a leptomeningitis when the circulating organisms were virulent within the meninges and were present in sufficient numbers. To add further data regarding the contention that the infection of the meninges in these cases was the result of lowered pressure of the spinal

fluid with associated vascular changes and not due to spread of the infection from the local point of puncture, it was thought desirable to devise some method of effecting this lowering of intracranial tension without the withdrawal of fluid by puncture.

Fortunately at this time an investigation of the effect of intravenous injections of hypertonic and hypotonic solutions upon the pressure of the cerebrospinal fluid was in progress in this laboratory. The results of this investigation are presented in detail in another place (Weed and McKibben). It has been found that intravenous injections of concentrated solutions of the common sodium salts (chloride, bicarbonate, sulfate) and of dextrose, bring about a prompt initial rise in the pressure of the cerebrospinal fluid, followed quickly by a marked fall to far below normal. With large doses of the salts, the pressure of the spinal fluid, as recorded in the manometer, has been observed to fall to below zero. Customarily only a marked lowering of pressure was recorded. The maximum effect occurred about 20 minutes after the injection. With analogous injections of Ringer's solution, no persisting alteration in the pressure of the cerebrospinal fluid was caused. Conversely, however, intravenous injections of hypotonic solutions (distilled water) brought about a prompt and enduring rise in the pressure of the fluid.

The profound fall in the pressure of the cerebrospinal fluid occasioned by the intravenous injection of hypertonic solutions suggested their use as a facilitating agent in the production of meningitis by inoculation into the blood stream. The procedure would not necessitate the puncture of the subarachnoid space and would result in a lowering of the fluid pressure analogous to that produced by withdrawal. Experiments leading to this end were immediately undertaken with the controls receiving the same or larger intravenous injections. Additional animals, to establish the virulence of the particular culture, were given the same intravenous injection, but with withdrawal of cerebrospinal fluid 2 minutes later. The concentrated solutions of the salts were usually administered a few minutes before the intravenous injection of organisms, in order that the maximal number of organisms should be circulating in the blood at the time of the lowest pressure of the cerebrospinal fluid. The animals given such initial intravenous injections of concen-

trated solutions of salts, followed by intravenous inoculation, almost without exception, developed a typical leptomeningitis, usually more severe than in the animals from which the fluid was withdrawn by puncture. Other control animals, included in every series and receiving the same injection of the concentrated salts, remained normal. Practically all the experiments were carried out on cats with *Bacillus lactis aerogenes*, and only the results of these observations will be presented.

Two protocols to show the effect of this initial injection of salts in the facilitation of the infection of the meninges are included here.

Series BK.

Cat 1,137.	Cat 1,155.
Aug. 29, 1918, 11.50 a.m. Intravenous injection of 0.75 cc. of a 24 hour brain broth culture of <i>B. lactis aerogenes</i> .	Aug. 29, 1918, 11.38 a.m. Ether. Intravenous injection of 7.0 cc. of 30 per cent solution of sodium chloride, sterile. 5 minutes later, intravenous injection of 0.5 cc. of same brain broth culture of <i>B. lactis aerogenes</i> .
5.00 p.m. Normal, active animal.	5.00 p.m. Cat is down on side, unable to stand. Ataxic and groggy. Retraction of head causes no reaction.
Aug. 30. Normal and active. Animal remained normal and active for 1 month. Killed Sept. 28. Heart's blood culture negative.	Aug. 30, 9.00 a.m. Found dead in cage. Culture of heart's blood positive for <i>B. lactis aerogenes</i> .
<i>Gross Pathological Diagnosis.</i> —Normal central nervous system and meninges.	<i>Gross Pathological Diagnosis.</i> —Cerebral leptomeningitis; hemorrhagic.
<i>Microscopic Examination.</i> —Normal central nervous system and meninges.	<i>Microscopic Examination.</i> —Exudative and hemorrhagic leptomeningitis, almost entirely in brain. Polymorphonuclear and mononuclear cells in equal numbers.

The injection of the hypertonic solution in the animal in the protocol above was rather large in volume for such a concentration. This large quantity of sodium chloride is not required for the proc-

ess of rendering easy the meningeal infection, for in the routine experiments injections of 5 cc. of the 30 per cent solution have proved equally efficacious. In many others this dose has been still further reduced; cats with intravenous injections of as little as 3 cc. of the 30 per cent solution of sodium chloride with the organisms have developed a typical leptomeningitis. Probably the physiological reaction of the animal in response to the injection of salt is the critical factor in the production of the infection, and the absolute quantity of the salt is important only when related to the physiological response in the individual.

The concentrated solutions of the other sodium salts have been shown to occasion an infection of the meninges from the blood stream almost equally as well as the sodium chloride. Most of the animals developed a typical leptomeningitis with the same regularity as did those from which cerebrospinal fluid was withdrawn shortly after the intravenous inoculation. The series with these other salts was always controlled by including an animal to which only the intravenous salt was given. Such animals promptly recovered from the injection and remained normal.

The protocols of two animals from a typical series are included below. The intravenous control is recorded in detail as well as a cat given the smaller intravenous inoculation after the injection of sodium sulfate. The animal, included for the control of the virulence of the culture, receiving the same intravenous dose (0.5 cc.) but with release of spinal fluid, was found dead in the cage the next morning. The other control (No. 1,194) with only the intravenous injection of 6 cc. of the same solution of sodium sulfate remained normal. The protocols are given below (Series BL, page 94).

The same experimental procedure but with the substitution of an intravenous injection of concentrated solutions of dextrose was tried out. The intravenous injection of 20 cc. of a sterile 30 per cent solution was combined with the injection of a suitable dose of organisms in two animals. Both these cats died, the first in 30 hours and the second in 6 days. The simple intravenous control for organisms in the series remained normal; the intravenous with release of spinal fluid died within 24 hours; the intravenous dextrose cat remained normal. At autopsy these animals which had received

Series BL.

Cat 1, 191.	Cat 1, 193.
Sept. 3, 1918, 10.00 a.m. Intravenous injection of 0.75 cc. of a 24 hour brain broth culture of <i>B. lactis aerogenes</i> .	Sept. 3, 1918, 10.30 a.m. Ether. Intravenous injection of 5 cc. of 30 per cent solution of sterile sodium sulfate. 1 minute later intravenous injection of 0.5 cc. of same culture of <i>B. lactis aerogenes</i> .
5.00 p.m. Normal, active animal.	5.00 p.m. A little sick and slow. Maintains normal postures.
Sept. 4, 9.00 a.m. Normal and active.	Sept. 4, 9.00 a.m. Still slow and sick. Dislikes moving about.
	11.15 a.m. Cat in acute mania, having extensor convulsions. Arches back and runs about cage in waltzing movement.
5.00 p.m. Normal and active.	5.00 p.m. Very sick. When undisturbed lies on side in extensor rigidity. When excited goes into acute mania.
Sept. 5, 9.00 a.m. Normal and active. Animal remained normal and active for a month. Killed Oct. 3, 1918. Heart's blood culture negative.	Sept. 5, 9.00 a.m. Dead in cage. Culture of heart's blood positive for <i>B. lactis aerogenes</i> .
<i>Gross Pathological Diagnosis</i> .—Normal central nervous system.	<i>Gross Pathological Diagnosis</i> .—Acute exudative hemorrhagic meningitis, of greatest intensity in brain.
<i>Microscopic Examination</i> .—Postfebrile meningeal reaction (few mononuclear cells).	<i>Microscopic Examination</i> .—Symmetrical exudative leptomeningitis, bacillary. Exudate chiefly polymorphonuclear.

the intravenous organisms plus the dextrose showed a mononuclear reaction in the meninges, but no marked exudative meningitis occurred. These two isolated cases do not afford a basis for deductions.

Summing up the results of the group of experiments in which the pressure of the cerebrospinal fluid has been lowered by intravenous injections of concentrated salts, it becomes evident that the procedure renders more certain the infection of the meninges from the blood stream. Whether this is the result of the primary lowering

of the pressure of the cerebrospinal fluid or is to be referred to the closely associated vascular changes cannot be told from these experiments. The facilitation of the infection of the meninges is obvious and the interpretation of the results coincides with the previously expressed hypothesis of the mechanism of infection of the meninges from the blood stream.

The raising of the pressure of the cerebrospinal fluid by intravenous injection of a hypotonic solution (distilled water) suggested its use in the prevention of infection of the meninges from the blood stream. Several cats were given intravenous injections of distilled water together with *Bacillus lactis aerogenes*; none of these animals developed meningitis but remained normal and active. Other experiments were undertaken to ascertain whether a positive protection of the meninges resulted from this intravenous water; no definite protection of the subarachnoid space from organisms within the blood stream has been recorded. It is felt, however, that further investigation along this line may prove of value.

SUBARACHNOID SERUM AS A FACTOR IN LOCALIZATION OF BLOOD STREAM INFECTION.

The critical experiments of Flexner and Amoss on the production of poliomyelitis by intravenous injection of the virus after the protective agencies of the central nervous system had been altered by subarachnoid injections of sera, etc., naturally suggested the application of this procedure to this study of meningitis from blood stream infection. And the more recent experiments of Austrian in causing a meningococcic meningitis in rabbits by intravenous inoculation after subarachnoid injection of serum, added support to the idea of the sensitization of the meninges by foreign proteins.

The production of a typical aseptic meningitis by subarachnoid injection of autologous, homologous, or heterologous sera is fairly well known. Experiments along this line were carried out during the past year in this laboratory, largely to check up cell counts of the cerebrospinal fluid and to determine the period of the height of the reaction. It was found that the maximal cellular reaction occurred in about 6 hours; after this a sharp decrease in the number of cells occurred for 48 hours with a slow return to normal in 120 hours.

The reactions to the different types of sera varied somewhat, but not markedly.

With the results of other methods of facilitation of an intravenous infection within the meninges markedly in favor of the hypothesis that the vascular changes associated with reductions in pressure of the spinal fluid largely accounted for the lodgment of bacteria there, it seemed not unlikely that in the presence of a sterile meningitis, such invasion of the meninges might occur. For with this aseptic inflammatory process was associated a hyperemia of the vessels in the membranes comparable, in respect to the alteration of the blood flow, to the passive changes induced by withdrawal of cerebrospinal fluid, by compression of the jugulars, and so forth. The results of these experiments on cats with intravenous injection of *Bacillus lactis aerogenes* following subarachnoid injections of sera have been positive in only a small proportion of the cases. The observations were carried out in series, and due to the varying reactions the results will be given in detail before generalizations are made.

In Series BR, the control animal (No. 1,295) received intravenously 0.75 cc. of a 24 hour meat infusion broth culture of *Bacillus lactis aerogenes*; it remained normal and active until killed 1 month later. To establish the virulence of the organisms, another cat (No. 1,281) was given 0.25 cc. of the same culture with release of cerebrospinal fluid in 2 minutes; this animal died of a typical leptomeningitis on the 2nd morning. Two other cats (Nos. 1,293 and 1,294) receiving intravenous hypertonic salt solution with the organisms, died of meningitis, thus confirming beyond question the virulence of the organisms. Three cats in this series were given preliminary subarachnoid injections of serum. The first of these animals (No. 1,299) received a subarachnoid injection of 3 cc. of autologous serum. The sterile inflammatory reaction was controlled by cell counts and the animal was given (5 days after the initial subarachnoid injection of serum) an intravenous injection of 0.25 cc. of the same culture used in the other animals of the series. This cat developed signs of a typical meningitis clinically and was dead on the 4th morning. An exudative cerebrospinal meningitis was verified macroscopically and microscopically. The second cat (No. 1,300) received an initial subarachnoid injection of 2.5 cc. of homologous serum; 5 days later it was given an intravenous injection

of 0.25 cc. of the same culture of *Bacillus lactis aerogenes*. It developed a chronic meningitis clinically and was killed on the 71st day; at autopsy the clinical diagnosis was confirmed. The third cat (No. 1,301), after the subarachnoid injection of 3 cc. of autologous serum 5 days previously, was given the same dose (0.25 cc.) of the same culture of *Bacillus lactis aerogenes*; it showed signs of sickness and weakness for 48 hours and was then killed. Pathologically, a cerebral meningitis was diagnosed in gross; microscopically a "mild acute meningeal reaction" was made out but no organisms were seen.

Series BT was planned in about the same manner. The control cat (No. 1,328) was given 0.75 cc. of a 24 hour meat infusion broth culture of *Bacillus lactis aerogenes*; it remained normal and active until killed 1 month later, when the meninges and nervous system were found to be normal. To determine the virulence of the culture, another cat (No. 1,326) was given 0.25 cc. of the same culture intravenously, and cerebrospinal fluid was withdrawn 2 minutes later; this animal died in 25 hours with typical signs of meningitis, and at autopsy an acute exudative meningitis was found. Two other cats (Nos. 1,320 and 1,321) received subarachnoid injections of 2 cc. of homologous serum 24 hours previously; on the day of experimentation each was inoculated intravenously with 0.25 cc. of the same culture. Both these animals showed signs of a mild meningitis for a couple of days and then became normal and active. This same reaction was observed in the third cat (No. 1,322) which received only the subarachnoid injection of homologous serum without the organism.

● If the two cats given subarachnoid serum and later an intravenous injection of organisms, in Series BV, only one developed meningitis. This animal (No. 1,340) was given a subarachnoid injection of 1.5 cc. of homologous serum 4 hours before the intravenous inoculation of 0.25 cc. of broth culture of *Bacillus lactis aerogenes*. The animal died in 50 hours with signs of meningitis; at autopsy an acute cerebrospinal meningitis was found. The other cat (No. 1,341) received the intravenous injection 6 hours after the subarachnoid; it remained normal and active.

In Series CC, the control cat (No. 1,417) received an intravenous injection of 0.5 cc. of a 24 hour meat infusion broth culture of *Bacillus lactis aerogenes*. It remained normal and active, and when killed

on the 18th day the nervous system was found to be normal. The virulence of the culture was shown by the development of a typical fatal meningitis in three other cats; two of the three (Nos. 1,432 and 1,434) received intravenous hypertonic salt solution and organisms, while the third (No. 1,431) was given the same routine intravenous inoculation (0.25 cc.) with release of spinal fluid. Two animals were given subarachnoid injections of 2 cc. of homologous serum, followed in 5 hours by an intravenous injection of 0.25 cc. of the same culture. One of these, (No. 1,430) was dead the next morning, showing at autopsy an acute leptomeningitis. The other (No. 1,443) developed a subacute meningitis and was killed on the 16th day. Likewise, of the two cats receiving preliminary subarachnoid injections of rabbit serum and 5 hours later the routine intravenous injection (0.25 cc.) of *Bacillus lactis aerogenes*, one (No. 1,424) died in less than 48 hours of an "acute generalized massive cerebrospinal meningitis," while the other (No. 1,442) showed signs of meningeal involvement for 72 hours and then became normal. When killed, the nervous system and membranes of this second cat were found to be within normal limits. The two other control animals in the series (Nos. 1,416 and 1,435) receiving only the subarachnoid injections of homologous and heterologous sera gave signs of a mild meningeal reaction for 24 hours and then became normal.

The two control cats (Nos. 1,464 and 1,465) in Series CF, receiving only the double unit dose (0.5 cc.) of *Bacillus lactis aerogenes*, remained normal, active animals; when killed they both had normal central nervous systems. The three cats used for the standardization of the virulence of the culture, receiving intravenous injections (0.25 cc. and 0.5 cc. of *Bacillus lactis aerogenes*) with release of cerebrospinal fluid, all died of an acute meningitis, the first (No. 1,466) over night, the second (No. 1,467) in 40 hours, and the third (No. 1,482) on the 6th day. Serum was injected into the subarachnoid space of eight other cats as a preliminary measure; each was given in 4 to 5 hours an intravenous dose of 0.5 cc. of the same culture of *Bacillus lactis aerogenes*. Two of these (Nos. 1,476 and 1,478) received subarachnoid rabbit serum; two (Nos. 1,479 and 1,481), anti-meningococcic serum; two (Nos. 1,474 and 1,475), cat serum; and two (Nos. 1,471 and 1,472), normal horse serum. All the animals were a

trifle sick and weak for a day or more, and then became normal in every respect. None developed an acute fatal meningitis, but showed the same reactions as the control animals which were subjected only to the injection of serum into the subarachnoid space.

The results in Series CG were somewhat similar. The intravenous control (No. 1,493) receiving 0.5 cc. of *Bacillus lactis aerogenes* remained normal, while the cat (No. 1,486) receiving 0.25 cc. of the same culture intravenously with release of spinal fluid died on the 3rd morning of an acute fulminating cerebrospinal meningitis. The other experimental animals were all given 2 cc. of either cat, horse, rabbit, guinea pig, or antimeningococcus serum by the occipito-atlantoid route 4 to 5 hours before the intravenous injection of 0.25 cc. of the same culture was made. Only one of the ten animals (No. 1,497) developed an acute fatal meningitis; the initial injection was of rabbit serum and the animal died on the 4th morning. The other animals showed only the customary signs of a mild meningeal irritation and then became normal.

In the last series, CH, two cats (Nos. 1,526 and 1,529) were given only the intravenous injection (1 cc. and 0.75 cc., respectively) of broth culture of *Bacillus lactis aerogenes*. Both remained normal and when killed both showed no abnormality in the central nervous system. The animal serving as control for the virulence of the growth (No. 1,520) was given 0.5 cc. of the same culture, followed in 2 minutes by release of spinal fluid; this cat was found dead in the cage on the 2nd morning. Not one of the ten animals given preliminary subarachnoid injections of various sera, followed in 5 to 6 hours by intravenous inoculations of 0.5 cc. of the same culture, developed a fatal meningitis, but all showed signs of mild irritation for a day or so. This same clinical picture was recorded in the animals which received only the subarachnoid serum.

It is difficult to sum up these findings with any degree of positiveness. In all, 39 cats were given preliminary subarachnoid injections of autologous, homologous, and heterologous sera; from 4 hours to 5 days later routine intravenous injections of suitable doses of *Bacillus lactis aerogenes* were made. In each series the virulence of the organism was established by the death of one or more cats from a meningitis caused by intravenous inoculation fol-

lowed by release of cerebrospinal fluid. Likewise, controls for the intravenous injection (usually receiving double the unit dose) and for the subarachnoid serum were included. Of the 39 cats subjected to this experimental procedure, only 6 developed an acute fatal meningitis. This proportion (6 out of 39) coincides with Austrian's record of the development of a fatal acute meningitis in 3 out of 20 rabbits, subjected to analogous procedures. Two others of his cases yielded meningococcus in films from the meninges, 1 hour after intravenous injection. The clinical reactions of the animals receiving the subarachnoid serum and the intravenous inoculation but not developing an acute fatal meningitis, are wholly similar to those of the animals which received only the subarachnoid serum; both types of reactions are temporary. The subacute and chronic reactions recorded in the foregoing paragraphs have not been included in the summary, because of difficulty of interpretation.

These results, then, seem to indicate that the reaction to the serum in the subarachnoid space facilitates, in a small proportion (6 out of 39) of the cases, infection of the meninges from the blood stream. The process of facilitation is by no means as invariable as is that resulting from release of cerebrospinal fluid or from any of the other procedures detailed in foregoing sections.

MENINGITIS PRODUCED BY INTRAVENOUS INOCULATION ALONE.

The question of the production of a meningitis by more or less massive intravenous doses of organisms was investigated soon after the beginning of these experiments with *Bacillus lactis aerogenes*. The early report of Netter recorded an unsuccessful attempt to produce meningitis in rabbits by indirect inoculation of pneumococci; it was only when the brain was injured by a cautery that he was able to cause localization of the organisms within the meninges. Bull's observations showed that by overwhelming doses of streptococci intravenously a marked encephalomeningitis could be caused in rabbits and dogs in a fairly large percentage of cases. Austrian's experiences with intravenous injections of meningococci demonstrated that no meningitis would result until associated with the subarachnoid injection of serum. The particular virulence of *Bacillus lactis aerogenes* for the meninges suggested that a typical

meningitis might be produced by simply increasing the intravenous dosages.

The earlier experiments, however, seemed to offer evidence against the view that simple intravenous inoculation with this organism might result in meningitis. Before the virulence of these cultures was fairly well standardized, several animals were killed by intravenous injections which were obviously too large. The animals usually died with no indication of meningeal involvement. Subsequently, another series of experiments was carried out and two animals were included to test out the possibility of a meningitis from a simple intravenous inoculation. Two protocols from this series (Nos. 463 and 467) are reproduced on page 66. One of these animals (No. 463) was given 0.5 cc. of a 24 hour broth culture of *Bacillus lactis aerogenes*; it remained normal and when killed a normal central nervous system was found. The other cat (No. 467) received one-half this intravenous injection of the same culture; but 2 minutes later 2 cc. of clear cerebrospinal fluid were removed. This animal died in 24 hours; both in gross and microscopically an acute leptomeningitis, chiefly of the brain, was found. Another cat in this series (No. 471) was given simply an intravenous injection of 2.5 cc. of the same culture (ten times the unit dose). The next day the animal was sick but without meningeal symptoms. On the 2nd day the animal was down on its side; on the 3rd day the cat was moribund; and on the 4th morning it was found dead in the cage. Heart's blood culture at death was positive. Microscopically an acute exudative leptomeningitis (with encephalitis and myelitis) was found. In the same series, another cat (No. 470) was given 5 cc. of the same culture intravenously; this quantity was twenty times the unit intravenous dose. The animal was dead in the cage the next morning; culture of the heart's blood at this time was positive. Microscopically the most striking feature of the nervous system was the extreme bacilleemia. In the subarachnoid space, an excess of mononuclear cells (febrile?) with infrequent polymorphonuclears was found. A few free bacilli without much exudation around them (post mortem?) were also demonstrated. The pathological findings in the central nervous system were not sufficient to account for death. The body tissues showed cloudy swelling, but no abscesses.

These two experiments indicated that a leptomeningitis could be produced simply by larger doses of *Bacillus lactis aerogenes*. But when analyzed carefully, other features of the process seemed to be worthy of consideration. In this series the animal (No. 467) subjected to withdrawal of spinal fluid following an intravenous injection of the unit dose (0.25 cc.) died of a meningitis in 24 hours; contrasted with this is the cat (No. 471) receiving ten unit doses (2.5 cc.) intravenously, but not dying until the 4th morning. Likewise the cat (No. 470) which received 20 times the unit dose (5 cc.) and which died over night had only minimal meningeal involvement, but the bacteremia was extreme. The margin of safety, however, of the procedure did not in this series seem great enough from the bacteriological standpoint, but many other observations have led to the view that with constant passage through the meninges, the margin of safety between the intravenous dosage of organisms necessary to produce meningitis when cerebrospinal fluid is withdrawn and the intravenous dosage necessary to produce meningitis alone becomes very large (at least 100 times). Thus, while meningitis may be easily produced by intravenous dilutions as low as 1:100 (1 cc.) provided cerebrospinal fluid is released at the height of a bacteremia with *Bacillus lactis aerogenes*, it requires many times this dosage to cause meningitis if only the intravenous injection is given. The margin of double the intravenous dose for the control animal has in scores of experiments been justified; the individual reactions of the animal have played no part in causing a meningitis in this dosage. The unit injection (0.25 cc.) has been kept purposely large in order that the meningitis resulting should be inevitable, acute, and fatal; even with such a comparatively large injection the margin of safety has proven wholly adequate.

The virulence of the strain of *Bacillus lactis aerogenes* has varied considerably when maintained solely on cultures. As pointed out in an early section, it was found necessary to pass the organisms periodically through the meninges of cats in order to maintain the subarachnoid virulence. This heightening of the intrameningeal virulence permitted larger intravenous dosage without involvement of the meninges. With such standard cultures it was possible in all cases to give doses of at least 1 cc. of the 24 hour culture without

danger of a persisting bacteremia. Such cultures practically always required an intravenous injection of over 2 cc. to kill an ordinary cat (2.5 kg.) in 48 hours. The margin of safety in all these cases was practically 6 to 8 times the customary unit intravenous dose. Compared with the higher dilutions (1:100) which would with a fair degree of certainty produce meningitis by intravenous injection with release of cerebrospinal fluid, the margin of safety becomes far greater.

But it must not be assumed that the intravenous injection of a lethal dose of *Bacillus lactis aerogenes* inevitably results in the production of a meningitis. The animals for the most part die as a result of the toxemia from the systemic infection, and at autopsy show no meningitis. Usually the subarachnoid space contains a few mononuclear cells, sometimes an isolated polymorphonuclear—the typical “febrile reaction.” When any widespread infection of the meninges does occur following such massive doses, the process is merely part of the generalized infection of all the tissues of the body. The production of meningitis by this means can hardly be considered as the selective localization of the infection in one organ or tissue, because of any particular virulence of the organisms for that particular tissue.

The summary of many observations on the subject of the intravenous injection of many cultures of *Bacillus lactis aerogenes* inclines one to the view that the margin of safety in the experiments detailed in previous sections is sufficient. Animals may be killed by the larger intravenous doses of the organism. Death in these instances is usually to be referred to the septicemia and not to the possible production of meningitis by this means, for the nervous system of most of these animals is normal; it is only in the exceptional case that a larger dose causes a real meningitis. And this localization within the meninges is in these animals to be looked upon merely as a part of a general process of infection of other tissues of the body.

DISCUSSION AND CORRELATION OF FINDINGS.

In the foregoing sections of this chapter the production of meningitis by intravenous injection of certain organisms has been described. Except when the number of organisms introduced was extreme, this intravenous inoculation alone did not result in a meningitis; the bacteria were promptly destroyed in the blood stream and the animal remained normal. If, however, certain experimental procedures were carried out during the bacteremia, leptomeningitis of typical pathology resulted with a remarkable degree of certainty. In the cases in which withdrawal of cerebrospinal fluid was accomplished at the height of the suitable bacteremia, meningitis was almost invariably produced; the same certainty of meningeal infection held also for those animals in which lowering of the pressure of the cerebrospinal fluid was accomplished by intravenous injection of hypertonic solutions. When, however, the alteration in the intracranial pressure relations was made by momentary cessation of the circulation, or by temporary cerebral congestion (compression of jugulars), only about one-half of the animals developed meningitis, even though the bacteremia was otherwise suitable. And when the protective mechanisms of the meninges had been altered by preliminary subarachnoid injections of serum, only about 15 per cent (6 out of 39) of the cats died from an acute meningitis, following similar intravenous inoculations.

The literature affords but few examples of the production of meningitis by intravenous inoculation. Netter in an attempt to explain the occurrence of meningitis in pneumonia, performed some critical experiments in 1887. This investigator could not find that section of the cervical sympathetic cords caused any inflammatory alteration in the meninges of rabbits suffering from an experimental pneumococcus septicemia, although this theory of localization had received great prominence (Goujon). Netter then produced meningitis by direct intradural injection of pneumococci mixed with urine and blood. Also lumbar subarachnoid injection of pneumococci after laminectomy resulted in meningitis, as well as the injection of these organisms through the occipito-atlantoid ligament. Netter also gives experiments in which pneumococci were introduced indirectly into the blood stream by lung injection. Before a meningitis resulted from this procedure, it was found necessary to create a local injury of the brain and meninges; Netter accomplished this by destroying, with the thermocautery, a portion of the left hemisphere in an animal. At autopsy, on the 2nd

morning, in a quoted protocol, there was an area of reddish softening where the cortex was cauterized, and over both hemispheres vascular injection and exudate. This meningeal exudate contained enormous quantities of pneumococci. Netter states:¹

“La presence du pneumocoque dans les vaisseaux de l'encéphale ne suffit pas à amener une meningite, plus qu'elle ne suffisait dans les cavités cardiaques pour amener une endocardite. Il faut, chez les animaux, modifier l'encéphale comme il était nécessaire d'agir sur le revêtement interne du coeur.”

This idea of a modification of the protective mechanism of the nervous system—not an outspoken injury necessarily—finds an analogy in the important experiments of Flexner and Amoss on poliomyelitis. These workers have demonstrated a remarkable delicacy of adjustment of the meningeal mechanisms (including choroid plexus) for preventing the invasion of the nervous system from without. In the paper published in 1914, record was made of experiments in which the power of the virus of poliomyelitis was augmented after intravenous inoculation through an aseptic meningitis induced by the intraspinal injection of horse serum. Their later paper (1917) contains the report of further experimental alteration of the protective agencies of the central nervous system. Intravenous inoculations of the virus gave fatal poliomyelitis when the monkeys had received previous subarachnoid injections of horse serum, monkey serum, and normal salt, Ringer's, or Locke's solution. These preliminary subarachnoid injections were made from 18 to 24 hours before the inoculation. The release of perfectly clear cerebrospinal fluid 18 hours previously did not impair the protective mechanisms of the meninges against invasion from the blood stream. Likewise, the withdrawal and return of autologous spinal fluid did not destroy the meningeal defenses, but in some cases the introduction of homologous fluid in similar replacements resulted in the subsequent occurrence of poliomyelitis. A slight complicating hemorrhage in such punctures promoted the infection of the nervous system, even when the intravenous inoculation was delayed for 18 hours. Subarachnoid injection of horse serum permitted the development of poliomyelitis after intranasal inoculation. Of all the irritant solutions tested, immune serum alone on subarachnoid injection was not followed by infection from the virus introduced into the blood stream. The protective action of this immune serum was capable of overcoming the promoting action of normal serum and of the other irritants used.

Austrian's experiments follow rather closely upon the ideas of Flexner and Amoss in altering the normal defenses of the meninges. Austrian, in addition to nasal inoculations, gave fifteen rabbits intravenous injections of a standard suspension of meningococci; no meningitis was produced and the organisms disappeared from the circulation in 15 to 75 minutes. Twenty other rabbits were

¹ Netter, A., *Arch. gén. méd.*, 1887, clix, 446.

then given preliminary intraspinal injection of 0.5 to 1 cc. of normal rabbit serum, and from 30 to 50 minutes later received an intravenous injection of the same standard suspension of meningococci.

Austrian states:²

"In 20 experiments of this type, eight of the animals died within 8 to 12 hours after injection without clinical evidence of meningeal irritation and at autopsy no meningitis was found. From the meninges of two animals killed one hour after intravenous injection, the meningococcus was identified in smears, and from the spinal canal of one of them the organism was obtained in culture. Three others of this series developed a typical fatal meningitis."

Elser and Huntoon reported, in 1909, the production of meningitis in the rabbit by intravenous injection of an organism resembling *Streptococcus mucosus*, at times in combination with the meningococcus. A purulent cerebrospinal meningitis (with also an acute nephritis) was found post mortem. There was especial localization of the pathological process at the base of the brain, but a simultaneous involvement of cerebral and spinal meninges was noted.

In 1916 Bull published the results of his studies on the disappearance of pneumococci from the blood stream after intravenous injection in the dog. Such injections of from 1 to 4 cc. per kilo of body weight of a bouillon culture of virulent pneumococci produced septicemia and meningitis in dogs. The injected bacteria left the blood stream rapidly and the blood became sterile, and a localization of the infection to the meninges occurred. All the fatal cases showed at autopsy a severe pneumococcic meningitis.

Bull's later observations are of interest in connection with the production of meningitis by intravenous injection. Cultures of streptococci were obtained from poliomyelitic patients and from an equal number of non-poliomyelitic cases. No infection was produced by injection of cultures of these strains in guinea pigs, and of four cats used only one died—without gross pathological involvement of the nervous system, though the surface of the brain showed a pure culture of streptococci. Sixteen dogs were given intravenously one-half to one culture of these organisms per kilo; one animal showed paralysis while four others developed meningitis. Of 78 rabbits inoculated with poliomyelitic strains of streptococci intravenously (usually about two slants), 43 died. Of the 43 dying, 14 developed a typical clinical meningitis; at autopsy focal lesions of the central nervous system were found associated with the meningitis. In similar experiments with non-poliomyelitic strains of streptococci 58 out of 76 rabbits died; of these 15 had meningitis. The lesions of the central nervous system could not be considered in any sense poliomyelitic. The pathological changes were those of a purulent meningitis with focal lesions (abscesses) in the nervous system. Perivascular involvement was common.

² Austrian, C. R., *Bull. Johns Hopkins Hosp.*, 1918, xxix, 185.

Detweiler and Maitland, in the course of experiments on the localization of *Streptococcus viridans* in rabbits, noted after intravenous inoculation with rather large doses, an occasional involvement of the central nervous system. The lesion was usually a hemorrhagic focus, or a perivascular round cell infiltration. In one photomicrograph meningeal infection is associated with the local perivascular lesion.

The findings recorded in the literature and here given in some detail are not numerous but they possess definite relationship to the work of which this paper forms the report. It seems that Netter's original dictum that an injury to the central nervous system must be done before that tissue can be invaded is hardly correct, yet it affords a fair basis for a conception of the process. Bull's production of an encephal meningitis in about 20 per cent of the rabbits receiving massive doses of streptococci demonstrates the final incorrectness of Netter's hypothesis, as does the production of meningitis by large intravenous injections of *Bacillus lactis aerogenes*. But the injection of these very large numbers of organisms is hardly to be compared with the experimental facilitation of the process of infection of the meninges in the presence of small numbers of circulating organisms.

The definite relationship, which seems demonstrated by the experiments recorded in this chapter, between the withdrawal of cerebrospinal fluid during a suitable bacteremia and the subsequent production of meningitis has not, as far as we have been able to determine, been recorded in the literature. The relationship finds its closest analogies in the experiments of Flexner and Amoss. The lodgment of the virus of poliomyelitis in the nervous system, after preliminary alterations of the meningeal protective mechanisms, may be considered, in certain respects, as equivalent to the production of a meningitis by organisms circulating within the blood stream. Similarly Austrian produced a fatal meningitis by intravenous injections of meningococci in 3 out of 20 cases, and in our analogous experiments a similar proportion of cases developed meningitis. In Austrian's experiments a period of only 30 to 50 minutes elapsed between the initial subarachnoid injection of serum and the intravenous inoculation; in Flexner and Amoss' series the interval was usually about 18 hours. In our experiments with

Bacillus lactis aerogenes the second intravenous injection followed the initial subarachnoid at various periods from 4 hours to 5 days. No essential difference in the percentages of positive meningeal infections due to differences in the interval between the two injections could be made out.

But compared with the efficacy of the preliminary subarachnoid injection of serum in causing localization of infection, the withdrawal of cerebrospinal fluid is a far more positive means of causing the intrameningeal lodgment of circulating organisms. The experiments with *Bacillus lactis aerogenes* on the laboratory mammals have demonstrated in positive fashion that the release of cerebrospinal fluid facilitates the infection of the meninges from the blood stream. Simple lumbar puncture, performed 18 hours previously, was found by Flexner and Amoss to have no influence on the production of poliomyelitis on intravenous inoculation; if blood contaminated the fluid the lodgment of the virus in the nervous system was aided. Our observations on the effect of such puncture coincide with these, for the withdrawal of cerebrospinal fluid 30 or more minutes before the intravenous inoculation has not resulted in the invasion of the meninges by *Bacillus lactis aerogenes*.

But it is not alone with *Bacillus lactis aerogenes* that the release of cerebrospinal fluid seems to render more certain the lodgment of organisms from the blood stream within the meninges. Observations detailed in the previous sections demonstrate that the facilitation is of biological significance for other organisms virulent within the meninges. Thus a typical meningitis has been produced in cats by this procedure with *Bacillus pyocyaneus* and *Bacillus paratyphosus* B, as well as with *Bacillus lactis aerogenes*; in rabbits the release of spinal fluid has brought about a meningitis from intravenous inoculation of streptococci and meningococci. Compared with the number of streptococci injected by Bull in his observations, the dilutions of the culture of streptococci used in our experiments are surprisingly great; in these a fatal meningitis was produced by intravenous injection of 1 cc. of dilutions up to 1:5,000, when combined with release of cerebrospinal fluid. The localization of infection here seemed a definite result of the experimental procedure.

It is difficult to reconcile the facilitation of meningeal infection from the blood stream, effected by the withdrawal of cerebrospinal fluid, to the hypothesis of Netter that injury is necessary to cause such localization. The preliminary injection of serum into the subarachnoid space might possibly be construed as constituting an injury, but the mere presence of the sterile inflammation seems more likely as a localizing factor. But neither of these explanations fairly meets the question of how the withdrawal of cerebrospinal fluid, cerebral venous congestion, and the other experimental procedures given in foregoing sections really bring about this critical lodgment in the meninges of organisms from the blood stream.

The explanation developed in part throughout this paper seems to meet the many factors favoring this invasion of the meninges from the blood stream; this explanation concerns the reduction of the pressure of the cerebrospinal fluid or associated vascular changes. The many experiments devised to test out the idea that this meningitis was not the result of a spread of infection from the local tract of the needle have all led to this conclusion. Thus it was shown that the same pathological type of meningitis was produced by withdrawal of fluid by occipito-atlantoid or by lumbar needle; that the mere opening of the meninges by a needle allowed sufficient escape of cerebrospinal fluid into the soft tissues to effect the apparently necessary reduction of the pressure of the fluid. Replacements of the cerebrospinal fluid before and during the intravenous injection have given some evidence that the facilitation of infection is the result of the lowered pressure of the cerebrospinal fluid, but reliance cannot be placed on these particular findings. The development of meningitis in 50 per cent of the animals receiving intravenous inoculations, followed by temporary compression of the jugulars or momentary cessation of the circulation, indicates that the slowing of the blood flow through the cerebrum may be a factor of importance in such invasion of the meninges. On a similar basis the compensatory dilatation of the cerebral veins to replace, as far as possible, the fluid withdrawn by puncture likewise results in the slowing of the blood stream. The occasional production of meningitis by intravenous inoculation in the presence of an aseptic inflammatory reaction in the subarachnoid space might well be explained by the favoring

influence of the active hyperemia of the membranes on the lodgment of circulating organisms. The other vascular processes, following alteration in cerebrospinal fluid pressure, cerebral congestion, etc., are passive, but are, however, apparently far more efficacious in bringing about infection of the meninges than are the active inflammatory changes. In addition, the lowering of the pressure of the cerebrospinal fluid by puncture or intravenous injections of concentrated saline solution, renders much more certain the invasion of the meninges; this reduction of the intracranial pressure must be considered an additional facilitating factor in the process.

When consideration is given to the production of meningitis by intravenous injection of organisms and of the hypertonic solutions, it seems hardly possible that the similar infection of the meninges, due to release of the cerebrospinal fluid, is due to a spread of the infective agents from the local tract of the needle. For the lowering of the pressure of the cerebrospinal fluid by means of the intravenous hypertonic solution is equivalent to the withdrawal of fluid, though without the puncture opening of the meninges. Likewise, the production of the meningitis by the cerebral congestion and stoppage of the circulation in about half the cases adds further evidence against the idea of a spread from the local point.

In this biological facilitation of the infection of the meninges by the various procedures detailed in foregoing sections, two factors stand forth as of greatest importance in the process. The first is the probable prime essential: the virulence of the organisms within the meninges must be marked. The bacteria when they gain access to the subarachnoid space must be capable of multiplication there, even when in small numbers and when the individual resistance of the animal to the particular strain of organisms is great. Experience has taught that the necessary virulence can be most readily maintained at a fair standard in the strain of *Bacillus lactis aerogenes*, but with streptococcus the virulence may easily be raised to the necessary degree for rabbits. The importance of the heightened intrameningeal virulence is not so much to be related to the need for many organisms in the initial infective process in the meninges as to the avoidance of the intravascular virulence, for with so many of the common organisms death is easily produced by the bacteremia. The heightening of the intrameningeal virulence merely

increases the margin of safety between the lethal intravenous dosage and the minimal intravenous requirement.

The second criterion for the successful invasion of the meninges from the blood stream relates to the number of organisms circulating at the time of the secondary experimental procedure. This number may be considered to be a wholly arbitrary one, dependent almost entirely upon the virulence of the particular culture used. With standard cultures, in these experiments, rather high dosage was customarily employed with *Bacillus lactis aerogenes*, resulting in the invariable production of a fatal meningitis. But much greater dilutions could have been easily used, as was shown by the fact that even when puncture was delayed 5 hours after the intravenous inoculation, the animal died of meningitis. While in the observations with this organism dilutions of only 1:100 in 1 cc. doses were the minimum, it was felt that a far smaller number of organisms could have been injected with the same certainty of meningeal infection. Much greater dilutions (1:5,000) were employed in the quoted experiments with streptococci in rabbits; the facilitation of the infection of the meninges by release of cerebrospinal fluid was certain in the series recorded. But it must be emphasized that the number of organisms circulating in the blood stream at the time of the additional facilitating procedures is of great importance in the determination of the infection of the meninges.

It seemed best, then, to view the facilitation of invasion of the meninges by organisms circulating within the blood stream as due to alterations in the blood flow (passive and at times active hyperemia) through the cerebral vessels, with the reduction in the pressure of the cerebrospinal fluid as an important additional facilitating factor.

SUMMARY.

An acute and fatal leptomeningitis has been produced in the common laboratory animals by intravenous injections of suitable numbers of *Bacillus lactis aerogenes*, followed by withdrawal of cerebrospinal fluid during the bacteremia. This procedure almost inevitably results in the development of a meningitis, as does also the reduction of pressure of the cerebrospinal fluid under similar circumstances by intravenous injection of hypertonic solutions of the common sodium salts. When the cerebral circulation is altered by

jugular compression or by stoppage of the heart during such a bacteremia, the meningitis occurs in only about one-half of the cases. Preliminary subarachnoid injection of serum, followed later by suitable intravenous inoculation of this organism, gave meningitis in only 6 out of 39 cases.

The release of cerebrospinal fluid has acted as a facilitating factor in producing meningitis when the circulating organisms in cats were also *Bacillus pyocyaneus* and *Bacillus paratyphosus* B; in rabbits, when the organisms were streptococci and meningococci. The facilitation of infection of the meninges from the blood stream by this withdrawal of cerebrospinal fluid seems therefore established as a biological factor. It is essential that the organism circulating within the blood stream should possess particular virulence within the meninges and be present in sufficient numbers.

EXPLANATION OF PLATES.

PLATE 10.

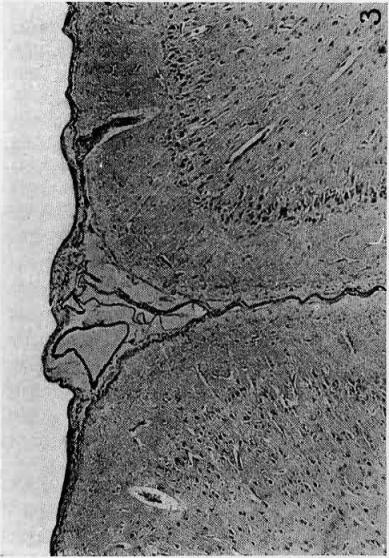
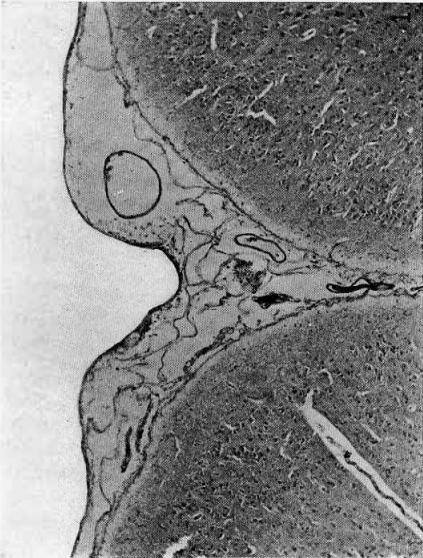
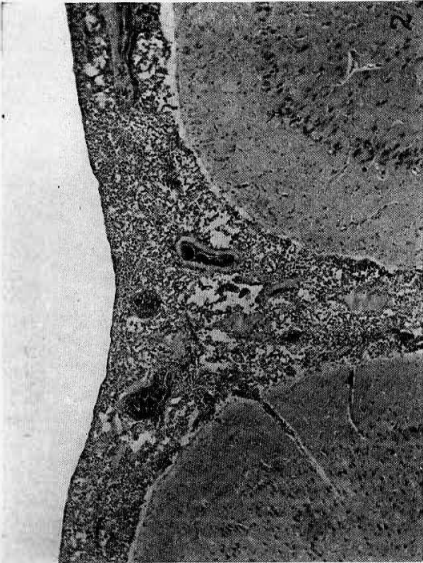
FIGS. 1 and 2. Photomicrographs of similar areas from the cerebral cortex of animals in Series V. Fig. 1 is from Cat 463 (intravenous injection of 0.5 cc broth culture of *B. lactis aerogenes*), killed at the end of a month; normal leptomeninges are shown. Fig. 2 from Cat 467 (intravenous injection of 0.25 cc. of the same culture but with release of cerebrospinal fluid); the acute meningitis of 24 hours duration is shown. Protocols in text. $\times 45$.

FIGS. 3 and 4. Photomicrographs of similar areas from the cerebral cortex of animals in Series AU. Fig. 3 is from the control, Cat 964, receiving only the intravenous injection; the leptomeninges are normal. Fig. 4 shows the typical meningitis resulting from intravenous inoculation, followed by withdrawal of lumbar spinal fluid (Cat 971). Protocols in text. $\times 45$.

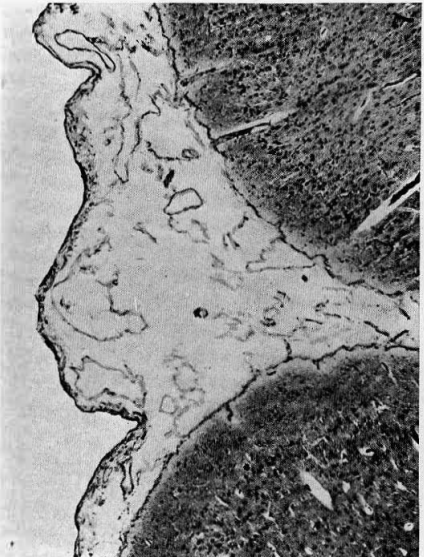
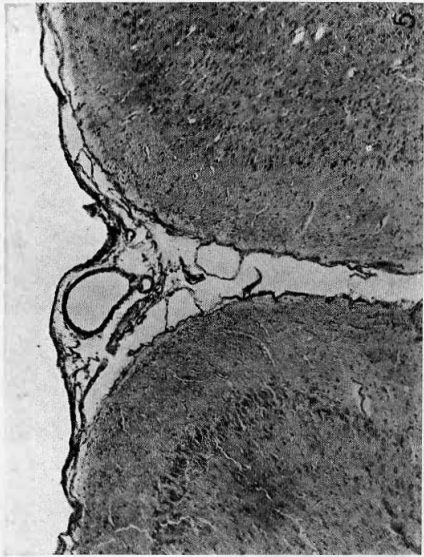
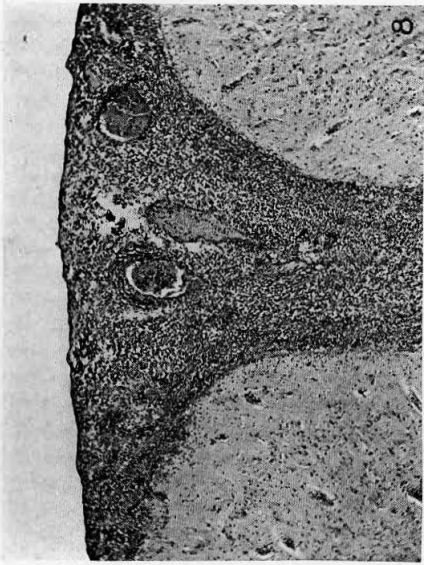
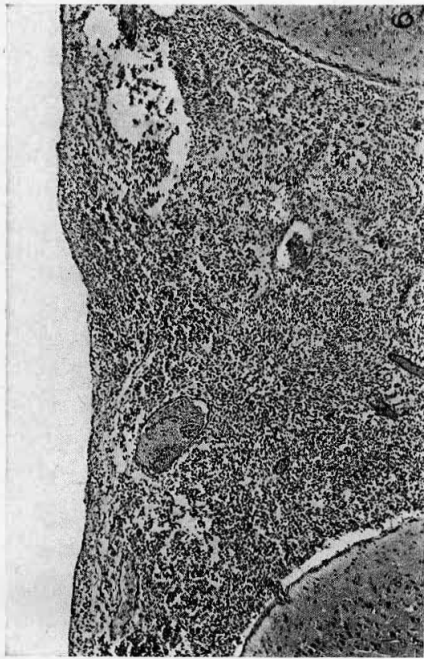
PLATE 11.

FIGS. 5 and 6. Photomicrographs of similar areas from the cerebral cortex of animals in Series Z. Fig. 5 is the control, Cat 500, with normal meninges. Fig. 6 shows the massive meningitis of 2 days duration caused by intravenous inoculation with release and replacement of cerebrospinal fluid (Cat 501). Protocols in text. $\times 45$.

FIGS. 7 and 8. Photomicrographs of similar areas from the cerebral cortex of animals in Series AV. Both animals received intravenous injections of 0.5 cc. of a broth culture of *B. lactis aerogenes*; this inoculation caused no reaction in the control (Cat 984) shown in Fig. 7. The other animal, Cat 987, was further subjected to cerebral congestion; a typical meningitis is shown in Fig. 8. Protocols in text. $\times 45$.



(Weed, Wegelorth, Ayer, and Felton: Meningitis by intravenous inoculation.)



(Weed, Wegforth, Ayer, and Felton: Meningitis by intravenous inoculation.)

V. EXPERIMENTAL ACUTE HEMATOGENOUS MENINGITIS.

A PATHOLOGICAL STUDY.

By JAMES B. AYER, M.D.

PLATES 12 TO 17.

This report represents a pathological analysis of 109 animals, approximately one-half of the cases upon which the experimental deductions of the foregoing chapter are based. The study was made with a number of points in view: first, to make a diagnosis; second, to determine, if possible, at what place organisms gain entrance to the meninges; third, to trace the spread of the meningitis; and lastly, to note any other matters of pathological interest. With regard to the first point, there can be no doubt as to the diagnosis of meningitis in these cases, and what is more, that we are dealing not merely with a reaction to a few organisms which have left the blood stream, but with a virile growth in the meninges. We feel that the second point, concerning the site of earliest meningeal involvement, is in part answered. Points of interest have also been obtained with reference to the spread of the infective process both in the central nervous system and beyond it.

While a number of types of meningitis in man are considered to be secondary to septicemia,—notably that caused by the pneumococcus,—inability to reproduce hematogenous meningitis in animals has naturally caused a certain skepticism as to this view, particularly when the possibility, however remote, of direct infection could be advanced. This has been particularly true of the epidemic form which today has adherents for both modes of infection. While the literature is not devoid of records of experimental meningitis produced from blood stream infection, a thorough search is rewarded with very few such cases, and no writer has been found who claims successful inoculation with certainty.

In 1887 Netter was unable to find meningitis in the rabbit in which he had produced an experimental pneumococcus endocarditis, although he had shown the organism to be virulent in the meninges by direct subarachnoid inoculation; after cauterizing the cortex, however, meningitis was precipitated from the blood stream infection.

In 1909 Elser and Huntoon injected the whole of an agar slant into the ear vein of a rabbit and produced a purulent meningitis in which innumerable cocci were to be seen. In another rabbit receiving both streptococci and meningococci intravenously, the result was the same, the former organism alone being recovered at autopsy. Both these animals died on the 4th day following inoculation; the authors believed that death was due to meningitis, and furthermore that the streptococci possessed a special virulence for the meninges. Nevertheless, further experiments along similar lines proved unsuccessful. While expressing their belief that meningococcus meningitis is hematogenous in origin, they admitted that "all attempts to produce meningitis in animals by inoculation of the meningococcus in parts more or less removed from the central nervous system have failed."¹

Bull in 1916, using massive intravenous injections of virile pneumococci (1 to 3 cc. of a bouillon culture per kilo of body weight) in dogs, noted that purulent meningitis was always present in fatal cases. This worker was investigating immunity factors in pneumococcus infection and showed clearly in his animals a rapid disappearance of bacteria from the circulation during the first hours after inoculation with subsequent increase in the severity of the septicemia until its climax on the 4th or 5th day; it was at this time that fatal meningitis so often supervened.

This same writer in 1917, using a large number of strains of streptococci, was able with some to produce meningitis in rabbits, more rarely in dogs, and in a monkey. Large doses were employed by him, usually an emulsion from two or three slants being given intravenously. The meningitis appeared to have been primary in some and secondary to small cerebral abscesses in others; also foci of infection were common in other organs of the body, thereby making it unlikely that there was a special affinity of these streptococci for the meninges.

Detweiler and Maitland, investigating the localizing properties of *Streptococcus viridans* from the blood stream, produced in six animals infection in the brain, but the associated meningitis seemed always to have been secondary to the perivascular abscesses. They also used large doses.

Flexner and Amoss in 1914 demonstrated that the virus of poliomyelitis when present in the blood could be localized in the meninges if the latter had previously been rendered more permeable by the production of an aseptic meningitis by means of a subarachnoid injection of serum.

¹ Elser, W. J., and Huntoon, F. M., *J. Med. Research*, 1909, xx, 521.

Austrian in 1918, evidently with Flexner and Amoss' technique in mind, was able to precipitate meningitis in the presence of meningococcus septicemia, but only in the presence of a sterile meningitis could this be accomplished. He succeeded in at least three out of twenty rabbits. Austrian's experiments are the only successful ones with which I am acquainted in which meningococcus has been localized in the meninges from the blood stream. His experiments showed clearly a selective action for the meninges, for the dosage was relatively small, being a fraction of a 20 hour slant.

While all these cases represent meningitis derived from the blood stream, there is evident a difference in them, and the following groups seem justified:

1. Meningitis secondary to focal brain abscess (Bull, 1916; Detweiler and Maitland).

2. Meningitis localized by trauma, with secondary general spread (Netter).

3. Meningitis as part of an overwhelming blood infection, in which meningitis is only one, and frequently the least conspicuous, lesion (Bull, 1917).

4. Meningitis generalized from the beginning, which appears to be the cause of death rather than the septicemia or its accompanying lesions (Austrian, Elser and Huntoon).

In this last type alone, a selective action upon the meninges seems justified.

The clinician will recognize that each of the above types has its analogue in man. It is, however, the fourth type which seems to play such an important part, and this type has been the most difficult to reproduce. It is this type which forms the subject of the present and the foregoing chapter.

Success in obtaining the desired result of an hematogenous meningitis, cumulative in effect and leading to a fatal result, was dependent upon three factors; first, a long and persistent search for organisms of sufficient virulence in the subarachnoid space, then increase or maintenance of such original virulence by successive passage through animals by subarachnoid injection, and lastly, finding of methods of lowering the resistance to the passage of the organisms from blood stream to subarachnoid space. In all, 102 strains of 21 organisms were experimented with, and of these only five yielded

the desired result, and of these only three gave unquestioned acute fulminating meningitis.

METHODS AND MATERIAL.

Of the five organisms with which we are concerned, a strain of *Bacillus lactis aerogenes*, obtained from the lung of a patient dying of pneumonia, was earliest found and most reliable. It consequently was the organism used in most of the cases here described. It was found to possess such virulence that a fatal meningitis was produced in cats by subarachnoid injection of 1 cc. of 1:10,000,000,000 dilution (approximately 20 bacilli) and meningitis was produced after inoculation of the blood stream with as little as 0.01 cc. of a 24 hour bouillon culture if followed by release of spinal fluid or other procedure employed for facilitating subarachnoid lodgment. This virulence was retained for a number of months, but later had to be increased by successive passage through cats by subarachnoid injection. Toward the close of our work an hemolytic streptococcus from the throat and a meningococcus (Type IV) were obtained, which on successive passage by subarachnoid inoculation in rabbits were found to give similar results in these animals.

A strain of *Bacillus pyocyaneus* and another of *Bacillus paratyphosus*, while exhibiting the same biological principle of subarachnoid localization, gave less acute forms of meningitis.

As a routine method, animals recently dead (never longer than over night) or killed because moribund or for the purpose of early pathological study, were embalmed as follows: the thorax was opened, the internal mammary vessels were clamped, and a cannula was tied into the aorta; through this a liter of 10 per cent formalin was allowed to run from a height of about 3 meters. Cranium and vertebral column were then removed, cerebral and cerebellar hemispheres and dorsal surface of cord exposed, and the whole was fixed in 10 per cent formalin for several days. Brain and cord with dural envelope were then completely removed in one piece from their bony casing and gross examination was made. Blocks for microscopic study were cut as follows: (1) from cortex about the middle of the sulcus lateralis, right and left; (2) from lateral cortex, near rhinic fissure, right and left; (3) transverse section to show third and lateral ventricles, to-

gether with thalamus, corpus callosum, fornix, etc. These blocks were embedded in paraffin, with pia attached, but without dura; (4) two transverse sections through medulla and cerebellum at different levels; (5) five levels of spinal cord, C2, C6, T4, T12, and L4. These seven sections were embedded in celloidin with dura attached. Thus twelve blocks were prepared from each case as routine in the cat, which was the animal chiefly employed; in rabbits, guinea pigs, and rats a smaller number of blocks sufficed. Sections were cut at 15μ and stained with hematoxylin and eosin and with toluidine blue.

I. MENINGITIS FOLLOWING INTRAVENOUS INOCULATION AND FACILITATING PROCEDURES.

1. *Experiments with Bacillus lactis aerogenes.*

As has been said above, and more thoroughly discussed in the preceding chapter, a bacteremia from this organism has been localized in the subarachnoid space by a number of methods,—following release of spinal fluid, by administration of hypertonic solutions intravenously and by alteration of the cerebral circulation by congestion and cardiac stasis, and also by the instigation of an aseptic meningitis. The pathological picture resulting is similar in all, with small differences.

(a) *Gross Findings.*—In normal cats after fixation as above described, the thin dura mater appears shiny and transparent, and through it the markings of the brain and cord are visible to the naked eye or with a low power hand lens. The convolutions, cerebral and cerebellar, are well rounded; vessels in the sulci are visible, whether empty or containing blood, and minute vessels running onto the convolutions are distinct; over the base the hypophysis and nerve roots up to their brain connections are perfectly clean cut. Throughout the cord, too, the dura is of pearly whiteness, and under it the posterior columns are seen to be elevated, and the individual root components, both ventral and dorsal, are perfectly distinct.

In a cat (No. 521) killed $4\frac{1}{2}$ hours after intravenous injection of 0.25 cc. of a 24 hour broth culture and followed by release of spinal fluid, the cord appears normal, the cisterna magna contains clear fluid, but over both cerebral hemispheres there is marked obscuring

of all the surface markings, especially anteriorly. On the left the frontal lobe is largely covered by a yellowish exudate which is surrounded by a zone of hyperemia and petechial hemorrhage; on the right smaller similar areas occupy the anterior two-thirds of the hemisphere.

Cats dying or killed between 6 and 8 hours after injection show, as a rule, a little flattening of the convolutions and a little obscuring of the cerebral markings, due, as microscopic examination of the fluid or tissues shows, to exudate.

When death occurs over night (8 to 20 hours) the picture is so constant that those in the laboratory who were examining routinely could recognize this group with considerable certainty. The brain is especially noteworthy, the color gray, brown, or red-brown, frequently with one or more areas of obvious hemorrhage situated over the anterior poles. The cortical markings are obscure, in places entirely hidden by the dirty gray overlying exudate; greatly engorged vessels are distinctly seen in the narrowed sulci, together with exudate and free blood, and the convolutions are flattened. The cerebellar hemispheres are usually flatter than normal but less obscure than the cerebral, and the region of the cisterna magna may be quite clear, as are also the markings of the base of the brain. The spinal dura is usually tense and dead white in color, and the markings of posterior columns and roots are indistinct. One gets the impression of an acute hemorrhagic and exudative process involving especially the cortex anteriorly, with base of brain, cerebellum, and cord relatively normal. For example, Case 566 shows:

"Brain tense. Convolutions flattened—entire left cerebral hemisphere as seen from above obscured by diffuse antemortem hemorrhage. Markings on right just visible through much clouded membranes. Cerebellar markings obscured. Entire base of brain normal in appearance. Cord throughout, normal.

"*Diagnosis.*—Hemorrhagic purulent leptomeningitis of cerebral cortex; slight of cerebellum. Normal cord."

Thus it is seen that within 20 hours the meningeal process is already well developed. Animals with such a process show marked signs of meningitis and many die at this time. If death does not occur until the 2nd day the process is found to be generalized as well as

more intense, with little or no indication of the area of earliest localization. The brain and cord of an animal dying after 48 hours appear a dirty yellow or brown throughout, usually with dark red blotches or points of hemorrhage over both brain and cord (Plate 12, Fig. 1). The dura throughout is lusterless. Evidence of great increase of pressure of brain and cord is given by the marked flattening of the cerebral convolutions and by transverse corrugations of the cord corresponding to the configuration of the vertebral canal. On closer examination all the finer markings of brain and cord are obscure or lost.

In animals dying after 2 days we are apt to find accumulations of pus in certain loci in addition to the generalized exudate, especially in the cisterns at the base of the brain, the cisterna magna, about the cervical cord, and in the lumbar sac. After 48 hours, too, the increased cerebral and cord pressure tends to become less.

Thus from the macroscopic examination it is seen that exudation is usually first apparent over the cerebral cortex, particularly its anterior poles, becomes rapidly generalized throughout brain and cord, and later is found in greatest amount in the various cisterns and culs-de-sac of the subarachnoid space. Of 25 cases dying or killed under 24 hours, 23, or 92 per cent, showed exudate chiefly in the cerebral subarachnoid space, usually over cortex, both on gross and microscopic examination.

The gross picture of meningitis from *Bacillus lactis aerogenes* as presented is similar for all methods of localization. When localization has been effected by means of intravenous injection of hypertonic solutions of sodium chloride, hemorrhage is more conspicuous. In such a cat (No. 1,124) dying within 18 hours of injection, the following note was made:

"Brain and cord of yellow-brown color with confluent hemorrhage in left frontal and right occipital pole. Convolutions considerably flattened, vessels in sulci well filled and in places there is free blood. Meninges opaque, rendering markings indistinct. Base marking a little clouded, less so than over convolutions. Cord: Dura tense throughout. Posterior column markings just visible, root markings for most part distinct, though in places, notably in lumbar cord, they appear matted together."

That considerable blood gains entrance to the cerebral subarachnoid space early in this infection is certain. That this blood is not a result of puncture is assured from its occurrence where no puncture has been performed. That it is not due to the technique of embalming is indicated by the fact that normal brains similarly treated do not show hemorrhage and also that there is microscopic evidence that it is antemortem.

Microscopic Examination.—We shall now consider in greater detail typical cases of meningitis produced by *Bacillus lactis aerogenes* at different time intervals.

Experiment 522.—Intravenous injection of 0.25 cc. of *B. lactis aerogenes*, followed in 2 minutes by occipito-atlantoid puncture. Killed in 6 hours.

11 a.m. Ether. Intravenous injection of 0.25 cc. of a 24 hour broth culture of *B. lactis aerogenes*. 11.02 a.m. Occipito-atlantoid puncture, 1.5 cc. of clear cerebrospinal fluid giving a few red cells and a negative culture. 5.00 p.m. Ether and a second puncture, which showed 8,000 cells, practically all polymorphonuclear leucocytes, and a positive culture for *B. lactis aerogenes*. Animal killed with ether. Heart's blood positive for same organism. Embalmed with formalin in manner described.

Gross Examination.—In the brain the sulci seem somewhat obscured and indefinite. No real swelling of the tissues. Very slight areas of extravasation throughout dura. Small petechial hemorrhages over cerebellum; all the cerebellar sulci seem slightly obscure. Spinal cord: Most of the markings on both dorsal and ventral surfaces can be made out but all are a little less clean cut than usual. Some swelling of cord in cervical and lumbar regions.

Impression.—Slight leptomeningitis; possibly more severe over cerebral cortex than in spinal region.

Microscopic Examination.—Findings are almost limited to the subarachnoid space. Throughout the brain, but especially in the sulci of the cortex there is a moderate amount of exudate, composed chiefly of polymorphonuclear leucocytes with a considerable number of large and small mononuclear cells, together with serum loosely packed in the trabeculated subarachnoid space, and to a less degree in the pial meshwork. The arachnoid membrane presents a smooth exterior, but its inner surface is spongy in character and its structural cells, as also those of the arachnoid trabeculae, are large and apparently actively growing cells. The velum interpositum and velum medullare—projections of the pia-arachnoid—are similarly infiltrated; perivascular spaces of the brain are not dilated or abnormal except that near the subarachnoid space a few polymorphonuclears are seen. Brain tissue is not remarkable except for a scattering of a few polymorphonuclears throughout the molecular zone. Ventricles are of normal size, free of exudate, and the ependyma, both parietal and choroidal, is intact

and of normal appearance. No abnormality of the choroid plexus is made out, either of lateral, third, or fourth ventricles.

The cord presents exudate of a similar type in the subarachnoid space throughout, most marked in the ventral fissure, but in no region or level to be compared in amount with that in the cortex. Two bacilli, the only ones seen, are here found in the exudate.

Blood vessels for the most part are empty and no organisms are seen in the vessels. (Plate 12, Figs. 2, 3, and 4.)

In this case after only 6 hours of infection, we have microscopic evidence of an exudative meningitis already established, clearly most marked in the cerebrum and probably most over the cortex. Yet the process is generalized throughout the subarachnoid space. It is noteworthy that there is at the same time no evidence of ventricular infection. A similar picture with cerebral distribution of exudate as in the above case was presented in a cat in which localization of the meningitis was effected by means of intravenous sodium chloride. The protocol in brief is as follows:

Experiment 1,358.—Intravenous injection of concentrated sodium chloride, followed by intravenous injection of 0.25 cc. of *B. lactis aerogenes*. Clinically, meningitis resulted. Killed in 6½ hours.

10.50 a.m. Ether. Intravenous injection of 3 cc. of 30 per cent solution of sodium chloride. 10.52 a.m. Injection of 0.25 cc. of a 24 hour broth culture of *B. lactis aerogenes* into another vein. 5.00 p.m. Convulsion and signs of acute meningitis. 5.08 p.m. Killed. Heart's blood positive for *B. lactis aerogenes*.

Gross Examination.—Brain and convolutions moderately flattened. Meninges less transparent than normal but all markings can be made out. Cerebellum same. Slightly more opaque at base. Vessels everywhere slightly congested and apparently some free blood in sulci. Cord: Dura more tense than normal. Roots and posterior columns a little obscure throughout.

Impression.—Generalized, slight degree of acute meningitis.

Microscopic Examination.—A well marked exudative and hemorrhagic meningitis of the brain is seen, with numerous bacilli free in the subarachnoid space. The large mononuclear cell predominates over the polymorphonuclear. In the cerebellar sulci there is free blood. In the cord only a small amount of exudate appears, without organisms. Ventricles contain a few bacilli, and some are seen lying under the parietal ependyma. The choroid plexus is, however, normal. Substance of brain and cord not abnormal. (Plate 14, Figs. 13, 14, and 15.)

We see from this experiment that when concentrated sodium chloride is employed the pathological picture is similar to that result-

ing from release of spinal fluid in the presence of bacteremia. There is somewhat more free blood in the meninges in cases where hypertonic saline solution is used, but in both, meningitis is well established in 6 hours and in both the greatest amount of exudate and the greatest number of organisms are seen over the cerebral cortex.

A considerable number of cats died during the night following inoculation. As the time interval in these is uncertain and as post-mortem changes are likely to add complicating factors, we shall consider next the pathology after 24 hours. It should be noted that there is no essential pathological difference between animals dying over night and after 24 hours.

Experiment 467.—Intravenous injection of 0.25 cc. of *B. lactis aerogenes* followed in 2 minutes by occipito-atlantoid puncture. Death in 24½ hours.

11.00 a.m. Intravenous injection of 0.25 cc. of a 24 hour broth culture of *B. lactis aerogenes*. 11.02 a.m. Occipito-atlantoid puncture with release of 2 cc. of clear fluid from which a sterile culture was obtained. 5.00 p.m. Cat almost normal. 7.45 p.m. It is weak and sick. 9.00 a.m. the following morning. Symptoms of meningitis, definite. 11.30 a.m. Death. Culture from heart's blood positive.

Gross Examination.—Brain hyperemic throughout, large and small vessels being filled. Membranes thickened, white and opaque, especially on dorsal surface of cerebellum and medulla. Small confluent hemorrhages near median line behind crucial sulcus and at occipital poles. Confluent hemorrhages over medulla oblongata, but dura here is too opaque to determine their boundaries. Spinal cord nearly normal, but with slight cloudiness of membranes over cervical and lumbar enlargements.

Impression.—Acute leptomeningitis, chiefly of brain.

Microscopic Examination.—Most conspicuous is the meningeal exudate, which is seen throughout the brain and cord; in quantity, massive over the cortex, much in the cerebellar sulci, moderate at base and cervical cord, slight in lumbar. The exudate is found largely confined to the subarachnoid space, the arachnoid membrane forming an efficient barrier to its extension into the subdural spaces. The inner layer of arachnoid has, however, proved less efficient in this case than in others, and the pial meshwork is seen to be greatly infiltrated. The exudate consists mostly of polymorphonuclear leucocytes and innumerable bacilli; there is a goodly number of large mononuclear cells, many of which contain granular material and a few of them inclusions of bacilli, and also a few mast cells. There is also much fibrin and a little free blood. Phagocytosis of bacilli by polymorphonuclear leucocytes is not seen.

Perivascular spaces of brain and cord are well filled with polymorphonuclear and mononuclear cells, but without bacilli for the most part. The most intense

perivascular infiltration is seen in the cortex of the brain and in the central gray of the cord, but the deeper vessels of the former and the superficial vessels of the latter are also as a rule affected to a less extent.

There is very slight disturbance of the substance of the brain. An invasion of the molecular zone of the cortex, obviously by direct extension, is seen in one place. Also there is a scattering of polymorphonuclears throughout the medulla and in the molecular zone, but without bacilli, except the one focus mentioned, and with no conspicuous changes in nerve cells. In the cord, however, and particularly the cervical cord, the invasion of the gray matter by polymorphonuclears is in places intense, and bacilli are here present in small numbers free in the tissue. Likewise in the cervical gray matter three small hemorrhages are seen. The nerve cells in regions of such infection present chromatolytic changes.

Turning to the ventricles we are struck by their comparatively normal appearance. They are of normal size; the lateral ventricles are entirely free of exudate, the third contains serum and a few ependymal cells only, the fourth and central canal of the cord contain a very few polymorphonuclear cells and a rare bacillus. The ependyma appears everywhere intact; there is, however, a slight cellular infiltration of the loose connective tissue between the choroidal ependyma and the somewhat dilated choroidal blood vessels, rendering the choroid plexus abnormally prominent. Thorough search, however, fails to show organisms within this infiltrated stroma, although the choroidal vessels contain a number of bacilli. This picture is true of the choroid of all the ventricles. In sharp contrast to this picture of ventricles and choroid is that presented by the velum medullare and velum interpositum, both of which are densely infiltrated with exudate similar to that in the subarachnoid space.

The blood vessels are for the most part engorged and contain few to numerous bacilli. No affection of the vessel walls is seen other than slight intimal cell proliferation.

In the short space of 24 hours we see in the above case a fully developed leptomeningitis, generalized, but rather more marked over the cortex than elsewhere. This exudate, fibrinopurulent in type, contains such large numbers of organisms that no doubt can be entertained as to their rapid growth in the subarachnoid space. Already we see invasion of the brain directly from the meninges and of the central gray matter of the cervical cord. At this period, however, the ventricles are either normal or show only the earliest signs of involvement.

In Figs. 9 to 12, Plate 13, the amount and distribution of the exudate is readily seen. For comparison are shown homologous areas from the control cats (Plate 13, Figs. 5 to 8), which received twice

the intravenous dose, but from which no spinal fluid was withdrawn and in which no meningitis occurred.

Animals living 2 to 3 days show a greater amount of exudate, usually massive, throughout brain and cord. Microscopic examination fails to show any qualitative difference, however, from the foregoing cases. But when we look within the brain and cord it is found that the ventricles usually contain pus, and it is the rule rather than the exception to find more or less extensive involvement of the brain and cord tissue. An example of this is the following, jugular compression being the facilitating agent employed in the production of meningitis.

Experiment 991.—Intravenous injection of *B. lactis aerogenes*, 0.5 cc., followed by jugular compression. Death from meningitis in about 40 hours.

July 25, 1918, 11.00 a.m. Ether. Intravenous injection of 0.5 cc. of a 24 hour broth culture of *B. lactis aerogenes*. 2 minutes afterwards compression of both jugulars with fingers for 30 seconds. 5.00 p.m. Normal, active animal. July 26, 9.00 a.m. Normal, active animal. 5.00 p.m. Cat is very sick. July 27, 9.00 a.m. Dead in cage. Heart's blood culture positive for *B. lactis aerogenes*.

Gross Autopsy.—Brain and cord of brownish tint. Cerebral vessels much engorged, but without obvious hemorrhage, convolutions moderately flattened, membranes of cerebrum and cerebellum of increased density. Cord markedly swollen, especially in cervical region where transverse striations are present. Throughout cord posterior column and root markings are almost invisible.

Diagnosis.—Cerebrospinal meningitis, acute, generalized.

Microscopic Examination.—There is a universal extensive and intensive fibrino-purulent exudate in the subarachnoid space, similar to the foregoing cases. Exudate extends in places through the outer arachnoid wall, and overlying dura also shows patches of exudate; this is particularly true where nerve roots have pierced the membrane carrying exudate from the subarachnoid space with them, in which event the roots are at times infiltrated; at these root zones also the dura is most frequently infiltrated, at times densely, with exudate; here also pus is seen lying epidurally. Posterior root ganglia are not infiltrated.

Throughout the brain and cord there is generally perivascular exudate, chiefly polymorphonuclears, but with few or no bacilli. Both superficial and deep vessels are thus affected, large and small. All ventricles are well filled with exudate, though not dilated, and their ependymal walls broken. In the loose tissue immediately subjacent to the ependyma of the lateral ventricles are seen foci of polymorphonuclear leucocytes and bacilli, especially in the neighborhood of vessels showing perivascular exudate. The choroid plexus itself in all the ventricles is swollen because of dilated vessels and infiltration of its stroma with polymorphonuclears and bacilli; the choroidal ependyma is distended and ruptured.

Besides the encroachment on the brain tissue about the lateral ventricles, the cerebellum presents a number of areas of involvement of its molecular layer, obviously by direct extension from the meninges. These areas of true infection with pus and organisms must not be confused with the scattered polymorphonuclear leucocytes which are present in this case, as in all, throughout the molecular zone of the cortex and molecular layer of cerebellum, a condition probably defensive in nature (see below). In the cord also the cervical segments in particular show an extensive infiltration of the central gray and neighboring posterior columns with polymorphonuclears and bacilli, a process arising, it would seem, from the central canal, which is dilated, filled with pus, and the lining wall of which is ruptured.

Blood vessels are for the most part engorged, but, except for evidence of proliferation of the intimal cells, no abnormality of their structure is made out. There are, however, several small hemorrhages in the gray matter of the cord.

It is remarkable that the nerve cells are little affected. Even those of the anterior horns at the level of extensive myelitic infection, large motor nerve cells on the floor of the pus-filled fourth ventricle and pyramidal cells of the cortex, between which are scattered polymorphonuclears, appear for the most part normal.

In this animal, dying on the 2nd day, we see that not only is the exudate widespread and excessive but that the ventricles and canal are filled with exudate, and invasion of the brain and cord is being effected about lateral ventricles, in the cerebellar cortex, and about the canal.

When meningitis has followed the release of spinal fluid or administration of concentrated saline solutions (Plate 15, Figs. 17 and 18), the pathological picture at this time cannot be told from that just described.

The meningitis produced by this organism is almost always fatal within 3 days, and the picture presented in the last description holds for the great majority of animals dying subsequent to 48 hours. Variations as to amount of exudate exist, but there is less variation as to its generalized distribution and less still in its character. Involvement of the central nervous tissue and of the internal fluid spaces, frequent in its occurrence, is, however, variable in its position and extent, and will be considered under a special heading.

2. Experiments with *Streptococcus*.

After prolonged search, an hemolytic streptococcus from throat culture was found which exhibited sufficient subarachnoid virulence to make it likely that it could be localized from the blood stream in a similar manner to *Bacillus lactis aerogenes*. After increasing still further its virulence by passage through rabbits to a point where 0.0005 cc. administered by subarachnoid injection would cause a fatal meningitis, a number of successful experiments were conducted in which an intravenous inoculation was localized in the subarachnoid space following release of spinal fluid in the same manner as in the case of *Bacillus lactis aerogenes*. Needless to say, the control animals remained normal. The tissues of three such rabbits have been examined.

Experiment 1,412.—Rabbit. Intravenous injection of 0.001 cc. of *Streptococcus hemolyticus* followed by release of spinal fluid. Death from meningitis in about 18 hours.

Nov. 15, 1918, 12.20 p.m. Ether. Intravenous injection of 1 cc. of 1:1,000 blood-Locke's solution, 18 hour culture of *Streptococcus hemolyticus* after passage through 17 rabbits by subarachnoid injection. 2 minutes afterward occipito-atlantoid puncture; slightly blood-tinged fluid. 5.00 p.m. Normal, active animal. Nov. 16, 9.00 a.m. Dead in cage. Heart's blood culture positive for *Streptococcus hemolyticus*.

Gross Autopsy.—Brain of dirty yellow color, vessels everywhere engorged. Creamy opacity obvious and definite over whole cerebral cortex. Cerebellar lobes muddy. Base of brain: all pontine and bulbar markings wholly obscured. Cord: Swollen throughout whole extent. Longitudinal fissure everywhere obliterated, no posterior root markings can be made out anywhere.

Diagnosis.—Acute exudative leptomeningitis of both brain and cord.

Microscopic Examination.—Throughout the brain and cord, but somewhat more in the brain, the subarachnoid space is well filled with an exudate (Plate 17, Fig. 27) made up chiefly of polymorphonuclear leucocytes and in places large numbers of streptococci. The pial projections into the brain are similarly infiltrated, but little exudate is seen in the ventricles. The third ventricle and canal contain a few cells and poorly staining cocci, but the lateral ventricle appears free. Choroid plexus of the third ventricle normal; that of the laterals is not included in the section. No invasion of the cortex and central ganglia is seen, but in the medulla there are several small hemorrhages in the white matter and a diffuse leucocytic invasion in this territory. No invasion of the cord is seen.

Exudate can be traced along nerve roots as far as the ganglia but the latter are not invaded and similar invasion of dura and epidural exudate at these root zones is to be seen.

Blood vessels are seen to contain a few cocci, but are otherwise normal in appearance.

Three rabbits similarly injected died under 20 hours. These show a picture (Plate 17, Fig. 28) comparable with that just described, which in turn is seen to be quite similar to that presented by the cats dying early with meningitis produced by *Bacillus lactis aerogenes*. Another rabbit under identical experimental conditions died in two weeks; focal lesions were found in the cerebral cortex (Plate 14, Fig. 16), in addition to the meningitis.

3. *Experiments with Meningococcus.*

Realizing the great importance which would be attached to experimental meningitis if produced by the meningococcus, numerous strains were tried unsuccessfully. Fortunately, toward the end of our studies one was obtained from Camp Jackson which, when passed through rabbits, exhibited the necessary virulence and we were enabled to reproduce the desired experiment with this organism.

Experiment 1,523.—Rabbit. Intravenous injection of 1.5 cc. of meningococcus, followed in 2 minutes by release of spinal fluid. Death from meningitis in 42 hours.

Dec. 1, 1918. Intravenous injection of 1 cc. of a 24 hour broth culture of meningococcus (Type IV), virulence raised by consecutive subarachnoid inoculations of other rabbits. 2 minutes later, occipito-atlantoid puncture; fluid clear. Dec. 2. Well defined signs of meningitis. Dec. 3. Death, 42 hours after original injection. Occipito-atlantoid puncture 30 minutes after death yielded 0.5 cc. turbid fluid containing a few red and many white corpuscles and bacteria.

Gross Autopsy.—Brain swollen; marked vascular stasis. Cortex, base of brain, and cisterns filled with brownish yellow opaque substance. Cord: Everywhere swollen and similarly discolored. Posterior root markings and longitudinal fissures are obliterated. Free blood apparently in subarachnoid space of lower thoracic region.

Diagnosis.—Acute generalized leptomeningitis.

Microscopic Examination.—Throughout the brain and cord there is a very considerable exudate in the meninges, of about equal distribution, chiefly polymorphonuclear in type but with many mononuclears. A moderate amount of blood is intimately mixed with the exudate and in one place a considerable amount

of free blood is seen lying between pia and cord. Numerous rather poorly staining cocci are seen, a few of them evidently diplococci, apparently free in the subarachnoid space. Perivascular spaces are well filled with exudate. Substance of brain and cord appears normal except for the usual polymorphonuclear infiltration of the molecular layer without organisms. Ventricles and canal all contain exudate; the stroma of choroid plexus contains polymorphonuclears but no organisms.

Diagnosis.—Acute exudative and hemorrhagic meningitis (Plate 16, Fig. 25 and Plate 17, Fig. 29).

4. *Experiments with Bacillus pyocyaneus and Bacillus paratyphosus B.*

As has been shown in the foregoing chapter, the biological principle of meningeal localization from bacteremia holds for *Bacillus pyocyaneus* and *Bacillus paratyphosus B.* It has also been intimated that the meningitis resulting is not fulminating and does not correspond to the type which we have just studied. The following protocol is from such an experiment.

Experiment 627.—Intravenous injection of 0.5 cc. of *B. pyocyaneus* followed in 2 minutes by cistern puncture. Death in 5 days.

12.20 p.m. Intravenous injection of 0.5 cc. of a 24 hour broth culture of *B. pyocyaneus*. 12.22 p.m. Occipito-atlantoid puncture, fluid obtained giving a negative culture. Cat remained normal and active for 2 days, then became weak in the hind legs and died on the 5th day after inoculation. Heart's blood culture positive.

Gross Autopsy.—There is a marked difference between the brain and spinal cord; the vessels of the former appear well filled and the convolutions flattened. The cortex presents a uniform gray color but markings are readily visible. Cord is white, but root and longitudinal markings a little obscure.

Diagnosis.—Meningitis, especially cerebral, of moderate degree.

Microscopic Examination.—There is a moderate infiltration of the subarachnoid space with large mononuclear cells, especially in brain, together with similar exudate in the cerebral perivascular spaces. The meningeal reaction is patchy in character, tending to settle in corners formed by convolitional irregularities and about nerve roots. In one such pocket a number of bacilli are seen. The vessels and parenchyma appear normal.

Bacillus paratyphosus.—After a sufficient subarachnoid virulence had been acquired by passage through cats (until 1 cc. of a 1:10,000 dilution of a 24 hour broth culture by subarachnoid inoculation would produce fatal meningitis in 24 hours), it was found that a mild

degree of meningitis could be produced with this organism in the presence of a bacteremia following release of spinal fluid. The pathological picture is similar to that described for *Bacillus pyocyaneus*.

With both these organisms, we obtain unquestioned mild meningitis, but with bacilli very few in number. Control animals given intravenous injections alone did not develop meningitis. It is obvious that death has not occurred in these cases primarily from meningitis; it is more likely that death is due to the accompanying septicemia.

While we must admit that the same biological principle of meningeal localization is here fulfilled, this type of meningitis from the pathological point of view differs fundamentally from the foregoing fulminating type.

II. MENINGITIS BY INTRAVENOUS INOCULATION ALONE.

With the doses used to control the above experiments, animals practically always remain well or are slightly sick for only a few hours, and do not show meningeal symptoms. The nervous systems of such animals show no abnormality or only slight changes which may be designated as a "febrile reaction." If, however, very large doses are given, meningitis may result.

1. *Normal Control Animals*.—As has been frequently said, the experiments were invariably controlled: an animal of approximately the same size and age received an intravenous injection of the same or double the dose given to the animals in which attempts at meningeal localization were planned.

Usually the control animal would be wholly unaffected, or slightly sick for a few hours, and then become normal. When killed, the brain and spinal cord of such animals have been found to be normal, both on gross and microscopic examination.

2. *Febrile Meningeal Reaction*.—Rarely an animal receiving what was usually a sublethal intravenous inoculation would die within 24 hours. In such cases the brain and cord appear normal or very nearly so, but microscopically a mild meningeal reaction is seen. The cerebral arachnoid membrane and its trabeculae are noticeably involved, the cells of which are seen evidently proliferating *in situ* and budding off into the subarachnoid space, where a number of

large mononuclear cells, some of which are phagocytic, are seen. Fibrin, free blood corpuscles, and polymorphonuclears are also present, but organisms are either absent or extremely rare. Death in such cases must be attributed to the septicemia; it cannot be thought to be due to this mild acute meningeal reaction (Plate 16, Figs. 22 and 23).

The animal receiving the customary sublethal intravenous inoculation practically always remained normal. When killed after a month, the central nervous system, if not entirely normal, may show a slight opacity of the cerebral meninges. Microscopic examination in such cases shows irregularly distributed small accumulations of lymphocytes and plasma cells with an occasional phagocytic and mast cell and rarely a giant cell. No organisms are present. These accumulations are most frequently seen in pocketed areas between medulla and cerebellum, and at corners formed between nerve roots and dura; a favorite site is the angle formed between brain or cord and nerve root.

It is believed that this mild meningeal reaction is analogous in its chronic form to the acute febrile reaction just described.

3. *Acute Exudative Meningitis*.—On a number of occasions large intravenous injections of bacteria have been given with resultant fulminating purulent meningitis. Sometimes death would occur with great rapidity, at other times not for several days. The following case closely resembles those described by Bull in which intravenous inoculation of pneumococci was followed by rapid destruction of organisms in the blood stream, to be subsequently followed by rapid increase in the septicemia ending with a fatal meningitis.

Experiment 1,132.—Intravenous injection of 0.75 cc. of *B. lactis aerogenes*. Death on 4th day.

Aug. 26. Intravenous injection of 0.75 cc. of a 24 hour brain broth culture of *B. lactis aerogenes*. The cat remained normal until the 2nd day, when it became a little slow in its movements and apparently sick. It was found dead in the cage on the 4th day. Culture from heart's blood positive for *B. lactis aerogenes*.

Gross Examination.—Brain and cord show a generalized purulent meningitis, most marked over cerebral convolutions. There is also an abscess of the abdominal wall.

Microscopic Examination.—Throughout the subarachnoid space of brain and cord is a very considerable exudate, consisting chiefly of polymorphonuclear leucocytes; also many large mononuclear cells, many of which contain granules and bacteria. Bacilli are present in large numbers. Perivascular spaces too are moderately filled with exudate. The ventricles and central canal contain pus, the latter being distended with it, and about the canal in thoracic and lumbar cord there is well marked abscess formation.

The pathological picture presented by this case is similar to that seen in the cases described in the first part of this paper. But as this meningitis occurs from intravenous inoculation alone, usually only when relatively large doses are used, and as animals receiving this large injection frequently die showing no meningitis or only a febrile reaction, it cannot be classed with cases in which meningitis has been produced by venous inoculation with as little as 0.01 cc. and followed by one or the other of the localizing agents used in this laboratory. The experiment recorded in the protocol above is unusual in that the intravenous dosage was not excessive; an individual susceptibility to such injections was occasionally encountered.

III. PATHOLOGY OF VISCERAL ORGANS.

In view of the fact that control animals did not develop meningitis and lived, study of the pathological lesions is of secondary importance. Nevertheless, organs from a considerable number of animals were examined, both from animals in which meningitis was localized and from animals which had received intravenous injections alone and in which there was no meningitis. Similar well marked lesions were regularly present in both types. Even in animals receiving the small dose of 0.01 cc. of a 24 hour broth culture of *Bacillus lactis aerogenes*, epithelial changes and hemorrhages are found in most of the organs in addition to the well marked meningitis. With the standard dose of 0.25 cc. used in most of the experiments, somewhat more marked reactions are seen in animals dying of the meningitis.

Kidney.—The kidney presents as the mildest lesion a cloudy swelling of its tubular epithelium; more often there is degeneration of its cells and filling of the lumina with epithelial exudate and blood elements. A general acute congestion and frequent small hemorrhages in cortex and medulla are seen in the more severe cases.

Liver.—The liver appears congested, the cells swollen and poorly staining.

Spleen and Pancreas.—The spleen and pancreas show acute congestion.

Adrenal.—The adrenal shows at times degeneration of the cells of both cortex and medulla.

Lung.—The lung may appear normal, more often congested, but frequently shows free blood and exudate in the alveoli, and in the longer cases a true bronchopneumonia.

In fact, the organs show throughout acute changes of such a degree that death of the animal probably depends in part upon them. These lesions are obviously not in themselves fatal. In some animals receiving overwhelming doses, *e.g.* 5 cc., death has occurred, evidently due to a combination of meningitis and severe visceral lesions.

We have thus far considered the pathology of meningitis as produced by a variety of procedures from blood stream infection in five different organisms, and have shown that with three of them an acute fulminating meningitis results. We have also seen (Part II) that intravenous injections of the same number of organisms, but not followed by procedures which facilitate subarachnoid lodgment, produce either no meningitis or only a mild meningeal irritation which has been termed a "febrile reaction."

IV. ORIGIN AND SPREAD OF MENINGITIS.

1. *Seat of Origin of Earliest Lesions.*—The earliest lesions are invariably seen in the cerebral subarachnoid space. As early as 2 hours after inoculation an occasional organism may be observed, together with serum and usually some free blood, over the vertex. By 6 hours, as we have already seen, a distinct exudate is here present, and although not absent elsewhere, the impression is gained that this is the area of earliest infection. Of 25 cats dying or killed under 24 hours, 23 (92 per cent) show the greatest amount of exudate in the cerebral meninges, almost invariably over the cortex.

2. *Subarachnoid Spread of Infection.*—Spread of infection to all parts of the subarachnoid space occurs with great rapidity. In cats dying over night, all show some involvement throughout, although

there is at this time a disproportion in favor of the brain. Up to 24 hours it is usually possible to distinguish this difference between brain and spinal cord, but by 48 hours the exudate becomes universally massive.

3. *Infection of Ventricles and Central Canal.*—In a few early cases, the ventricles are normal, although meningitis is well established (Plate 12, Fig. 4). As a rule, however, infection of the ventricles and canal takes place very early. Never has it been seen prior to, or more intense than the process in the meninges. We have already seen (Cat 1,358) that in 6 hours free organisms were found in the ventricle. By 18 hours it is common to find not only organisms, but some exudate; of 37 cats dying or killed under 24 hours, 65 per cent presented ventricular infection, while for those over 24 hours, involvement was demonstrated in 90 per cent. Ventricular infection may then be considered the rule rather than the exception—in fact almost constant in a well developed case of meningitis. Not all the ventricles are affected to the same degree or at the same time. The impression is that the ventricles show involvement in the following order: fourth and canal, third, and laterals. There may be a small amount of exudate in the ventricle with normal appearance of wall and choroid plexus; more often, however, the parietal ependyma appears elevated and broken, and bacilli are seen lying free in the loose subependymal meshwork. When ventricular infection exists, the velum is uniformly infiltrated with exudate, but the choroid plexus may early be entirely normal in appearance. Soon, however, the choroidal vessels appear engorged, the stroma is infiltrated with polymorphonuclear leucocytes, and the choroidal ependyma distended; but even when infection of the ventricle is well established no break in the choroid is seen, nor indeed are organisms usually seen outside of its vessels (Plate 16, Fig. 24). Later the choroid, as also the walls of the ventricle, frequently become involved in an intense purulent process.

4. *Invasion of the Parenchyma of the Central Nervous System.*—If ventricular infection occurs early, so also does invasion of the central nervous system itself. In the series of 37 cases examined of less than 24 hours infection, nearly one-half (48 per cent) show thus early involvement of brain and cord. Sixty-two cases of over 24 hours

duration show invasion in 71 per cent. So that, as in the case of ventricular infection, invasion of the brain and cord must be looked upon as the rule and not the exception. By far the greater number of cases present invasion about the lateral ventricle. The earliest stage of this process may be seen in the bacilli underlying the parietal ependyma; next we see exudate still in a subependymal position, and later extension into the surrounding white matter. At this stage the ventricle becomes transformed into a pus pocket. An early and frequent invasion of the cord is also seen, cervical levels being earliest attacked. The process, like that just described, commences either from the canal or immediately subjacent; at first, organisms are seen spreading into the central gray matter with polymorphonuclear cells coming in very soon (Plate 15, Fig. 21). Thence the spread is usually posteriorly into the white matter and laterally into the gray. As seen in Case 467, this process may be extensive even in 24 hours.

The above types of invasion are by far the most frequent and likewise the most extensive. While their origin may be questioned, little doubt exists in the mind of the writer that the ventricles and canal, already described as infected even earlier and more frequently than the nervous tissue, form the starting points for invasion of brain and cord.

A number of cases show invasion of the nervous system in punctate arrangement, suggesting invasion from a vessel, and at times organisms have been seen apparently radiating into the nervous tissue from an infected perivascular space. The vessels in the neighborhood thought to be the source are usually small and sometimes show so many cells and bacteria in their lumina as to suggest a thrombotic process, but vessels jammed with bacilli were seen only in cats dying and found dead in the cage after several hours and can hardly be considered as antemortem thrombi. These infections attributable to perivascular infection are seen especially in the gray, but also in the white matter of the brain and cord; at first diffuse in character they tend to resolve into foci (cf. Plate 14, Fig. 16). This type of invasion occurs in all stages of infection—it was seen in five of the 37 cases of less than 24 hours duration and in about the same proportion in longer infections. It was seen in 11

cases in the brain and 9 in the cord, distinctly less frequently than the previous type, but when it is remembered how small an area of the total nervous system comes under the microscope it is reasonable to think that this type of lesion must also occur with great constancy in brain and cord.

A third type of infection was seen, infrequent and late in appearance; namely, direct invasion from the pia. This marginal infection is of interest in spite of its relative infrequency (seen twice in brain and twice in cord) because from its location, the large surface of brain and cord would seem to be exposed earliest and to the most virulent infection. Histological examination suggests three protective factors. First, it is noted that in early infection and even later when there is considerable exudate, organisms appear to be retained in the subarachnoid space. The pial meshwork becomes swollen and filled with polymorphonuclear leucocytes and mononuclear cells, but organisms are fewer in number in its meshes than in the subarachnoid space. This suggests that the inner wall of the subarachnoid space serves an important function in limiting infection inward, just as we have already seen that its outer wall excludes infection from the subdural space. A second defense is possibly offered by the dense layer of connective tissue which forms the inner portion of the pia mater. Probably a third type of defense is seen in the cortex itself,—very early, indeed, almost constantly, the molecular zone of the cerebral cortex and the molecular layer of the cerebellum are seen to contain polymorphonuclear cells scattered free in the tissue. With increase in meningeal infection this infiltration becomes denser near the surface, and is seen working back into the layers of pyramidal cells. In extreme cases this leucocytic infiltration is very striking; while invariably generalized, the density of such accumulations varies and in places almost suggests abscess formation. That this process is protective in function rather than a form of diffuse infection rests upon the following observations: In spite of the extent and density reached, no organisms are ever seen. Moreover, when infection does finally break down the barriers and invades the cortex its progress is evidently slow, as if met by defense. Also when attacked from the rear as in abscess, this area, rich in leucocytes, is seen to stand out longest against invasion (cf. Essick).

Perhaps the best argument, however, lies in the simple fact that abscess formation in this situation, early or late, is rare.

Besides invasion of the nervous substance by organisms and exudate, hemorrhages occasionally appear; these occur more often in the gray matter of the cord than elsewhere. However, they are not common and are usually small.

As our study was directed chiefly toward the question of distribution and invasion of exudate, no special stains were employed for the study of nerve cells or fibers, but with toluidine blue a good idea of the former was obtained. It is surprising how little the nerve cells are affected. With meningitis properly confined to the subarachnoid space, as in the early cases, no abnormality of the nerve cells is seen. Even when invasion of the gray matter occurs, many of the cells in the neighborhood preserve their staining qualities perfectly; only after some time is extensive degeneration noted.

5. *Extension Outward from the Subarachnoid Space.*—Very early in many instances are seen collections of polymorphonuclear cells in the dura, but not so early or in such position as to suggest a primary focus in this membrane. Moreover, organisms are usually absent in such areas. Histological examination shows two methods of production of acute pachymeningitis by direct extension. Not infrequently, as incident to the infection of the subarachnoid space, the outer layer of the arachnoid is seen apparently attached to the dura, and the latter membrane at this point swollen and infiltrated with leucocytes and sometimes with bacilli. A more common site of extension, however, is where the dura is "pierced" by the nerve roots. A root carries with it its arachnoid envelope and subarachnoid exudate; this exudate is seen with great frequency to accompany both anterior and posterior roots outward for a short distance, sometimes infiltrating the nerve bundles (Plate 16, Fig. 26). In the case of the posterior root the exudate at times goes up to and partially surrounds the ganglion, but was never seen to invade it (Plate 16, Fig. 25). It is at these root zones that the dura becomes with great frequency invaded by exudate, and small amounts are frequently seen on the external surface of the dura at this point.

6. *Phagocytosis.*—We have made many examinations of film preparations of the cerebrospinal fluids of animals with meningeal infec-

tions. Attention was particularly devoted to the study of the phagocytosis of the infecting organisms by the cells of the fluid, as an index of one of the protective agencies of the body. An interesting phenomenon has seemed indicated by these observations on the fluids of animals infected with *Bacillus lactis aerogenes*. In those animals in which the meningeal infection was of a fulminating character (death in 18 to 24 hours), phagocytosis of the bacilli by the cells of the cerebrospinal fluid was almost never observed (Plate 15, Fig. 19). When, however, the meningeal infection was of a less severe nature with death ensuing only after 4 to 5 days, film preparations revealed phagocytosis of the bacilli by the cells of the cerebrospinal fluid (Plate 15, Fig. 20). In the first group, the infection may be assumed to be of such virulence that phagocytosis was limited or nullified.

SUMMARY AND DISCUSSION.

From the massive exudative picture described no doubt can be entertained but that with *Bacillus lactis aerogenes*, streptococcus, and meningococcus, a true hematogenous meningitis has been produced, when certain facilitating measures have been employed. That these organisms have a natural pathogenicity within the meninges is assured by the large number of bacteria found at autopsy, increasing with the duration of the meningitis. That the meningitis itself is fatal is evidenced by the fact that animals receiving similar or greater intravenous inoculations alone do not succumb. Objection may naturally be raised that with the release of spinal fluid the needle has carried the organisms into the subarachnoid space. Such contamination is rendered unlikely by finding the earliest lesions distant from the point of puncture, and frequently the site of the puncture unaffected until the process is elsewhere well established. Moreover, this objection cannot be entertained in meningitis which has developed subsequent to intravenous injections of concentrated saline solutions, to congestion, to stoppage of the heart, or in the presence of aseptic meningitis.

Meningitis similarly produced with *Bacillus paratyphosus* and *Bacillus pyocyaneus* is not of the same character. There is pathological evidence that it is a true meningitis; but that the meningitis

of itself is fatal in these cases is less certain, and that there is virile growth of these organisms in the subarachnoid space is unlikely. The most plausible explanation for the picture presented is that insufficient organisms gain entrance to the meninges, for the virulence is known to have been sufficient to produce a massive purulent meningitis when enough organisms were inoculated directly into the subarachnoid space.

A large number of cases has been examined with the view to differentiating the types of meningitis following the various provocative measures employed; except that the cats in which concentrated sodium chloride was employed show a more hemorrhagic type of exudate than the others, no conspicuous difference is apparent.

While meningitis may be occasioned from the blood stream by means of relatively small inoculations (as little as 0.01 cc. of a 24 hour broth culture) when facilitating measures are adopted, a similar pathological picture may be obtained from a bacteremia alone, but only when a much larger number of organisms is involved. With the larger inoculations, moreover, death frequently occurs without any, or only the slightest, infection of the meninges. We must therefore regard the two types of fulminating meningitis as biologically different, although pathologically similar. The distinction between the two types should be looked for in the difference in the reaction of the body organs, on which an exhaustive study has not been made.

In dealing with the site of localization of the organisms within the central nervous system, we should not be too dogmatic when we realize how rapidly infection spreads in the previously unaltered subarachnoid space. However, evidence appears overwhelmingly to favor the cortical meninges as the site of earliest infection, with rapid spread from there to other portions of the subarachnoid space and ventricles. So rapid is this spread that one would be almost justified in assuming a synchronous appearance of the organisms in other places, were it not for the impression gained that spread is by continuity and for the fact that in cases of direct subarachnoid inoculation the spread of organisms and exudate is strikingly similar.

How do the organisms gain entrance into the meninges from the blood stream? An apparent common effect of all facilitating pro-

cedures is a change in the pressure relations between venous circulation and cerebrospinal fluid, and the most reliable of these procedures (injection of concentrated saline solution and release of cerebrospinal fluid) are clearly shown to lower fluid pressure (Weed and McKibben). The capillaries and small vessels should be most noticeably affected by such change and we should expect an increased permeability of these to account for the presence of organisms in the subarachnoid space. Unfortunately for this theory, capillaries have not been anatomically demonstrated in the arachnoid membrane. Capillaries in the brain seemed most likely anatomically as the place of invasion, but the rare presence of organisms in their perivascular spaces connecting with subarachnoid space renders this hypothesis impossible of demonstration. The smaller vessels of the pia, constantly dilated and engorged in these cases, are perhaps culpable, and the delicate arachnoid villi should be considered as possibly the seat of interchange. It may be noted in passing that in man a type of meningitis is recognized which involves essentially the territory occupied by the arachnoid villi (Klippel).

There is no microscopic evidence for primary invasion through the choroid plexus in these cases. The stroma of the plexus early becomes swollen with serum and polymorphonuclear leucocytes, but organisms are not seen outside the choroidal vessels until later, and the choroidal ependyma seems to remain intact in spite of considerable stretching. So early are organisms seen lying under the parietal ependyma, however, that the question arises whether they could perhaps have emigrated through subependymal capillaries, thus reaching the ventricles.

The early invasion of the ventricles should be emphasized. Bacteria at first appear alone, but exudate quickly forms. Infection of the ventricles and canal is to be expected early and is almost always present in a well established meningitis. This fact is of interest because of the impression held by many that in man, infection of the ventricles is an unusual complication, whereas in the experimental animal it is found almost constantly to accompany the meningitic process. The clinical significance of this universal ventricular involvement is obvious in connection with complications such as hydrocephalus and abscess.

As might be expected, infection of ventricles and canal leads to infection of brain and cord. Very early in many cases there is unquestioned evidence of direct invasion of the surrounding nervous tissue from these fluid spaces, and later it is common. Of a total of 109 cases examined with this point in mind, obvious infection of brain or cord, or both, as evidenced by exudate and organisms, was found in approximately one-half. Invasion of brain or cord from foci in white or gray matter occurs less frequently, but it is seen often; the method of invasion is less certain than that from the ventricles, but it appears to be by extension from infected perivascular spaces. Direct extension from the meninges, theoretically easy, is infrequently seen. Invasion of the nervous tissue of brain and cord from all sources is the rule rather than the exception in these fulminating cases. Of the 109 cases analyzed, 60 per cent show some form of invasion.

CONCLUSIONS.

1. Meningitis has been produced by experimental bacteremia, followed by withdrawal of spinal fluid and other procedures.
2. With the strains of *Bacillus lactis aerogenes*, meningococcus, and streptococcus employed, an acute hemorrhagic purulent meningitis has been produced, in which the bacteria appear to be rapidly increasing in number.

With *Bacillus pyocyaneus* and *Bacillus paratyphosus* B, a mild acute meningitis results, with very few organisms to be seen.

3. Control animals, receiving intravenous inoculations alone, show no pathological lesion of the central nervous system, or, rarely, merely a mild "febrile reaction," recognizable only microscopically. With intravenous inoculations of large numbers of organisms, a massive meningitis may be produced, but such inoculation frequently kills the animal without causing a meningitis.

4. In experimental hematogenous meningitis there is evidence that organisms enter the subarachnoid space, probably over the cerebral cortex, very soon after inoculation.

5. In fulminating cases the organism rapidly proliferates and spreads quickly throughout the subarachnoid space and into ventricles and canal; infection of these fluid spaces being almost constantly present.

6. Invasion of the brain and spinal cord, most frequently by direct extension from ventricles and canal, occurs in the majority of cases, and is frequently seen within 24 hours.

The writer wishes to acknowledge cooperation in the examination of tissues by Captain George B. Hassin, Medical Corps, U. S. Army, and Contract Surgeon Charles C. Macklin, U. S. Army.

EXPLANATION OF PLATES.

PLATE 12.

FIG. 1. Monkey. *B. lactis aerogenes*, 0.75 cc. (1:100) intravenously, followed by withdrawal of spinal fluid. Death about 42 hours later. Shows massive hemorrhagic exudative meningitis, especially over parietal and frontal cortex. Spinal cord and cisterna magna (region of puncture) show much less exudate. $\times \frac{2}{3}$.

FIG. 2. Cat 522. *B. lactis aerogenes*, 0.25 cc. of broth culture by intravenous inoculation followed by occipito-atlantoid puncture. Killed in 6 hours. Shows early but well marked meningitis over parietal cortex. $\times 70$.

FIG. 3. Same case. Ventral fissure of cervical cord. Very few exudative cells in meninges in contrast to well marked exudate in cortex. $\times 70$.

FIG. 4. Same case. Third ventricle and choroid plexus appear normal. $\times 70$.

PLATE 13.

FIG. 5. Cat 463. Received 0.5 cc. of broth culture of *B. lactis aerogenes* intravenously. Killed as control to Cat 467. Parietal cortex and meninges normal. $\times 36$.

FIG. 6. Same case. Cervical cord. $\times 40$.

FIG. 7. Same case. Lumbar cord. $\times 40$.

FIG. 8. Same case. Third ventricle. Choroid plexus normal. $\times 600$.

FIG. 9. Cat 467. Received 0.25 cc. of broth culture of *B. lactis aerogenes* intravenously, followed by occipito-atlantoid puncture. Death in 24 hours. Parietal cortex, massive exudative meningitis. $\times 36$.

FIG. 10. Same case. Cervical cord. Shows moderate amount of exudate. $\times 40$.

FIG. 11. Same case. Lumbar cord. Shows moderate amount of exudate, but less than in cortex. $\times 40$.

FIG. 12. Same case. Third ventricle. Stroma of choroid plexus is slightly distended but contains no bacteria. Bacilli seen only in blood vessels. $\times 600$.

PLATE 14.

FIG. 13. Cat 1,358. Received 3 cc. of sodium chloride, 30 per cent, intravenously, followed by intravenous inoculation of 0.25 cc. of broth culture of *B. lactis aerogenes*. Killed in 6½ hours. Cortical meninges show moderate amount of exudate. $\times 40$.

FIG. 14. Same case. High power of meninges shown in Fig. 13. Exudate consists of polymorphonuclear leucocytes and mononuclear cells, with many bacilli. $\times 720$.

FIG. 15. Same case. Cervical cord (C2) shows very slight degree of meningitis, much less than over cortex. $\times 40$.

FIG. 16. Rabbit 1,413. Received intravenous inoculation of 1 cc. of 1:2,000 streptococci 18 hour blood-Locke's solution culture, followed by occipito-atlantoid puncture. Death in 2 weeks. Abscess in cortex, presumably from infected perivascular space. $\times 40$.

PLATE 15.

FIG. 17. Cat 1,336. Received intravenous inoculation of 0.25 cc. of *B. lactis aerogenes*, 24 hour broth culture, followed by 5 cc. of sodium chloride, 30 per cent, intravenously. Death in about 42 hours. Cortex shows massive exudative meningitis. $\times 40$.

FIG. 18. Same case. Cervical cord (C4). Shows moderate amount of exudate in ventral fissure. Shows arachnoid loosely adherent to dura. $\times 40$.

FIG. 19. Cat 393. Received intravenous inoculation of 0.2 cc. of a broth culture of *B. lactis aerogenes*, followed by release of spinal fluid. Death in 21 hours. Film preparation from spinal fluid to show polymorphonuclear type of exudate and large number of encapsulated bacilli. No evidence of phagocytosis. $\times 1,170$.

FIG. 20. Similar case to preceding, with death on 5th day. Film from spinal fluid on 4th day shows phagocytosis of bacilli. $\times 1,170$.

FIG. 21. Cat 1,196. Received intravenous inoculation of 0.5 cc. of 24 hour broth culture of *B. lactis aerogenes*, followed by occipito-atlantoid puncture. Death in 40 hours. Central canal of cord filled with exudate and beginning invasion of spinal cord. $\times 100$.

PLATE 16.

FIG. 22. Cat 470. Received intravenous inoculation of 5 cc. of 24 hour broth culture of *B. lactis aerogenes*. Death from septicemia in 18 hours. Cortical meninges show a slight increase in mononuclear cells only. $\times 100$.

FIG. 23. Cat 1,173. Received intravenous inoculation of 1 cc. of 24 hour broth culture of *B. lactis aerogenes*. Killed for control in 1 month. Shows only exudate seen in meninges; an accumulation of polymorphonuclear and mononuclear cells, but without organisms in cervical cord. $\times 220$.

FIG. 24. Cat 1,294. Received intravenous inoculation of sodium chloride, 5 cc., 30 per cent solution, followed by *B. lactis aerogenes*, 0.25 cc., 1:1,000,000,000 meat infusion broth. Death in 4 days. Shows massive exudate in lateral ventricle; also cellular infiltration and dilatation of choroid plexus with extreme thinning of choroidal ependyma. $\times 100$.

FIG. 25. Rabbit 1,523. Received intravenous inoculation of 1.5 cc. of meningococcus culture (Type IV) followed by release of spinal fluid. Death in about 42 hours. Shows massive exudate in meninges of cord, extending out with and infiltrating nerve root; surrounding but not invading root ganglion. $\times 40$.

FIG. 26. Cat 1,281. Received intravenous inoculation of *B. lactis aerogenes*, 0.25 cc., 1:1,000,000,000 24 hour meat infusion broth culture. Death in about 40 hours. Shows exudate leaving subarachnoid space with and infiltrating nerve root. Slight infiltration of dura at this point. $\times 50$.

PLATE 17.

FIG. 27. Rabbit 1,412. Received intravenous inoculation of streptococcus, 1 cc., 1:1,000 18 hour blood-Locke's solution culture, followed by occipito-atlanto-puncture. Death in about 18 hours. Shows small area of normal cortex (below) with massive exudate of polymorphonuclear type with many cocci. Note comparative freedom of organisms in pia directly overlying cortex. $\times 1,560$.

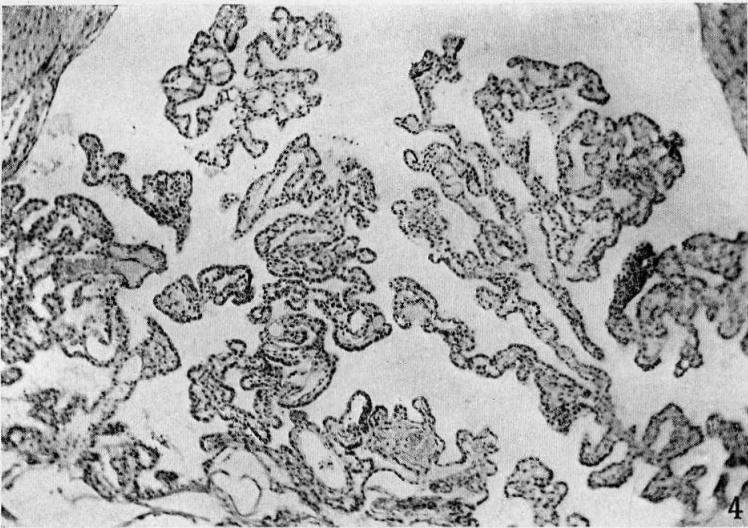
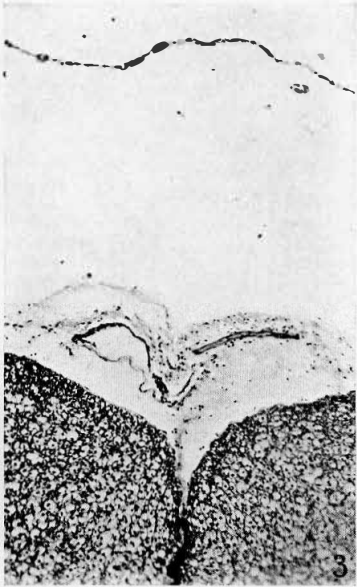
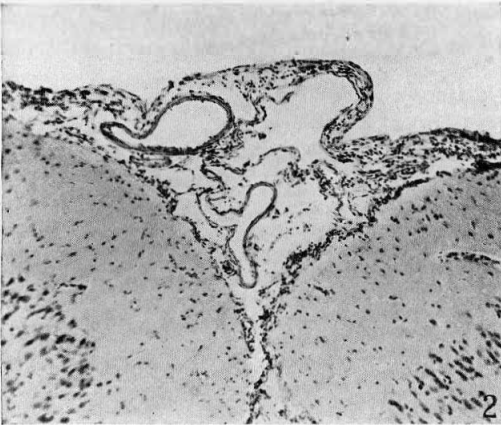
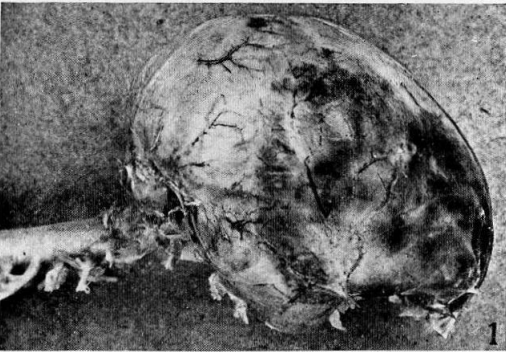
FIG. 28. Rabbit 261. Inoculation with release of cerebrospinal fluid. Same as in preceding case. Death in 15 hours. Shows exudate in meninges of medulla oblongata. Note freedom from infection in part of fourth ventricle, the lateral recess of which appears in the upper left hand corner. $\times 112$.

FIG. 29. Rabbit 1,523. Received intravenous inoculation of meningococcus, 1.5 cc., followed by release of cerebrospinal fluid. Death in 42 hours. Shows massive exudate in meninges of cervical cord. $\times 107$.

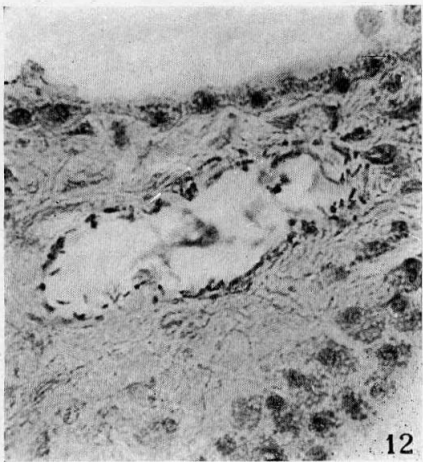
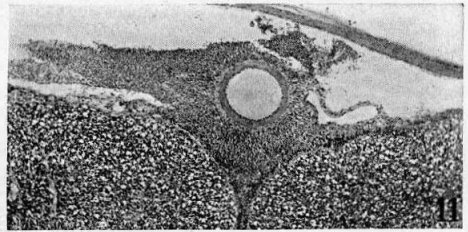
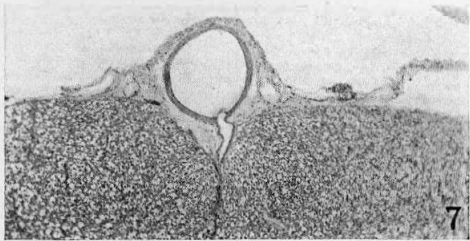
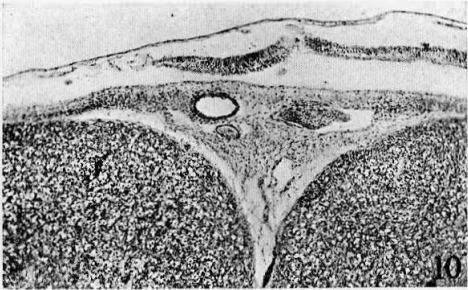
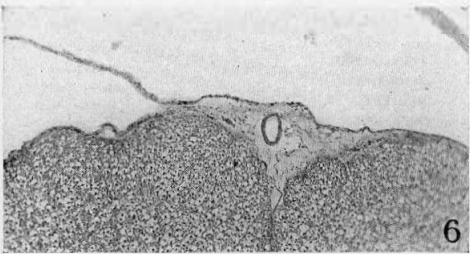
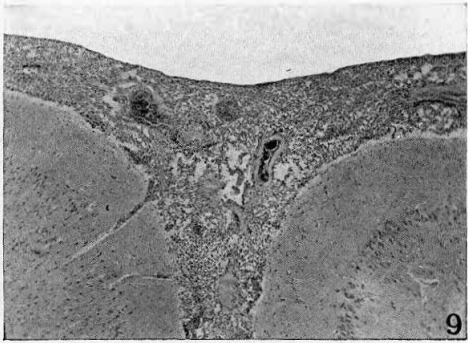
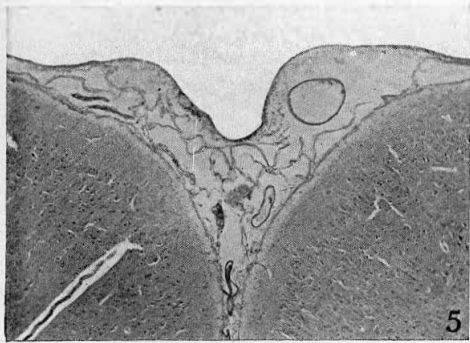
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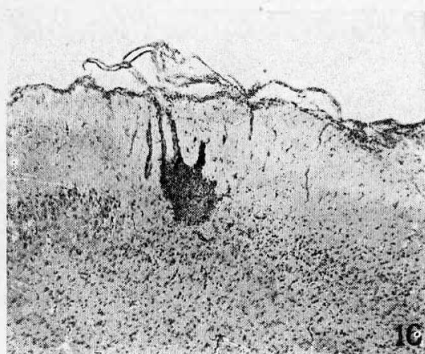
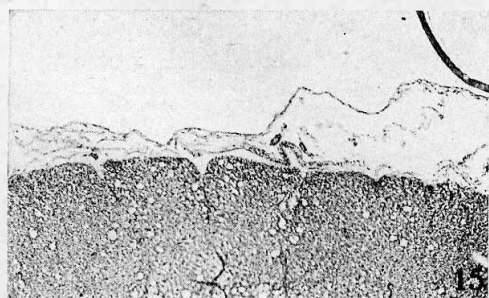
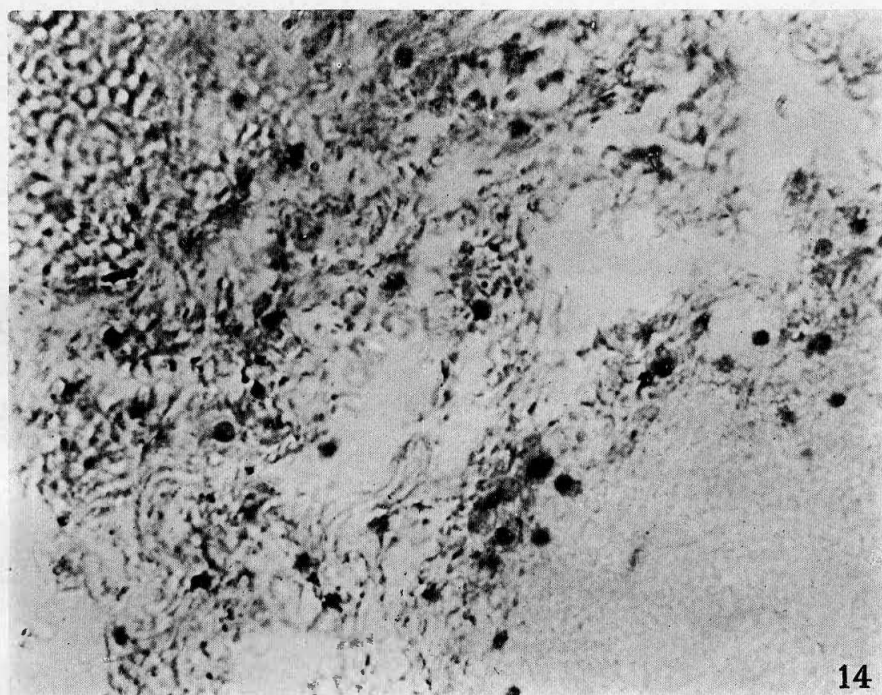
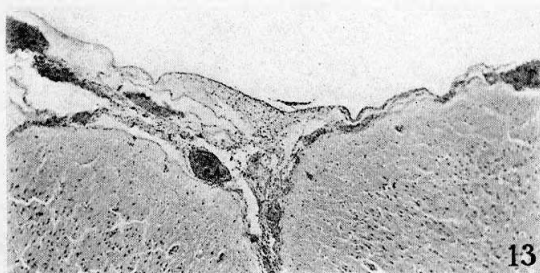
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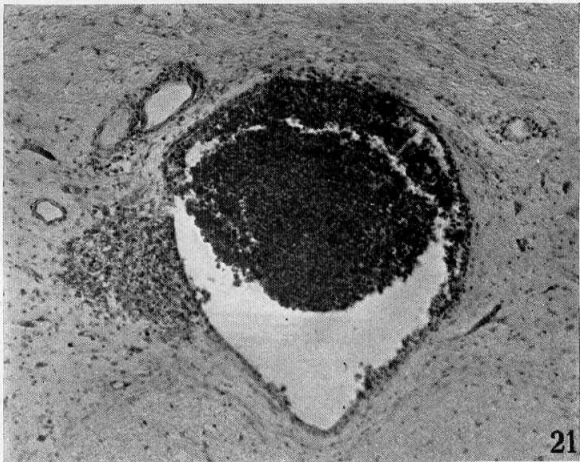
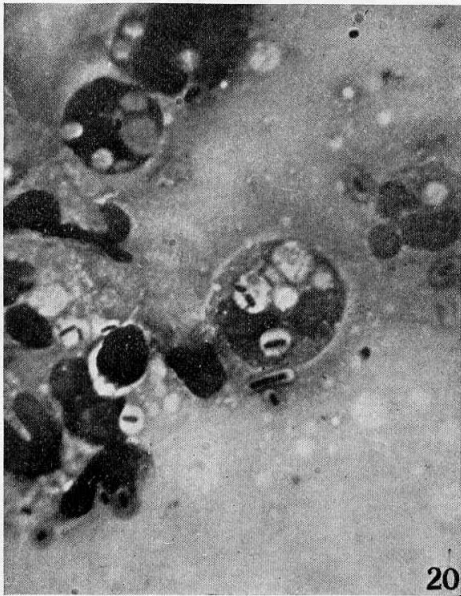
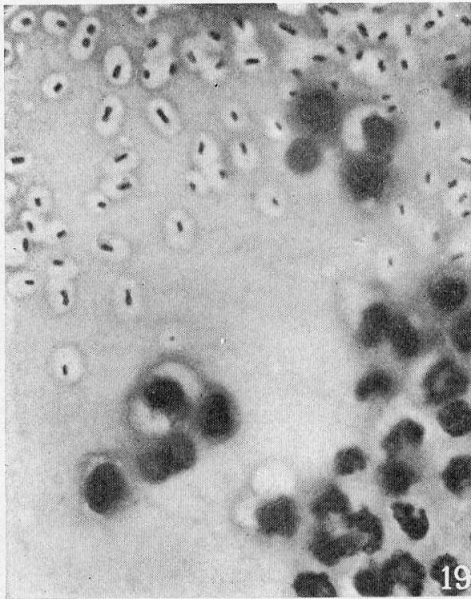
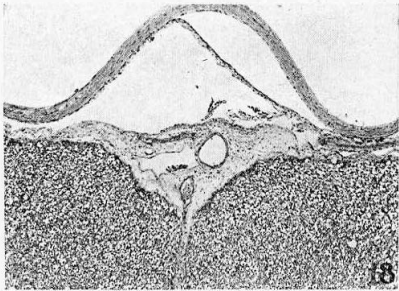
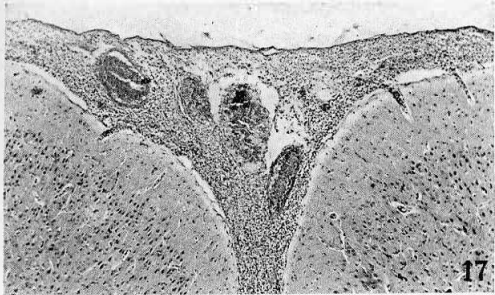
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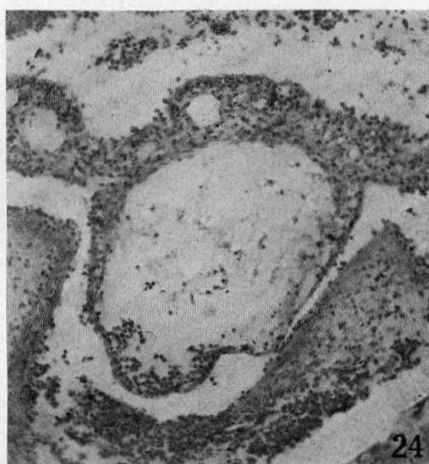
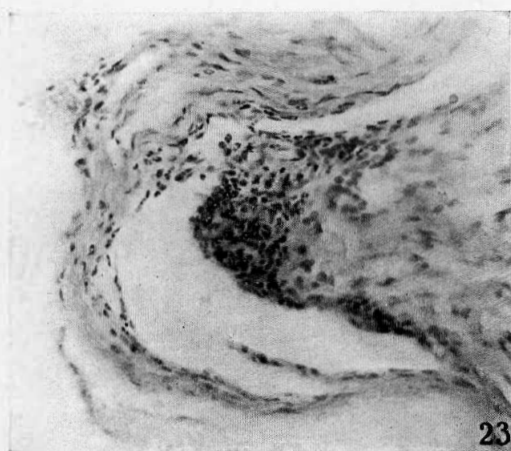
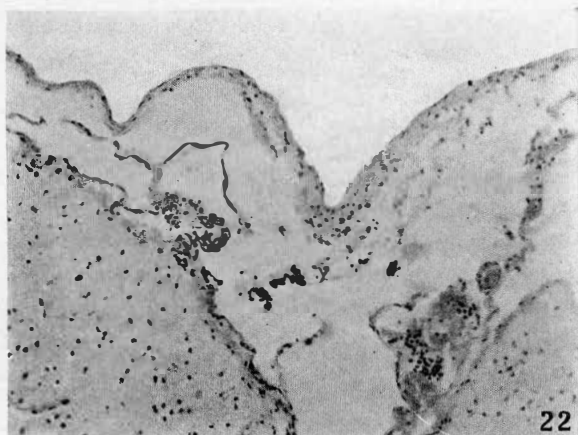
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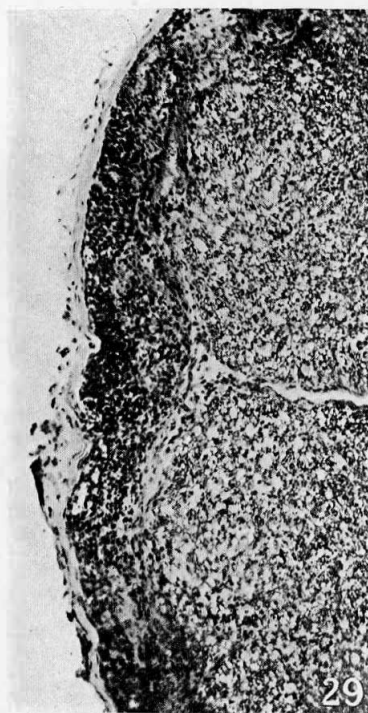
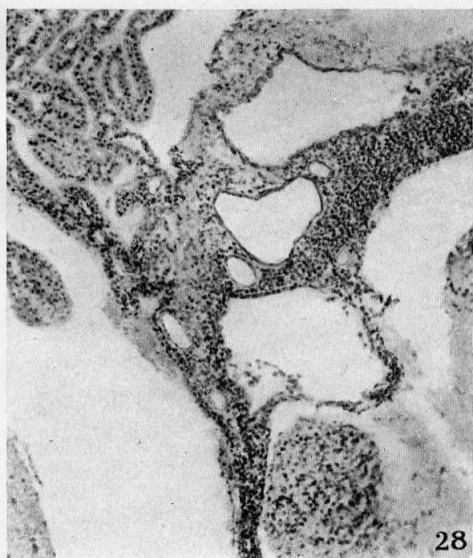
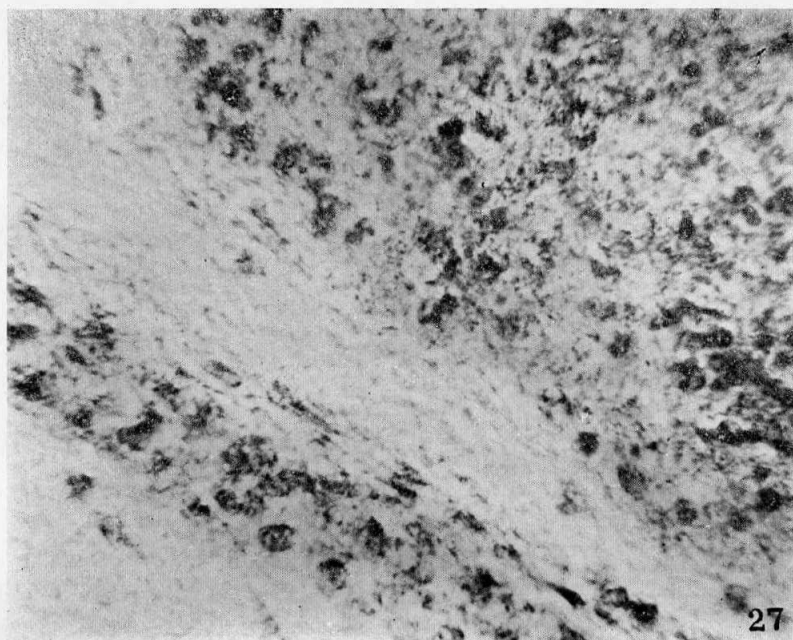


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