

## SERUM TREATMENT OF INFLUENZAL MENINGITIS.\*

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Our precise knowledge of the bacteriological causes of acute cerebrospinal meningitis has been greatly promoted by the employment of lumbar puncture as an aid to the diagnosis and to the treatment of that condition. We are now in a position to define quite accurately the several bacterial forms of acute meningitis and to state their relative frequency and degree of fatality. Among the highly important gains in knowledge of acute meningitis is the fact that the influenza bacillus is by no means an infrequent cause of that condition, and, further, that influenzal meningitis has terminated fatally in all but six of the fifty-eight pure cases thus far reported. The number of reported cases of influenzal meningitis is increasing rapidly, probably because their frequency and importance are becoming more impressive as the bacteriological diagnosis of cerebrospinal meningitis is becoming more widely known and more commonly performed.<sup>1</sup>

The frequency and severity of influenzal meningitis were impressed upon us at the Rockefeller Institute, where, for a period of several years, large numbers of specimens of cerebrospinal fluids have been examined bacteriologically. These fluids were sent for examination on the supposition that they were obtained from cases of epidemic meningitis, so the diagnosis was unexpected. All the cases diagnosed by us as influenzal meningitis terminated fatally.

The impulse to study influenzal meningitis experimentally came in part from the circumstance of its high fatality, and in part

\*Received for publication, May 18, 1911.

<sup>1</sup>Wollstein, Influenzal Meningitis and Its Experimental Production, *Am. Jour. Dis. Child.*, 1911, i, 42; Dunn, Cerebrospinal Meningitis, Its Etiology, Diagnosis, Prognosis and Treatment, *ibid.*, 85; Davis, Influenzal Meningitis, with Especial Reference to Its Pathology and Bacteriology, *ibid.*, 249.

constituted a feature of a more general investigation that has been conducted, under Dr. Flexner's supervision, for several years, on the control of localized infectious processes within the body. The first step in this experimental study was, obviously, the production experimentally of a condition closely resembling influenzal meningitis in man.

First, a word as to influenzal meningitis in human beings. The disease is more frequent among infants and children than among adults. It sometimes follows upon undoubted influenzal disease of the respiratory tract, and sometimes develops independently of obvious disease of that tract. Since the influenza bacillus is often present in the secretions of the respiratory mucous membrane in children suffering from a variety of diseases, during the wide prevalence of influenza, it is probable that the infection of the meninges is always secondary to respiratory infection and is accomplished chiefly through the blood current. Direct infection from the nose cannot be excluded and should be considered as a possibility. The probability of an ascending infection of the meninges from the nasal mucosa is supported by the observation that in monkeys the influenza bacillus appears in the nasal mucus after being injected into the subdural space of the spinal canal by lumbar puncture. However, all or nearly all cases of spontaneous influenzal meningitis in human beings are examples of influenzal bacteremia, since the bacilli have been cultivated in large numbers, before and after death, from the heart's blood. The same general fact is true of experimental influenzal meningitis in the monkey. When the condition is produced by means of a subdural injection of a virulent culture, the bacilli can be cultivated during life and at autopsy from the heart's blood.

We have been successful, as was stated briefly in a former publication,<sup>2</sup> in producing a condition of acute meningitis in monkeys that resembles the spontaneous disease occurring in human beings in respect to the clinical course and symptoms and the pathological effects. But not all strains of influenza bacilli suffice to set up a severe and fatal meningitis in monkeys when cultures are injected into the subdural space by lumbar puncture. The conditions under which virulent strains have been secured are themselves important

<sup>2</sup> Wollstein, *loc. cit.*

and informing in respect to the significance of the influenzal bacillus infections in human beings.

#### EXPERIMENTAL INFLUENZAL MENINGITIS.

The first step in the experimental work is the finding of a virulent strain of *Bacillus influenzae*. The selections are first made by tests upon white mice, guinea pigs, and rabbits. The bacilli which are isolated from the respiratory organs during life or after death are, as a rule, virulent for mice and guinea pigs. Of sixty strains so tested, only three were devoid of all virulence. The case is quite different for the rabbit, since only one strain derived from the lungs showed virulence, and in the human patient from which it was obtained, a blood invasion had taken place.

To be contrasted with this finding is the next one, namely, that all the meningeal strains except one (six in number) tested upon young rabbits, proved to be virulent. The exceptional strain was inoculated into a green monkey (*Cercopithecus callitrichus*) by lumbar puncture, and was found to be non-virulent. This result indicates in a general way, that the rabbit and the monkey react similarly to non-virulent strains of *Bacillus influenzae*. The correspondence is, however, not exact. We have noted that a strain of *Bacillus influenzae*, derived from a case of human meningitis, which was at first pathogenic for rabbits and monkeys, might after frequent transplantation, remain actively virulent for the rabbit and yet lose a considerable part of its power to infect monkeys. It also happens that a virulent strain may lose, after a long series of transfers outside the body, all pathogenicity for rabbits and monkeys.

The successful production of influenzal meningitis in the monkey depends upon the selection of a virulent culture and the maintenance of the pathogenicity. We have endeavored to maintain the virulence by frequent passage through the rabbit and occasional passage through the monkey. Such pathogenic cultures are grown upon blood agar slants for twenty-four hours, when they are washed down with salt solution. The effective dose consists of two cultures suspended in one or two cubic centimeters of this fluid and injected into the spinal canal in the lumbar region.

The effects of the subdural injection begin to be apparent in about five hours after the inoculation. The first noticeable effect is a disinclination to move about actively, although the monkey may not show severe symptoms for twelve or even twenty-four hours. Death may result as early as thirty-six hours after the inoculation, or it may be delayed for three or four days. A characteristic example of the longer period is as follows:—

*Experiment IV.*—A monkey (*Macacus rhesus*) received two slant cultures of *B. influenza* subdurally in the lumbar region. No immediate effect. Five hours later the monkey was unusually quiet and disposed to sit on the bottom of the cage. On being disturbed it moved about actively. After twenty hours it was still quiet and less disposed to be active. No food taken. Lumbar puncture yielded a turbid fluid containing many polymorphonuclear leucocytes and influenza bacilli. Almost all the extracellular bacilli grew profusely in culture. The symptoms grew progressively severer during the day. On the second day, the animal lay curled up, refused food, but noticed surrounding objects. Lumbar puncture yielded a fluid more turbid than before, containing an increased number of bacilli and many leucocytes. A degree of phagocytosis occurred. Cultures grew profusely. The symptoms progressed during the next twenty-four hours and the fluid obtained by lumbar puncture had become highly purulent. Death took place on the fourth day after inoculation.

*Autopsy.*—The brain showed purulent exudate over the cortex of the hemispheres, and the cord showed a similar exudate in the lumbar region. The pia arachnoid was everywhere cloudy. Pure cultures of *B. influenza* were obtained from the brain and cord. Outside the region of the central nervous system two foci of influenzal infection occurred; a lobular pneumonia of the lower lobe of the right lung over which the pleura showed a fibrino-purulent exudate, and a fibrino-purulent exudate over the anterior border of the right lobe of the liver. The influenza bacillus was present in pure culture. Sections prepared from the brain and cord showed a fibrino-purulent inflammation limited to the pia arachnoid. The heart's blood contained the bacilli.

The monkey that died thirty-six hours after inoculation showed inflammation of the pia arachnoid of the brain and cord, but no secondary foci of infection. Cultures from all parts of the central nervous system and from the upper nasal mucosa gave *B. influenza*.

#### THERAPEUTIC EXPERIMENTS.

That an acutely fatal form of influenzal meningitis can be produced experimentally in monkeys had now been established. The establishment of this fact was essential to the next step; namely, the attempt to control the condition therapeutically. With this purpose in mind, we had begun some months earlier to immunize a goat with the influenza bacillus, so that an immune goat serum was avail-

able for the experiments. Our plans involved two lines of procedure: (1) to determine the value of the immune serum as such, and (2) to attempt to intensify its activity by the addition of bactericidal chemical agents. Of the latter we had in mind, in view of Lamar's<sup>3</sup> studies on the pneumococcus, the use of certain bactericidal soaps. Preliminary experiments quickly showed, however, that oleate soaps in strengths below 1 per cent. exerted little effect on cultures of *Bacillus influenzae* and, also, that the soap and immune serum mixtures were of no avail in restraining the progress of experimental influenzal infections in the monkey, but tended rather to hasten the development of the meningitis when injected into the subdural space, by reducing the local resistance of the tissues. There remained, therefore, the immune serum alone to be tried.

Throughout this study we have had in mind the treatment of the infection by local application of the therapeutic agent. The convincing demonstration of the value of the antimeningitis serum when applied in this manner and its want of all action when introduced into the blood, directed our efforts.<sup>4</sup> It is a very simple matter to bring the serum into the cerebrospinal membranes by direct injection, and quite impossible to cause it to be secreted there from the blood.

In influenzal meningitis, whether spontaneously acquired in human beings or produced experimentally in animals, we have to deal not only with an extensive local infection and inflammation about the sensitive nervous organs, but with a bacteremia as well. The question therefore arose, whether the control of the local infection could be effective in view of the general infection. We have viewed this question as of subordinate importance, on the supposition that the invasion of the blood by the bacilli was produced by their local development in the meninges, the restraint of which would be followed by their disappearance from the circulation. That this view

<sup>3</sup>Lamar, Chemo-Immunological Studies on Localized Infections. Lysis of the Pneumococcus and Hemolysis by Certain Fatty Acids and Their Alkali Soaps, *Jour. Exper. Med.*, 1911, xiii, 380.

<sup>4</sup>Flexner, Concerning a Serum-Therapy for Experimental Infection with *Diplococcus Intracellularis*, *Jour. Exper. Med.*, 1907, ix, 168; Flexner and Jobling, An Analysis of Four Hundred Cases of Epidemic Meningitis Treated with the Antimeningitis Serum, *Jour. Exper. Med.*, 1908, x, 690.

is well founded, the results of the therapeutic experiments proved. On the other hand, there can be no real objection to the introduction of serum into the blood either by subcutaneous or direct intravenous injection, in order to assist in the control of the bacteremia. While it has not been necessary to resort to this measure in the monkeys, it might be desirable to do so in the case of human beings.

The goat that supplied the immune serum was injected at intervals with living cultures of *Bacillus influenzae*, for a period of about eighteen months. We determined that normal goat serum was devoid of agglutinin and opsonin for the influenza bacillus. The first cultures employed came from the respiratory tract of children, were non-virulent for animals, and produced no reaction in the goat. After two months, the blood reactions had not changed. At this time, a virulent strain of the bacillus obtained from the respiratory tract became available for injection. A reaction was immediately set up, and agglutinins and opsonins began to appear in the blood. After eight months' treatment, the agglutinins measured 1 to 80 and the opsonins 1 to 2,000. The first therapeutic test was made with the serum taken at this time (experiment V). At the end of eighteen months' treatment, the opsonic value had risen to 1 to 5,000. The serum is not bactericidal *in vitro* and does not give rise to complement fixation in dilutions greater than 1 to 100. The later experiments with monkeys were all made with the serum taken from the goat after one and one half year's immunization.

Experiment V supplied the indication that an immune serum might, under favorable conditions, control an experimental influenza bacillus infection of the meninges in the monkey. The fact is first to be observed that the interval elapsing between the infection and beginning of treatment was only one hour, and second, that the animal passed through a severe illness from which recovery appeared to follow, but that in spite of the abolition of the meningeal infection, the animal succumbed to an influenzal lobular pneumonia attended by empyema.

*Experiment V.*—A monkey (*Macacus rhesus*) received by lumbar puncture, two slant cultures of *B. influenzae*, and one hour later 2 c.c. of immune goat's serum. Twenty-four hours later the animal was very ill. Lumbar puncture yielded turbid fluid containing many bacilli; culture pure and abundant. Two

cubic centimeters of serum injected. During the next twenty-four hours the symptoms were abating. Lumbar puncture gave turbid fluid still containing bacilli but showing increased phagocytosis. A third injection of the serum was given, which was followed by rapid improvement and what appeared to be final recovery. The third specimen of cerebrospinal fluid was nearly clear and contained few bacilli, which, however, still grew in cultures. A clear sterile fluid was secured on the fourteenth day. Death occurred on the twenty-fourth day. The membranes about the brain and spinal cord were normal; the lungs showed lobular foci of pneumonia and pus in the pleural cavities. Cultures from the central nervous system were sterile; but the pleural pus yielded *B. influenzae*.

The experiments were unavoidably interrupted at this period, to be resumed some months later. The next series was carried out with a virulent culture of *Bacillus influenzae* obtained from the meninges of a fatal case of influenzal meningitis, and with the immune goat's serum after eighteen months of treatment. Two slant cultures injected subdurally in the control monkey produced meningitis with the attending characteristic symptoms and effects that ended fatally in three and one half days.

*Experiment VI.—Macacus rhesus.* Usual inoculation. First serum injection one hour later. Animal ill after twenty-four hours. After second serum injection improvement began. Three serum injections given. From the third day on, the animal was lively. The animal survived. The spinal fluid one day after inoculation was turbid and contained many bacilli and leucocytes; little phagocytosis. The next day the fluid was turbid, but phagocytosis was more pronounced; very few colonies developed in culture. On the third day, the spinal fluid was nearly clear and no bacilli were found in films; two colonies grew in culture.

This experiment, although far from being a severe test, was much more successful than the similar one made with the goat's serum nine months earlier. The striking difference is seen in the rapidity with which, under the influence of the serum injections, the cerebrospinal fluid had lost its turbidity; the multiplications of the bacilli had been arrested—probably through the promotion of phagocytosis—and cultures were sterile.

*Experiment VII.—*Usual inoculation. First serum injection of 2.5 c.c. three hours later. At this injection the spinal fluid was already turbid from bacilli and leucocytes. The next day, the animal was very ill. Second serum injection given. Specimen of spinal fluid removed showed many bacilli and leucocytes, the former almost entirely within the cells. Third day, animal still ill; 2.5 c.c. serum injected. Fluid removed was turbid and showed leucocytes,

many of which contained bacilli; no free bacilli seen. Culture positive. Fourth day, animal recovering; 1 c.c. serum injected. Fluid still somewhat turbid; few leucocytes containing bacilli; no free organisms; slight growth in culture; Fifth day, fluid clear; no bacilli; culture negative. Recovery complete.

This animal was desperately ill on the second day and it seemed hardly possible that recovery could be secured. The course of the disease resembled that often seen in human beings, and yet terminated favorably. The striking effect was the rapidity with which phagocytosis of the bacilli set in and the growth of the bacilli became arrested, to which circumstances we attribute the recovery of the animal. A certain degree of phagocytosis occurs in human cases of influenzal meningitis, but the great number of bacilli remain outside of cells and are readily cultivated at all periods of the disease.

In the next experiment, the interval between infection and treatment was lengthened to six hours, and in the next, to nine and one half hours. Both terminated successfully. The interval was now increased to twenty-four hours, resulting, as will be seen, in perfect recovery.

*Experiment XI.—Macacus rhesus.* Usual inoculation, and twenty-four hours later, at which time the animal was very ill, 4 c.c. of serum injected. The highly turbid fluid withdrawn contained many bacilli and leucocytes, the former practically all outside of cells. Abundant cultures. The next day (forty-eight hours after inoculation), the monkey was greatly improved; 3 c.c. of serum injected. The fluid withdrawn was turbid and showed few bacilli, but they were profuse in culture. Third day, animal lively; 3 c.c. of serum injected; spinal fluid less turbid; very few bacilli; three colonies in culture. Fourth day, spinal fluid clear. Recovery complete.

The interval has not, up to the present, been increased beyond twenty-four hours. Doubtless animals could be saved at still later periods, but in view of the fact that individual peculiarities play a part in monkeys as in human beings in determining the period of survival, which may be as brief as twenty-four to thirty-six hours, and which in monkeys does not exceed three to four days, while in human beings it may extend to six or seven days, we have concluded that the longer period employed is great enough to establish the value of a local serum therapy in the experimental influenzal form of meningitis. In view of the severe conditions surrounding



influenzal meningitis in human beings, it would seem desirable to apply the serum to the treatment of the spontaneous disease. If this should be done, then every effort should be directed to the making of the bacteriological diagnosis at the earliest possible moment, and the employment of a serum that has been prepared with virulent influenza bacilli and shows a high degree of opsonic value.

We have not discussed the mechanism of action of the immune serum. That it is not bacteriolytic has been stated; but whether its whole activity depends upon its opsonic content and value, we have not undertaken to ascertain. In this paper we have confined ourselves to the presentation of certain facts which lend a hope for the better solution of the problem surrounding the condition of influenzal meningitis in man.

We have made one experiment for the purpose of determining whether influenzal meningitis in monkeys is an infection or merely an intoxication. Thus, the usual quantity of culture of *Bacillus influenzae*, killed at 56° C., was injected into the spinal canal of a rhesus monkey. The injection produced no marked symptoms; from which it was concluded that the fatal effects of influenzal meningitis in monkeys, as in man, depend upon multiplication of the influenza bacilli and the gradual intoxication thus produced.

#### CONCLUSIONS.

The injection of virulent cultures of *Bacillus influenzae* into the subdural space of several species of lower monkeys is followed by the development of an acute inflammation of the meninges, corresponding in clinical, bacteriological, and pathological effects with influenzal cerebrospinal meningitis in human beings.

Experimental influenzal meningitis in the monkey is a lethal disease which terminates fatally in from thirty-six hours to four days after the inoculation.

The injected influenza bacilli produce their effects through multiplication in the course of which they penetrate from the subdural space into the general blood current, from which they may be recovered during life and at autopsy, as is also true of the spontaneous form of influenzal meningitis in man.

By repeated injection, over a period of many months, of living virulent cultures of *Bacillus influenzae* into the goat, an immune serum possessing moderate agglutinating and high opsonic power may be produced, which is capable, when injected into the subdural space, of arresting the progress of an experimental influenzal meningitis, and of bringing about recovery in monkeys thus affected.

As a result of the serum injections, the influenza bacilli in the meninges are more freely englobed by phagocytes, their number is reduced, their capacity of growth diminished, and the eruption into the blood arrested. Along with these effects go, hand in hand, cessation of the local inflammatory process and progressive amelioration of the symptoms of illness, to be followed usually by rapid restoration of health.

In view of the highly fatal character of influenzal meningitis in human beings, the employment of an immune serum by subdural injection is recommended. Undoubtedly it will be necessary to apply the serum early and by repeated injections, by means of lumbar puncture, to secure beneficial results. The early application will, in turn, be dependent upon prompt bacteriological diagnosis, which can be made, as a rule, by the immediate microscopical examination of the cerebrospinal fluid without the employment of cultural methods. When possible, the microscopical diagnosis should be confirmed by cultural tests.