EXPERIMENTS ON THE NASAL ROUTE OF INFECTION IN POLIOMYELITIS.

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In this paper we shall describe experiments bearing on the intranasal route of infection in poliomyelitis. That the inciting microorganism or virus of poliomyelitis enters the central nervous system by way of the nasal passages is now generally believed. Moreover, it has been shown that this virus may be present on the nasal mucosa without inducing any signs of disease.

Both healthy and so called chronic carriers of the virus of poliomyelitis occur in man. Wide diversity of view prevails as to the frequency with which carriage of the virus arises and as to the period of persistence of the virus in the carriers. According to one group of observers (Wickman,[1] and Kling, Pettersson, and Wernstedt[2]), healthy and chronic carriers arise numerously during epidemics of poliomyelitis and actually exceed, possibly even many fold, the number of actual cases of the disease. Moreover, the virus may be very persistent in carriers who have recovered from an attack of the disease and be detectable by animal inoculation several months after all the acute symptoms have subsided (Kling, Pettersson, and Wernstedt). However, it should be remarked here that the virus is supposed to undergo gradual deterioration and thus fail in producing typical experimental poliomyelitis, although it is still capable of exciting atypical symptoms and lesions.

Another group of experimenters has come to quite opposite conclusions. Thus Flexner and Amoss[3] who employed excised tonsillar and adenoid tissue for inoculation did not find either the great frequency of occurrence or the long survival of the virus in convalescents implied in the preceding statements. On the contrary, while they found the tonsillar and other tissues infective for monkeys during the early period of the disease in man, they observed no effects, as a rule, from the inoculation of the tissues taken after the acute symptoms had subsided.

[1] Wickman, I., Beiträge zur Kenntnis der Heine-Medinschen Krankheit, Berlin, 1907.

[2] Kling, C., Pettersson, A., and Wernstedt, W., *Communications Inst. méd. État Stockholm*, 1912, iii, 5.

[3] Flexner, S., and Amoss, H. L., *J. Exp. Med.*, 1919, xxix, 379.

The point of difference involved is important, not only on the general basis of public health considerations, but also because of its bearing on our conceptions concerning the epidemiology of poliomyelitis. The morbidity of poliomyelitis even during severe epidemics is low. This circumstance has been accounted for by assuming either that a relative insusceptibility to the disease exists among general populations, which is no explanation at all, or that because of the wide dissemination of the virus during an epidemic and even in interepidemic periods, an unperceived active immunization of the community takes place. Insusceptibility then is due to specific protection or immunity.

In all instances in man and monkey in which an experimental inquiry has been made, it has been found that when active immunity exists, the blood carries neutralizing or destructive bodies for the poliomyelitic virus. No systematic study of the blood of exposed persons who have remained free from obvious poliomyelitis has been made. We conducted a number of tests of the blood of nurses, doctors, and others who had been repeatedly exposed during the severe epidemic in New York State in 1916, without, however, obtaining any clear and decisive results.

The kind of assumed protection just indicated would be general and specific. But experiments of Amoss and Taylor [4] have shown that another kind of potential protective mechanism is demonstrable in man. This device is local and depends upon the presence in the nasal membrane and its secretions of a substance, not yet defined, which possesses the power of neutralizing or otherwise destroying the virus of poliomyelitis. It is suggested that this local process may play an important part in determining the morbidity of epidemic poliomyelitis.

The experiments to be described in this paper relate to several aspects of the problem of intranasal infection in poliomyelitis and bear, therefore, on the preceding discussion. The first experiments to be given concern the question of the power of the nasal mucosa of the monkey to suppress the virus of poliomyelitis directly applied to it; or, in other words, the ascertaining of the period of survival of the virus on the nasal membrane.

[4] Amoss, H. L., and Taylor, E., *J. Exp. Med.*, 1917, xxv, 507.

Fate of the Virus Applied to the Nasal Mucosa.

An effective means of inducing infection in *Macacus* monkeys is to apply an active poliomyelitis virus to the nasal membrane on a cotton pledget. By virus in this connection is meant the comminuted spinal cord and medulla of a monkey which has suffered from acute experimental poliomyelitis.

Not all monkeys so treated acquire infection; indeed, the percentage of successful inoculations by the nasal route is smaller than by the brain or intracerebral route. The first question to present itself, therefore, is the fate of the virus in animals which may not succumb to intranasal inoculation.

It has already been shown that given an active virus, it can be detected in the nasal membrane by means of subinoculation. That is, if the nasal membrane carrying the virus is excised, ground with sterile sand, suspended in isotonic saline solution, and filtered through a Berkefeld candle, the filtrate will set up poliomyelitis in another monkey into which it is injected intracerebrally and intraperitoneally. The experiments to follow show that with a constant sample of the virus and a uniform mode of inoculation, the survival of the virus on the nasal membrane is irregular and individual.

The virus was one which has long been kept active by monkey passages, and the mode of application was by cotton pledget, which was allowed to remain in a naris for 2 or more hours. Upon removal, the animals were kept under close observation for varying lengths of time, their condition was noted, and the excised nasal membrane, after etherization of the selected animals, employed for obtaining the filtrate, as described above, for purposes of inoculating other monkeys.

Experiment 1.—May 25. *Macacus rhesus* A. Cotton pledget carrying the virus remained in a naris over night. 60 hours after the removal of the plug, the animal was killed with ether and the nasal mucosa excised. The filtrate prepared from this membrane was inoculated into *Macacus rhesus* B.

May 28. *Macacus rhesus* B. Received 2 cc. of the filtrate by intracerebral and 5 cc. by intraperitoneal injection. This animal remained well until June 3, when it showed excitement and an ataxic, uncertain gait. The symptoms extended rapidly, paralysis occurred, and the animal died on June 6. The lesions present in the spinal cord and medulla were typical of poliomyelitis.

This experiment shows, therefore, that it is possible to detect the virus by the methods employed at least 60 hours after its application to the nasal membrane. But other tests carried out simultaneously or subsequently on other monkeys killed at the expiration of 40 and 60 hours and 8 days after the removal of the pledget resulted negatively. Hence this experiment may be taken to indicate that the nasal mucous membrane of the *Macacus rhesus* possesses in some instances striking power of destroying or eliminating the virus of poliomyelitis energetically applied to it.

The property of the nasal mucosa to render ineffective, under certain circumstances, an otherwise efficient dose of the virus may be shown in still another way.

The manner of invasion of the central nervous system by the virus of poliomyelitis is still an open question. In view of the difficulties surrounding experimental infection by way of the blood, and the relative ease with which it is accomplished by way of the brain, nasal membrane, sciatic nerve, peritoneum, eye, and even subcutaneous tissue, Flexner suggested that in all instances the passage of the virus from the periphery to the center is ultimately by way of the nerves. According to this view, the virus applied to the nasal mucosa extends along the short olfactory nerve fibers to the brain and spinal cord. A certain amount of support for this mode of infection is supplied by the experiments of Landsteiner and Levaditi[5] and of Flexner and Clark,[6] in which after an intranasal inoculation the brain and cord of the monkeys were removed before any symptoms appeared and injected separately into other monkeys. Flexner and Clark noted that 48 hours after an intranasal inoculation the olfactory lobes but not the medulla and spinal cord might be infectious.

But illuminating as this experimental result is, it must be regarded as the exception rather than the rule. It happens also and perhaps much more frequently that after an intranasal inoculation the virus cannot be detected either in the mucous membrane or in any portion of the central nervous system. The following protocol illustrates this point.

[5] Landsteiner, K., and Levaditi, C., *Ann. Inst. Pasteur*, 1910, xxiv, 833.

[6] Flexner, S., and Clark, P. F., *Proc. Soc. Exp. Biol. and Med.*, 1912–13, x, 1.

Experiment 2.—Macacus rhesus. June 1. The cotton pledget carrying the active virus was permitted to remain in the naris for 24 hours. No symptoms had developed by June 5, when the animal was etherized, 88 hours after the tampon had been removed. The right middle turbinate, at the site of the tampon, showed a small hemorrhage into the mucous membrane.

The nasal mucosa and heavy suspensions of the olfactory lobes, postrolandic convolutions, medulla, and cervical and lumbar spinal cord were inoculated separately into the brains of other *rhesus* monkeys. In no instance was infection secured. The control *Macacus rhesus* in which the pledget remained for the same period became paralyzed on the 7th and died on the 9th day. The lesions of the central nervous organs were typical of poliomyelitis.

The virus used in this experiment was active and the procedure adequate. The difference in the results may be attributed to the power of the nasal mucosa in the one and not in the other animal to destroy the virus. This is the more probable explanation, although it is, of course, possible that at the expiration of the 88 hour period the increase of the virus was insufficient to flood the central nervous system so as to be detectable by the inoculation test.

The first view given is, however, supported by another experiment in which the cotton plug carrying the active virus was permitted to remain in the naris only 2 hours. One of the *Macacus rhesus* monkeys developed symptoms, became paralyzed, and the nervous organs showed typical lesions of poliomyelitis. The other showed no symptoms and was etherized on the 16th day. The nasal mucosa, olfactory lobes, and medulla were injected intracerebrally into three *rhesus* monkeys of which none developed symptoms.

Effects of Antiseptics.

The innate destructive property possessed by the nasal membrane for the virus of poliomyelitis may be regarded as a valuable defensive mechanism. The question has often been raised whether, during an epidemic of poliomyelitis, the application of antiseptics to the nasal mucosa is to be recommended. In the case of chronic meningococcus carriers, the suppression of that microorganism by the introduction of antiseptics directly into the nasopharynx has not been notably successful; and the meningococcus is apparently a much more fragile organism than the microbe of poliomyelitis.

There is a further important consideration. Now that it has been shown that the nasal membranes are themselves defensive, account needs to be taken of the action of antiseptic drugs upon the chemical substances in the membranes upon which their protective function depends. It is fortunate that in the case of poliomyelitis the effects of chemical antiseptics on the virus of poliomyelitis implanted on the nasal mucosa can be directly tested experimentally.

We already possess fair data of the effects of disinfectants on the virus *in vitro*. The effective chemicals chiefly studied are hydrogen peroxide (Flexner and Lewis), formaldehyde (Römer), thymol, potassium permanganate (Landsteiner and Levaditi), and still others. In the experiments to be given the only antiseptics employed were chloramine-T and the oily solution of dichloramine-T, as devised by Dakin and Dunham.[7, 8]

Two protocols only of this series of experiments will be given. In a few instances in which the virus was applied to the nasal mucous membranes, monkeys treated with the dichloramine-T did not become ill or paralyzed, but as in these cases the control animals also failed to come down, it was considered probable that the particular sample of the virus employed for inoculation was ineffective, or that all the animals used were refractory.

Experiment 3.—Macacus rhesus. Apr. 16, 5 p.m. Inserted tampon with virus into left naris. Apr. 17, 10 a.m. Removed tampon which was slightly blood-stained. Both nares washed with 1:1,000 chloramine-T solution in water, after which the dichloramine-T in oil was sprayed into the nostrils; twenty-five successive expulsions of the oily solution by hand pressure were made for each side. The spraying was repeated at 12 m. and 2, 4, and 6 p.m. Apr. 18. Spray every 2 hours from 8 a.m. until 6 p.m. Apr. 23. No symptoms had appeared until this date on which the animal showed excitement and paralysis of the left arm. Apr. 25. Animal generally paralyzed and prostrate. Apr. 27. Animal dying; etherized. The lesions in the central nervous organs were typical of poliomyelitis.

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[7] Dakin, H. D., and Dunham, E. K., A handbook on antiseptics, New York, 1917, 33.

[8] We are indebted to Dr. E. K. Dunham for the preparation used in our experiments and for advice as to the procedure to follow.

The conditions of the above experiment are severe. The tampon was left in the naris for 17 hours, and injury, as indicated by the blood staining, had been inflicted on the mucous membrane. In the next experiment the conditions are less severe, but the result was not essentially different.

Experiment 4.—Macacus rhesus. May 25. Oily dichloramine-T solution sprayed into nares at 2, 4, and 6 p.m. May 26, 8 a.m. Spray as before. 10 a.m. Inserted cotton plug carrying the virus into the left naris. 12 m. Removed tampon and applied the oily spray. Repeated the spray at 2, 4, and 6 p.m. May 29. Animal protects the left leg. June 3. Ataxic; excited. June 4. Extensive paralysis; prostrate. June 8. Dead. The spinal cord and medulla showed typical lesions of poliomyelitis.

The two experiments given do not, of course, show conclusively that the application of antiseptic fluids to the nasopharynx exercises no restraining influence on the multiplication and pathogenic action of the virus of poliomyelitis present there. The conditions of the experiments may well have been too severe to be readily comparable with those arising in man. But account must also be taken of the fact that monkeys sometimes resist the introduction of the virus by means of tampons without any aid to the defensive powers of the nasal membranes whatsoever. If so active an antiseptic agent as the chloramines may thus be ineffective, it would seem that even less could be expected of the indiscriminate chemical solutions often applied by sprays to the nasopharynx.

Blocking Infection via the Nasal Mucosa.

The mere plugging of the naris of a *Macacus rhesus* with a tampon carrying the active virus of poliomyelitis may not suffice to set up infection. The outcome is determined not only by the degree of activity of the sample of the virus, but also by the strength of the defensive mechanisms possessed by the particular animal. It has been shown that the nasal mucosa is protective, but it appears also that other and deeper mechanisms play a part in supporting or reinforcing the nasal defenses.

One of the deeper mechanisms is the meningeal-choroidal plexus complex, as pointed out by Flexner and Amoss.[9] The latter ascertained that an otherwise ineffective virus tampon could be rendered effective if the integrity of this complex was disturbed as, for example, by setting up within it a temporary chemical inflammation. Various mild chemical irritants suffice for this purpose, but sterile alien serum is highly satisfactory.[9] But the particular point which the next experiments illustrate is not so much the fact of the promotion of nasal infection by the method indicated as the means employed to block infection by way of the nares.

Flexner and Amoss[9,10] have shown also that the introduction of immune poliomyelitic serum by lumbar puncture into the subarachnoid space in monkeys suffices to prevent infection by way of the meninges, blood, naris, etc. The question which was now investigated was whether blocking of the nasal infection could be secured by means of the immune serum injected into the blood.

The first protocol given is that of an unsuccessful attempt to block infection by way of the nares by means of hexamethyleneamine. This drug does display some power of destroying or of inhibiting the development of the virus of poliomyelitis *in vivo* (Flexner and Clark[11]). But its inferiority to immune serum is great, and the experiment which follows can be viewed in that light and also as another control observation.

Experiment 5.—Macacus rhesus. Mar. 6. 2 cc. of sterile normal horse serum injected intraspinally. Mar. 7. 2 hour virus-carrying cotton tampon in naris. Mar. 9. 0.5 gm. of hexamethyleneamine in 10 cc. of water given by stomach tube twice a day. Treatment repeated daily for 6 days. Mar. 15. No symptoms. Mar. 16. Animal excited, somewhat ataxic, and protects the right arm. Mar. 22. Condition remained stationary until this date, when the paralysis involved both arms, back, and right leg. Mar. 28. Dead. The lesions present in the central nervous organs were typical of poliomyelitis.

Experiment 6. (a) Control.—Macacus rhesus. Feb. 8, 4 p.m. 2 cc. of normal horse serum injected intraspinally. Feb. 9. 2 hour nasal plug carrying the virus. Feb. 18. Arms paralyzed; back and right leg weak. Feb. 19. Pros-

[9] Flexner, S., and Amoss, H. L., *J. Exp. Med.*, 1917, xxv, 525.
[10] Flexner, S., and Amoss, H. L., *J. Exp. Med.*, 1914, xx, 249.
[11] Flexner, S., and Clark, P. F., *J. Am. Med. Assn.*, 1911, lvi, 585.

trate. Feb. 20. Etherized. The medulla and spinal cord showed typical lesions of poliomyelitis.

(*b*) *Test.—Macacus rhesus.* Feb. 8, 4 p.m. 2 cc. of normal horse serum intraspinally. Feb. 9. 2 hour nasal plug carrying the virus. On removal the plug was slightly blood-stained. Feb. 12. 5 cc. of immune monkey serum, obtained by pooling the blood from several animals, injected intravenously. This animal was kept under close observation for 2 months during which time no symptoms arose.

Experiment 7. (*a*) *Control.—Macacus rhesus.* May 14. 2 cc. of normal horse serum injected intraspinally. May 15. 2 hour virus-carrying plug in naris. May 16. 2 cc. of normal horse serum intraspinally. May 17. Repeated 2 hour nasal plug. May 18. 2 cc. of normal horse serum intraspinally. May 19. Repeated 2 hour nasal plug. May 23. Left facial and right arm paralysis. May 24. Prostrate. May 26. Etherized. The medulla and spinal cord showed typical lesions of poliomyelitis.

(*b*) *Test.—Macacus rhesus.* May 14. 2 cc. of normal horse serum injected intraspinally. May 15. 2 hour cotton plug carrying the virus in naris; 10 cc. of pooled monkey poliomyelitic immune serum intravenously. May 16. 2 cc. of normal horse serum intraspinally. May 17. 2 hour plug with virus in naris; 10 cc. of pooled monkey immune serum intravenously. May 18. 2 cc. of normal horse serum intraspinally. May 19. 2 hour plug with virus in naris; 10 cc. of pooled monkey immune serum intravenously. This animal developed no symptoms whatever during several months observation.

Experiment 8. (*a*) *Control.—Macacus rhesus.* June 4. 2 cc. of normal horse serum intraspinally. June 5. 2 hour cotton plug carrying virus in naris. June 6. 2 cc. of normal horse serum intraspinally. June 7. 2 hour virus-containing nasal plug. June 8. 2 cc. of normal horse serum intraspinally. June 9. 2 hour virus-carrying nasal plug. June 13. Animal weak; no definite paralysis. June 14. Dead. The medulla and spinal cord showed lesions of poliomyelitis.

(*b*) *Tests.*—Two *Macacus rhesus* monkeys. Procedure identical in both. June 4.2 cc. of normal horse serum intraspinally. June 5. 2 hour virus-carrying plug in naris. June 6. 10 cc. of pooled monkey poliomyelitic serum intravenously and 2 cc. of normal horse serum intraspinally. June 7. 2 hour virus-carrying nasal plug. June 8. 10 cc. of pooled immune serum intravenously and 2 cc. of normal horse serum intraspinally. June 9. 2 hour nasal plug with virus. June 10. 10 cc. of pooled immune serum intravenously. No symptoms of poliomyelitis developed during the period of observation which extended over several months.

The results of this series of experiments are clear and definite, and show conclusively that even under highly favorable conditions of susceptibility the infection of monkeys with the virus of poliomyelitis applied to the nasal mucosa can be prevented by passive immuniza-

tion of the body by way of the general blood. By this means, therefore, the effective passage of the virus from the nasal mucosa to the central nervous organs can be blocked.

The precise point at which the blocking takes place is in doubt. Two or three possibilities exist: First, after passage of the virus into the blood itself *en route* to the brain and spinal cord. This possibility is small, inasmuch as all the available evidence is against the virus of poliomyelitis reaching the nervous organs from the general blood (Flexner and Amoss[10]). Next, in the nasal mucosa itself, as the blood carrying the immune serum circulates through. There is no way of readily affirming or excluding this idea. It seems improbable, however, that the virus in the interstices of the tissue and especially in the olfactory nerves themselves would have been brought under sufficient influence of the immune serum to have been prevented from multiplying and inducing infection. Third, in the central nervous system itself. In our opinion, this last is the more probable site. All the conditions of the experiments are favorable to the passage of a certain amount of the immune serum into the subarachnoid space. Under the influence of the chemical irritant, both the choroid plexus and the meningeal vessels become more pervious to protein substances and hence to the immune bodies (Flexner and Amoss[12]). Once the immune bodies reach the subarachnoid space and mingle with the cerebrospinal fluid, infection with the virus of poliomyelitis injected into the blood or meninges themselves is prevented.

There is little doubt that the quantity of immune serum employed in some of the experiments was excessive. Experiment 6 shows that a single intravenous injection suffices to block the development of the virus. But in the experiments in which repeated nasal tampons were employed and in which several injections of normal horse serum were given, it was deemed advisable to maintain the concentration of the immune serum in the general blood. After all, the answer sought by the experiments was whether under conditions of severe inoculation and a highly favorable degree of susceptibility of the animal tested, blocking of the infection could be secured surely by way of the passively immunized general blood.

[12] Flexner, S., and Amoss, H. L., *J. Exp. Med.*, 1917, xxv, 499; 1918, xxviii, 11.

SUMMARY.

1. The experiments given in this paper, notwithstanding their seeming diversity, relate to the conditions underlying the states of susceptibility and refractoriness to infection with the virus of poliomyelitis applied to the nasal mucosa.

2. Certain monkeys are highly refractory to inoculation *via* the nares with the virus of poliomyelitis, apparently in virtue of a power possessed by the nasal mucous membrane to destroy or otherwise render ineffective the virus applied to it.

3. This property of the nasal mucosa appears to be distinct from any specific protective substance active upon the virus which may occur in the blood.

4. An effective nasal mucous membrane prevents the passage of the energetically applied virus to the brain and spinal cord.

5. The virus of poliomyelitis energetically applied to the nasal mucosa will survive for an undetermined period of time upon an ineffective, but for a relatively brief period of time upon an effective membrane.

6. The protective power possessed by the nasal mucosa is not in itself adequate to prevent infection with the virus introduced upon it, since slight injury to such independent structures as the meningeal-choroid plexus complex favors the passage of the virus from the nose to the central nervous organs.

7. The normal nasal mucosa is, therefore, an invaluable defense against infection with the virus of poliomyelitis; and the number of healthy and chronic carriers of the virus is probably determined and kept down through the protective activities of this membrane.

8. Antiseptic chemicals applied to the nasal mucosa upon which the virus has been deposited exhibit no great protective action and are of doubtful value. Indeed, it is not impossible that to the extent to which they may affect unfavorably the destructive properties of the nasal mucosa, they may be even objectionable.

9. Infection with the virus of poliomyelitis applied to the nasal mucosa under conditions favorable to the extension to the central nervous organs and multiplication there may be blocked or prevented by the injection of poliomyelitic immune serum into the blood.

While the exact manner and site of attack of the immune serum upon the virus is somewhat conjectural, when all the available data are considered it seems probable that the meeting place of the virus and immune serum is in the subarachnoid space.