

## THE RELATION OF THE MENINGES AND CHOROID PLEXUS TO POLIOMYELITIC INFECTION.

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The prevention or promotion of an infectious disease is determined by the interaction of several factors. This may be assumed to be true of epidemic poliomyelitis. We know that the presence of the microorganism, or virus, of that disease upon the mucous membrane of the nose and throat does not necessarily lead to infection. This form of contamination apparently gives rise to a class of healthy carriers of the microbic cause of poliomyelitis. The prevailing view, based on analogy with other infectious diseases in which the portal of entry of the causative microorganism is the upper respiratory mucous membrane, notably epidemic meningitis, is to the effect that these carriers are several times as numerous as actual cases of infection. Obviously, therefore, mechanisms exist to protect the contaminated persons from the pathogenic action of the virus which they carry.

The incidence of poliomyelitis in communities in which it has prevailed has been low. Thus the case incidence of the Greater New York epidemic (1916) was 1.59 per 1,000 of the population.<sup>1</sup> From this fact a high degree of individual insusceptibility has been inferred,<sup>2</sup> and the basis of the condition has been sought in the notion that epidemic poliomyelitis is a far more common condition than has been supposed, and in very mild form may prevail extensively without being recognized as that disease.<sup>3</sup> Since even a slight attack leaves an enduring protection,<sup>4</sup> a state of general immunity has been created

<sup>1</sup> Emerson, H., *Am. J. Med. Sc.*, 1917, cliii, 160.

<sup>2</sup> Flexner, S., *J. Am. Med. Assn.*, 1916, lxvii, 279.

<sup>3</sup> Wernstedt, W., *Communications Inst. méd. État à Stockholm*, 1912, iii, 235.

<sup>4</sup> Flexner, S., and Lewis, P. A., *J. Am. Med. Assn.*, 1910, liv, 45; *J. Exp. Med.*, 1910, xii, 227.

which manifests itself later during severe epidemics attended with paralysis and high mortality. While our present knowledge does not permit a final statement as to the applicability of this hypothesis even to Northern European countries in which poliomyelitis has been endemic for the past 30 years, it seems entirely inapplicable to America and the other countries which have only recently come widely under the influence of the epidemic disease.

The discovery of the main portals by which the virus enters the body has focused attention on the conditions which favor or hinder its entrance.<sup>5,6</sup> Having reached the upper respiratory mucosa the virus may take either of two routes in invading the central nervous organs. It may penetrate first into the local blood vessels and be carried into the circulation and thence to the nervous tissues, or it may pass into the lymphatic vessels surrounding the olfactory nerves and ascend more directly to the brain, medulla, and spinal cord.

Experimental evidence suggests the latter route.<sup>7</sup> It is difficult to infect monkeys with highly active poliomyelitic virus by way of the blood; and, conversely, it is easier to infect them by way of the nasal mucosa.<sup>6</sup> It is the function of the choroid plexus and the pial lymphatic vessels to exclude the virus present in the blood from the nervous tissues. Once these protective structures are injured, the exclusion ceases and infection can be made to follow readily. Flexner and Amoss<sup>8</sup> found that the injection of normal horse serum into the meninges, through which a transient aseptic meningitis is produced, is an effective way of overcoming the obstacle interposed by the structures mentioned. Intactness or permeability may conceivably play a part in determining whether infection is to ensue or not. When the virus enters directly from the nasal mucosa to the brain, the medulla and lastly the spinal cord become infected,<sup>7</sup> from which it would appear that the virus entering the central nervous organs by way of

<sup>5</sup> Flexner and Lewis, *J. Am. Med. Assn.*, 1910, liv, 535.

<sup>6</sup> Levaditi, C., and Landsteiner, K., *Compt. rend. Soc. biol.*, 1910, lxxviii, 417. Flexner and Lewis, *J. Am. Med. Assn.*, 1910, liv, 1140.

<sup>7</sup> Flexner, S., and Clark, P. F., *Proc. Soc. Exp. Biol. and Med.*, 1912-13, x, 1. Flexner, *Lancet*, 1912, ii, 1271; *Science*, 1912, xxxvi, 685; *Brit. Med. J.*, 1912, ii, 1261.

<sup>8</sup> Flexner, S., and Amoss, H. L., *J. Exp. Med.*, 1914, xx, 249.

the olfactory nerves permeates the organs continuously and is not distributed by the general circulation.

In discussing the defensive mechanisms, it is desirable in the first place to consider the ultimate fate of the virus which reaches the nasal mucosa as it does in many more persons than develop poliomyelitis. Amoss and Taylor<sup>9</sup> have found that the secretions of the mucous membrane are capable of neutralizing or inactivating the virus; and their experiments show also that this neutralizing property is absent altogether from the secretions of some persons and fluctuates in those of others, being present at one time and not at another. We may, indeed, view this destructive power as a means by which the number of persons becoming contaminated with the virus in the course of epidemics is diminished, as well as a mechanism through which infection may actually be prevented. Hence it may constitute one factor in the complex state to which the term susceptibility is applied. That still other factors enter into the perfection of this state is indicated by the experiments to be described.

#### *Meningeal Disturbances and Intravenous Inoculation.*

The routes through which infection with the poliomyelitic virus may be accomplished in monkeys have been closely studied. In order of ease and constancy they are: the intracerebral, intranasal, intraperitoneal, intraneural,<sup>10</sup> subcutaneous, and intravenous routes. The virus may also enter by other and extraordinary channels which have been little studied; namely, after intraocular injection<sup>11, 8</sup> and after feeding in animals narcotized with opium.<sup>12</sup> The intracerebral route gives not only the most constant results, but it exceeds the others in delicacy of response. This fact has been emphasized in a previous paper as has the power of the virus to be augmented after an intravenous inoculation through an aseptic meningitis induced by the intraspinal injection of normal horse serum.<sup>8</sup> Since then we have

<sup>9</sup> Amoss, H. L., and Taylor, E., *J. Exp. Med.*, 1917, xxv, 507.

<sup>10</sup> Some authors regard intrasciatic inoculation as next to intracerebral in effectiveness; in our experience it constitutes an uncertain portal of entry of the virus.

<sup>11</sup> Landsteiner and Levaditi, *Compt. rend. Soc. biol.*, 1909, lxxvii, 787.

<sup>12</sup> Leiner, C., and von Wiesner, R., *Wien. klin. Woch.*, 1910, xxiii, 91.

ascertained that an intranasal application of the virus, otherwise ineffective, may be made infectious by a similar aseptic inflammation of the meninges. The meningeal mechanism, which includes the choroid plexus, has indeed proved to be not only determinative in respect to the effect of an inoculation of the virus, but also of remarkable delicacy of adjustment. Pathologic changes of almost incredibly slight character may set aside its protective function.

*Horse Serum.*—The intraspinal injection of normal horse serum brings about in monkeys profound changes in the meningeal mechanism.<sup>8</sup> Besides producing a rich cellular, protein exudate, the epithelial coverings of the choroid plexus are rendered more permeable. Under these circumstances an otherwise ineffective quantity of the virus introduced into the circulation becomes infectious. But even so the quantity of virus effective by way of the circulation exceeds considerably that necessary to produce infection by intracerebral injection.

*Experiment 1 (Control).*—Dec. 13, 1916. A *Macacus rhesus* received an intravenous injection of 50 cc. of a centrifuged 5 per cent suspension of active monkey spinal cord and medulla,<sup>13</sup> 0.1 cc. of a Berkefeld filtrate of which was infectious by intracerebral inoculation. The monkey remained well.

*Experiment 2.*—Dec. 21, 1916. 4 p.m. 2 cc. of normal horse serum were injected intraspinally into a *Macacus rhesus*. Dec. 22, 11 a.m. 1 cc. of centrifuged virus as in Experiment 1 was injected intravenously. The monkey remained well.

*Experiment 3.*—The same as Experiment 2, with 5 cc. of the centrifuged virus. The monkey remained well.

*Experiment 4.*—The same as Experiments 2 and 3, with 10 cc. of the centrifuged virus. Paralysis on the 6th day and death on the 9th day following the intravenous injection.

The greater efficacy of the intracerebral route of inoculation is brought out by the experiments. The discrepancy is greater even than it appears to be because the filtration of the centrifuged suspension removes a large part of the protein content and with it, of course, much, and perhaps most of the virus.

In the past the intracerebral mode of inoculation has been relied

<sup>13</sup> In order to avoid immediate toxic effects, the emulsion must be made from nervous organs freshly removed from the paralyzed monkeys. Specimens kept at 4°C. or in 50 per cent glycerol are not satisfactory. Their injection is often followed in a short time by a drop in blood pressure and respiratory failure.

upon in therapeutic and disinfection tests with the poliomyelitic virus because it alone gave constant results. This was a disadvantage since, in the first place, the reaction of monkeys to an intracerebral inoculation of the adapted virus is far more severe than the response of human beings to the infection in the ordinary way. With the active virus employed in this manner, the mortality among the inoculated monkeys is approximately 100 per cent. In the next place the choice of disinfecting agents was necessarily limited to those which could be introduced into the cerebrum along with the virus. These disadvantages have been overcome through the promoting action of horse serum and other agents, some of which were hitherto regarded as indifferent, introduced into the meninges.

*Normal Monkey Serum.*—An homologous serum injected into the meninges sets up an aseptic meningitis, of lighter grade than that induced by a heterologous serum. Normal monkey serum was tested for its power to promote infection following an intravenous injection of the poliomyelitic virus.

*Experiment 5.*—Dec. 12, 1916. A *Macacus rhesus* received an intraspinal injection of 2 cc. of clear, hemoglobin-free normal monkey serum. Dec. 13. Intravenous injection of 50 cc. of a centrifuged 5 per cent suspension of active spinal cord and medulla. Dec. 18. Animal moves somewhat stiffly; ataxia; tremor of head; ptosis; shoulder muscles weak. Dec. 19. Died.

The autopsy showed typical lesions of poliomyelitis.

As this experiment shows, normal monkey serum resembles normal horse serum in promoting infection from an intravenous injection of the poliomyelitic virus. Experiments 6, 7, 8, and 9 indicate that even a less degree of inflammatory reaction than that induced by normal serum suffices to open the way for the virus present in the blood to reach the nervous organs.

*Salt, Ringer's, and Locke's Solutions.*—Of the three fluids mentioned, those which physiologically are most indifferent are Ringer's and Locke's solutions. As far as visible responses are concerned, all produce less inflammation of the meninges than either horse serum or monkey serum. And yet they are clearly proved by the reactions to be foreign and in a degree injurious to the meningeal structures.

*Experiment 6.*—Dec. 19, 1916. 5 p.m. 2 cc. of sterile isotonic salt solution were injected intraspinally into a *Macacus rhesus*. Dec. 20, 11 a.m. 50 cc. of a centri-

fused 5 per cent suspension of active spinal cord and medulla were injected intravenously. Dec. 26. Ataxia; tremor of head; double ptosis. Dec. 27. Died.

The autopsy showed typical and marked lesions of poliomyelitis.

*Experiment 7.*—Dec. 19, 1916. 5 p.m. 2 cc. of sterile Ringer's solution were injected intraspinaly into a *Macacus rhesus*. Dec. 20, 11 a.m. 50 cc. of centrifuged fluid similar to that in Experiment 6 were injected intravenously. Dec. 29. Ataxia; tremor of head. Dec. 30. Shoulder and back muscles weak. Jan. 2, 1917. Paralysis extended. Died.

The autopsy showed typical lesions of poliomyelitis.

*Experiment 8.*—*Macacus rhesus*. This experiment was exactly like the preceding one, except that sterile Locke's solution was used. Jan. 5, 1917. Intraspinal injection of Locke's solution. Jan. 11. The first symptoms appeared. Jan. 15. Died.

The autopsy showed typical poliomyelitic lesions.

In the next experiment, Locke's solution plus 0.5 per cent gelatin were employed.<sup>14</sup>

*Experiment 9.*—Jan. 23, 1917. 4 p.m. 2 cc. of sterile Locke's solution containing 0.5 per cent gelatin were injected intraspinaly into a *Macacus rhesus*. Jan. 24, 10 a.m. 50 cc. of a centrifuged 5 per cent suspension of spinal cord and medulla were injected intravenously. Feb. 2. Paralysis of legs; shoulders weak. Feb. 4. Prostrate. Feb. 5. Died.

The autopsy showed typical lesions of poliomyelitis.

It is obvious that even when the irritative and inflammatory changes induced in the meningeal structures are actually very slight, they suffice to remove the power of the intact organs to exclude the virus from the interstices of the central nervous tissues. These observations, in themselves important, unexpectedly confirm and extend an earlier experiment in which the injection of salt solution into a cerebral hemisphere promoted infection with a virus, otherwise ineffective by that route, introduced into the veins.<sup>8</sup>

#### *Homologous and Autologous Cerebrospinal Fluid and Simple Lumbar Puncture.*

Since it now appeared that very slight changes in the meningeal structures enabled the virus circulating in the blood to penetrate and

<sup>14</sup> Rous and Turner (*J. Exp. Med.*, 1916, xxiii, 219) observed that a fluid of that composition was an excellent medium for preserving red corpuscles intact for a considerable period and surpassed in that respect the other artificial physiologically balanced fluids tested.

multiply within the central nervous organs, the question arose as to the limit of the irritative process sufficing for this purpose. The next experiments related to (1) simple lumbar puncture, (2) the removal and return of the cerebrospinal fluid in the same monkey, and (3) the withdrawal of the fluid from one monkey and its replacement with the fluid taken from other monkeys.

*Simple Lumbar Puncture.*—By this term is meant the removal of fluid by lumbar puncture under conditions in which no blood whatever is drawn by the operation. If a trace of blood entered the needle, the animal was not regarded as suitable for the test.

*Experiment 10.*—Dec. 21, 1916. 4 p.m. 2 cc. of clear fluid were withdrawn from a *Macacus rhesus* by lumbar puncture. Dec. 22, 12 noon. Intravenous injection of 50 cc. of a centrifuged 5 per cent suspension of active spinal cord and medulla. No symptoms developed.

*Withdrawal and Return of Autologous Fluid.*—In this experiment, as in the preceding one, the merest tinge of blood in the withdrawn fluid renders the animal unsuitable.

*Experiment 11.*—Jan. 15, 1917. 4 p.m. 2 cc. of clear fluid were withdrawn from a *Macacus rhesus* by lumbar puncture. 5 p.m. The same fluid was returned by lumbar puncture. Jan. 16, 10.30 a.m. Intravenous injection of 50 cc. of a centrifuged 5 per cent suspension of active spinal cord and medulla. No symptoms developed.

*Introduction of Homologous Fluid.*—The same precautions were followed in this experiment to avoid the slightest hemorrhage in performing the lumbar puncture.

*Experiment 12.*—Jan. 5, 1917. 6 p.m. 2 cc. of clear cerebrospinal fluid were withdrawn from a *Macacus rhesus* by lumbar puncture and replaced by 2 cc. of clear fluid withdrawn 3 minutes earlier from a normal monkey of the same species. Jan. 6, 11 a.m. Intravenous injection of 50 cc. of a centrifuged 5 per cent suspension of active spinal cord and medulla. Jan. 14. Legs, arm, and back weak. Jan. 15. Prostrate. Feb. 2. Died.

The autopsy showed characteristic lesions of poliomyelitis.

*Experiment 13.*—Feb. 8, 1917. 4 p.m. 2 cc. of clear cerebrospinal fluid removed 30 minutes before from two normal *Macacus rhesus* monkeys were injected intraspinally into a monkey of the same species. Feb. 9, 10 a.m. Intravenous injection of 50 cc. of a centrifuged 5 per cent suspension of active spinal cord and medulla. Feb. 14. Paralysis of legs. Feb. 15. Prostrate.

The autopsy showed typical lesions of poliomyelitis.

This experiment has not succeeded in every instance.

That the production of even slight hemorrhage in withdrawing the cerebrospinal fluid may seriously alter the result is shown by the next experiment.

*Experiment 14.*—Feb. 15, 1917. 4 p.m. 1 cc. of cerebrospinal fluid slightly tinged with blood was withdrawn from a *Macacus rhesus* by lumbar puncture. The quantity of blood present in the fluid, estimated on the basis of red corpuscles present, was perhaps one-fiftieth of the total volume of the fluid. Feb. 16, 10 a.m. Intravenous injection of 50 cc. of a centrifuged 5 per cent suspension of active virus. Feb. 20, a.m. Ataxia; tonic convulsions. p.m. Prostrate. Feb. 21. Died.

The autopsy showed typical lesions of poliomyelitis.

The preceding experiments indicate that (1) simple uncomplicated lumbar puncture does not lead to infection after an intravenous inoculation; (2) the simple removal and return of the same cerebrospinal fluid in a given animal is likewise without effect; while (3) the interchange of fluid from one animal to another does lead to infection, although not in every instance. Moreover, injury to the blood vessels of the meninges also promotes the infection. The infection in the last example may be due either to the escape directly of the inoculated virus from the blood through the injured vessels into the meninges, or what is more probable, to the blood's entering the subarachnoid space and setting up a mild inflammatory reaction, which, as we have seen, suffices to promote the infection. Sometimes the interchange of fluids fails to be followed by an infection. This failure may be due to one of several causes: the two fluids interchanged may be essentially so alike in composition as to constitute practical identity; the test then becomes equivalent to the autologous experiment; or the meningeal structures in certain monkeys may be less responsive to such a mild irritant and resist its action altogether.

It is more remarkable that the irritative response should occur than that it should in some instances fail to take place. The experiments emphasize the extraordinary sensitiveness of the meningeal protective adjustment through which exclusion of the poliomyelitic virus, and doubtless other injurious substances, from the interior of the nervous organs, is accomplished. They suggest that if alterations as slight as these suffice to produce infection, the essential meningeal mechanism may have to be regarded as in no small part determinative of the development of poliomyelitic disease.



*Serum Promotion of Intranasal and Subcutaneous Infection.*

Following Flexner and Lewis's<sup>5</sup> demonstration that the virus of poliomyelitis introduced into the cerebrum escapes by way of the nasal mucosa, Levaditi and Landsteiner<sup>6</sup> first determined that the application of the virus to the nasal mucosa would lead to infection. As already stated, the intranasal method is a less constant means of producing infection than the intracerebral and requires somewhat greater quantities of the virus.

In the following experiments inoculation was accomplished by packing the left naris with a plug of absorbent cotton saturated with crushed spinal cord of a recently paralyzed monkey. The plug was left in position for 2 hours and then removed, and not infrequently it showed slight tingeing with blood.

We have found that packing for 2 hours is insufficient to lead to infection in normal monkeys. But when monkeys have previously received an intraspinal injection of horse serum, infection results.

*Experiment 15.*—Jan. 2, 1917. 5 p.m. 2 cc. of normal horse serum were injected intraspinally into a *Macacus rhesus*. Jan. 3, 11 a.m. A pledget of cotton soaked with fresh poliomyelitic spinal cord of a monkey was packed in the left naris. When removed 2 hours later it was slightly stained with blood. Jan. 9. Protects right arm. Jan. 11. Tremor of head; ataxia; considerable spasticity of legs and weakness of deltoids. Cerebrospinal fluid contains 2,680 cells per c.mm. and increased globulin. Jan. 12. Prostrate. Jan. 14. Died.

The autopsy showed characteristic lesions of poliomyelitis.

*Experiment 16.*—Feb. 8, 1917. 4 p.m. A *Macacus rhesus* received an intraspinal injection of 2 cc. of normal horse serum. Feb. 9, 10.30 a.m. A nasal plug saturated with poliomyelitic spinal cord and medulla was introduced and left in the left naris for 2 hours. Feb. 18. Paralysis in left arm; weakness of back. Feb. 19. Prostrate. Feb. 20. Etherized.

The autopsy showed typical lesions of poliomyelitis.

The same method of promoting infection was used in the instances of intrasciatic and of subcutaneous inoculation. In two trials no result was accomplished in the former; while in the latter infection took place.

*Experiment 17.*—Jan. 9, 1917. 5 p.m. 2 cc. of normal horse serum were injected intraspinally into a *Macacus rhesus*. Jan. 10, 2 p.m. Subcutaneous injection of 1 cc. of a 5 per cent suspension of spinal cord and medulla of a poliomye-

litic monkey. Jan. 20. Arms paralyzed; back weak; facial asymmetry. Jan. 21. Died.

The autopsy showed typical lesions of poliomyelitis.

A control animal in which no horse serum had been inoculated did not respond to the subcutaneous inoculation alone.

This series of experiments indicates that preexisting pathologic conditions in the meningeal structures promote infection in poliomyelitis not only when the virus is present in the blood, but also when it reaches the central nervous organs by way of the nerves, as in the case of intranasal inoculation and probably also of subcutaneous inoculation. The promoting influence of the serum in intranasal inoculation acquires special significance in view of the fact that the nasal mucosa is the usual portal of entry of the virus into the nervous organs in human beings;<sup>15</sup> that is, the intact meninges are a protection from nasal as from intravenous inoculation with the virus of poliomyelitis, as, conversely, the pathologically altered meninges are a cause of heightened susceptibility to the action of the virus present in either place.

#### *Immune Serum and Heightened Susceptibility.*

The experiments described show that not only normal horse and monkey serum, but fluids of far less irritative nature promote, when introduced into the meninges, infection with the virus of poliomyelitis. Earlier experiments, on the other hand, have shown that infection with the virus may be prevented by an immune serum even when the inoculation is made into the cerebrum.<sup>16</sup> Would infection therefore be prevented if an immune serum was injected into the meninges previous to the intravenous administration of the virus? For if infection does not occur, the difference would be attributable only to the presence within the immune serum of the neutralizing substances for the virus.

*Experiment 18.*—Dec. 21, 1916. 5 p.m. 2 cc. of immune monkey serum pooled from three animals which had recovered from an attack of experimental poliomyelitis were injected intraspinaly into a *Macacus rhesus*. Dec. 22, 11 a.m. 50 cc. of a centrifuged 5 per cent suspension of active spinal cord and medulla of a paralyzed monkey were injected intravenously. No symptoms appeared.

<sup>15</sup> Flexner, S., *J. Am. Med. Assn.*, 1910, lv, 1105.

<sup>16</sup> Flexner and Lewis, *J. Am. Med. Assn.*, 1910, liv, 1780; lv, 662.

This experiment is conclusive in that it indicates that an immune serum alone of all the irritative fluids injected into the meninges prevents the infection. The experiment has a bearing also on the serum therapy of human poliomyelitis and upon the question of the employment for intraspinal injection of normal sera and other fluids of more or less irritative character.

The next series of experiments relates to the protecting power of an immune serum when it is employed to overcome the effect on the infection of an intraspinal injection, either of normal monkey serum or of normal salt solution. That protection would thus be afforded by the immune serum was regarded as certain, in view of the power which it had already displayed to hold up an infection by means of the intravenous administration of the centrifuged virus following the intraspinal injection of horse serum.<sup>8</sup>

*Experiment 19.*—Dec. 19, 1916. 4.30 p.m. 2 cc. of normal monkey serum were injected intraspinally into a *Macacus rhesus*. Dec. 20, 1 p.m. 50 cc. of a centrifuged 5 per cent suspension of active spinal cord and medulla were injected intravenously. 1.30 p.m. Intraspinally injection of 3 cc. of pooled immune monkey serum. Dec. 26. Ataxia; head tremor; ptosis. Dec. 27. Symptoms more marked. Dec. 28. Widespread weakness of muscles. Dec. 30. Prostrate. This animal slowly recovered with marked residual paralysis.

*Experiment 20.*—Dec. 19, 1916. 2 cc. of normal monkey serum were injected intraspinally into a *Macacus rhesus*. Dec. 20, 12 noon. 50 cc. of a centrifuged 5 per cent suspension similar to that used in the previous experiment were injected intravenously. 12.20 p.m. 3 cc. of pooled immune serum were injected intraspinally. The immune serum was given on the next 3 successive days, and after a 2 days' rest on 3 more successive days. No symptoms developed.

*Experiment 21.*—This experiment was an exact repetition of Experiment 20, and yielded the same results.

The inflammatory changes induced by normal monkey serum similar to those brought about by normal horse serum require, apparently, more than a single injection of the immune serum to prevent infection after intravenous injection. Two or three injections at 24 hour intervals would suffice, for within that period the defect would have probably been healed and the major part of the virus neutralized. Moreover, it has been shown<sup>17</sup> that large doses of the virus injected into the blood begin to disappear in 96 hours and the virus

<sup>17</sup> Flexner and Amoss, *J. Exp. Med.*, 1914, xix, 411.

no longer circulates in detectable quantities after 120 hours. After inflammation has been produced in the meninges by normal monkey serum a single intraspinal injection of the immune serum mitigated the severity of the infection, since, contrary to the rule, the paralyzed animal lived.

The last experiment of this series was made with normal salt solution. It repeats exactly the one in which normal monkey serum was injected intraspinally, the centrifuged virus intravenously, and one dose of immune serum intraspinally. In conformity with the smaller damage inflicted upon the meningeal structures, a single dose of the immune serum is observed to prevent infection.

*Experiment 22.*—Jan. 15, 1917. 3.30 p.m. 2 cc. of normal salt solution were injected intraspinally into a *Macacus rhesus*. Jan. 16, 10 a.m. 50 cc. of a centrifuged 5 per cent suspension of active spinal cord and medulla were injected intravenously. 10.20 a.m. Intraspinally injection of 3 cc. of pooled immune serum. No symptoms developed.

This group of experiments confirms and extends somewhat the earlier ones made with normal horse serum. It now appears not only that an immune serum injected intraspinally is protective, but that the degree of its efficiency is more or less proportional to the injury which the meningeal structures have suffered from the irritating substances employed to increase their permeability. The slighter the inflammation, the more readily and quickly the injury is repaired; with isotonic salt solution a single dose of the immune serum suffices to prevent infection, while with monkey and horse serum several doses are required.

#### CONCLUSIONS.

Among the mechanisms which defend the body from infection with the virus of poliomyelitis is the meningeal-choroid plexus complex, which normally is capable of excluding the circulating virus from the central nervous organs. The complex plays a part also in preventing infection from virus present upon the nasal mucosa.

Aseptic fluids which irritate, inflame, or even slightly alter the integrity of the meninges and choroid plexus diminish or remove their protective function.

Normal monkey or horse serum, isotonic salt solution, and Ringer's

and Locke's solutions, when injected into the meninges, promote infection with the virus of poliomyelitis introduced into the blood, the nose, or the subcutaneous tissues.

Simple lumbar puncture and the withdrawal and return of the cerebrospinal fluid in normal monkeys, hemorrhage having been absolutely avoided, do not promote infection with virus injected into the blood; while the replacement of the cerebrospinal fluid of one monkey with that of another does in some instances lead to infection. Simple lumbar puncture attended with even very slight hemorrhage opens the way for the passage of the virus from the blood into the central nervous tissues, and thus promotes infection.

Hence, changes in the structure or function of the meningeal-choroid plexus complex, too slight to be detected by chemical and cellular changes in the cerebrospinal fluid or by morphological alterations, suffice to diminish in an essential manner its protective powers.

Of all the irritant fluids tested, immune serum alone injected into the meninges is not succeeded by infection from the virus introduced into the blood.

The protective property of the immune serum is capable of overcoming the promoting action of normal monkey and horse serum and the other irritants mentioned.

The importance first of the meningeal-choroid plexus complex in preventing infection with the virus of poliomyelitis, and next of immune serum in offsetting the disadvantages and dangers arising from defects in the mechanism is apparent, as is the bearing of the experiments reported on the serum therapy of epidemic poliomyelitis.