PERSISTENCE OF THE VIRUS OF POLIOMYELITIS IN THE NASOPHARYNX.

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This paper is intended as a contribution to the epidemiology of poliomyelitis. Present knowledge places that disease among the infections in which the specific cause is carried in the nasopharynx, and present belief is to the effect that the virus is conveyed from one person to another through the medium of the nasopharyngeal secretions. Indeed, the virus has been detected in these secretions by the inoculation test in three sets of conditions; (1) coincidentally with an attack of the disease incited by it; (2) a considerable period after the attack of the acute disease has abated; and (3) in healthy persons who have been in contact with cases of poliomyelitis.

This determination having been made, detailed information covering the frequency with which and the conditions under which the virus can be discovered in the nasopharynx is greatly to be desired. This information is essential to the working out of the principles of the method of control of epidemic poliomyelitis.

Account must be taken, at the outset, of the difficulties surrounding, at the present time, the demonstration of the virus. The only reliable means of its detection is the inoculation test. When an active virus is injected intracerebrally or even otherwise into monkeys a train of symptoms tends to be set up in these animals which closely resembles the symptoms present in man, the subject of poliomyelitis. Indeed, the analogy is even closer than this, because the histological changes arising in the central nervous organs of the monkeys are an exact counterpart of those present in fatal cases of the disease in human beings. We can now state unreservedly that when typical symptoms appear in the inoculated monkeys typical lesions will occur in their nervous organs.

There is no division of opinion among observers regarding the clinical and histological evidences of poliomyelitis in monkeys in instances in which the experimental disease conforms to frank cases of poliomyelitis occurring in man. There is, however, lack of agreement respecting a less typical experimental condition described by Kling, Pettersson, and Wernstedt.[1] The questions raised by these experimenters, who have introduced entirely new criteria into the subject, are of far reaching significance, because they affect the character of the evidence acceptable as indicating the presence of the virus of poliomyelitis in the nasopharynx. This aspect of the general subject will come under consideration in connection with the experiments to be reported and discussed in the present paper.

Review of the Literature.

In order that the experiments to be described may take their proper place among the studies dealing with the mode of infection in poliomyelitis, a brief review of the literature, especially of that stressing the nasopharynx as the portal of entry and of exit of the virus, will be given.

That the virus, the existence of which at that time was merely suspected, is communicated by personal contact was the thesis which Wickman's[2] studies served to emphasize. But until Landsteiner and Popper[3] communicated the disease to monkeys no more precise definition of the mode of infection could be given. It will be recalled that Landsteiner and Popper originally conveyed the infection from man to monkey by the intraperitoneal route of inoculation and were thus able to reproduce poliomyelitis only in the first series of injected animals. The employment of the intracerebral route of inoculation by Flexner and Lewis[4] led to the discovery that the virus could be transmitted from monkey to monkey through an indefinite series, in course of which its activity or virulence for monkeys increased many fold. Moreover, still other portals of experimental infection were successively disclosed, such as the large nerves, subcutis, subarachnoid space, nasal mucosa, eye, and, although with far greater difficulty, the general blood.

[1] Kling, C., Pettersson, A., and Wernstedt, W., *Communications Inst. méd. État Stockholm*, 1912, iii, 4.

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

[3] Landsteiner, K., and Popper, E., *Z. Immunitätsforsch., Orig.*, 1909, ii, 377.

[4] Flexner, S., and Lewis, P. A., *J. Am. Med. Assn.*, 1909, liii, 1639.

In addition, it was found by Leiner and von Wiesner[5] that the virus would penetrate the mucous membrane of the gastrointestinal tract of monkeys in which the motions of the digestive organs were for a time arrested by means of opium. It is noteworthy that aside from the intracerebral route the other modes of infection give more or less inconstant results, and that the external portal which is most favorable to the attack of the virus is the nasal mucous membranes.

The particularly favorable position afforded by the nasal route to infection is a fact the demonstration of which was indicated by circumstances surrounding the epidemiology of poliomyelitis in man. Flexner[6] pointed out that the not infrequent confusion of epidemics of poliomyelitis with those of cerebrospinal meningitis argued for a similarity not only in certain cardinal symptoms but also in the conditions under which the two diseases arise. Thus each disease attacked by preference infants and young children, although not wholly sparing older children and adults. Usually a single case appears in a family or home, but sometimes two cases, and less often three or more appear. These resemblances came to be further emphasized by the detection of the meningococcus carrier on the one hand and the demonstration of the ambulant and abortive cases of poliomyelitis on the other. Indeed, the single striking epidemiological point of difference relates to the seasonal prevalence which for poliomyelitis tends to be summer and autumn and for cerebrospinal meningitis winter and spring. Even this distinction is not absolute; we now know that winter epidemics of poliomyelitis occur, and circumstances favoring epidemics of cerebrospinal meningitis arise in the warm months of the year. The meningococcus carriers have been known for more than a decade; it is important to ascertain whether corresponding poliomyelitic virus carriers exist.

Experience having shown that a highly potent poliomyelitic virus may be secured by successive passages through monkeys, the way was opened for an experimental study of the nasopharynx as the portal of its entry into and exit from the body. The filterability of the virus, moreover, permitted its entire separation from the bacteria present in that locality. Flexner and Lewis,[7] therefore, crushed and extracted in salt solution the excised nasal mucous membrane removed from monkeys which succumbed to intracerebral inoculation of the virus and after filtration through a Berkefeld filter injected the fluid into other *rhesus* monkeys, thus inducing infection and paralysis. The corollary to this experiment, namely the infection of monkeys by direct inoculation of the virus into the nasal mucosa, was quickly supplied by Landsteiner and Levaditi.[8] The last test has been carried out successfully in several ways: by applying the virus to the abraded mucous membrane, by introducing it upon cotton tampons, and

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[5] Leiner, C., and von Wiesner, R., *Wien. klin. Woch.*, 1910, xxiii, 91.

[6] Flexner, S., *J. Am. Med. Assn.*, 1910, lv, 1105.

[7] Flexner, S., and Lewis, P. A., *J. Am. Med. Assn.*, 1910, liv, 535.

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

finally by Leiner and von Wiesner[9] by merely pencilling the mucosa without causing any demonstrable lesion whatever. It should be stated, also, that one or the other of the modes of inoculation succeeds or not according, apparently, to the initial potency of the virus and the species of monkey chosen. Finally, some experimental evidence has been adduced to show that the nasal membranes are better suited to convey the virus than the tonsillar tissue. Levaditi and Danulesco[10] made submucous injections into the two regions and induced infection in the one case and not in the other.

The virus of poliomyelitis exists not only in the tissues but also in the secretions of the nasopharynx of monkeys, as shown by Landsteiner, Levaditi, and Danulesco,[11] who inserted cotton plugs into the nares of paralyzed animals and found that after several hours the absorbed fluid sufficed, when injected, to infect other monkeys; while Thomsen[12] ascertained also that merely rubbing the mucosa with the tampon was sufficient to incite infection. In this connection mention may be made of the fact that Landsteiner and Levaditi[8] consider that they have traced the passage of the virus along the olfactory nerves to the olfactory lobe of the brain, and Flexner and Clark[13] likewise have found the olfactory lobes infective, and the spinal cord and medulla non-infective, 48 hours after an intranasal inoculation of the virus. The last observation indicates that the penetration of the virus from the surface to the interior of the nasal membrane and thence to the brain occurs quickly, a point borne out by certain experiments made with antiseptic drugs to be reported elsewhere.

The foregoing experimental results provide a basis for considering the inoculations which have been made directly with human materials derived from the nasopharynx. These materials may be divided into three classes as follows: (1) washings, (2) tissues (tonsils and adenoids) removed during life, and (3) tissues (tonsils, pharyngeal mucosa, nasal mucosa) recovered after death. As regards the first, numerous tests have been made. It will be of doubtful value to note in this place the failure to induce infection. The negative results are, in the light of present knowledge, without real significance. The limits of activity for monkeys of virus immediately obtained from human beings are quickly reached. Hence the dilute filtered washings of the nasopharynx could be expected to succeed only rarely. We have ourselves failed to incite infection with a filtrate prepared from the spinal cord of human cases of poliomyelitis when the unfiltered suspensions were active.

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[9] Leiner, C., and von Wiesner, R., *Wien. klin. Woch.*, 1910, xxiii, 323.

[10] Levaditi, C., and Danulesco, V., *Compt. rend. Soc. biol.*, 1912, lxxii, 606.

[11] Landsteiner, Levaditi, and Danulesco, *Compt. rend. Soc. biol.*, 1911, lxxi, 558.

[12] Thomsen, O., *Berl. klin. Woch.*, 1912, xlix, 63.

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

However, Kling, Pettersson, and Wernstedt[1] have brought indubitable proof that washings obtained from the nasopharynx both during life from typical cases of poliomyelitis and after death from persons who succumbed to the disease, may carry the virus in a form and quantity capable of inciting infection in monkeys. If we confine ourselves strictly to the instances in which the washings conveyed typical experimental poliomyelitis, it may be said that while the number of failures to infect exceeds the successful inoculations, yet the latter comprise a convincing series. While opinion on this point can hardly be divided, the same cannot be stated for the interpretation which they place upon the results of the inoculation of washings from abortive cases, mere contacts, and from recovered persons, to which fuller reference will be made presently.

The first to detect the virus in tonsillar and pharyngeal mucosa obtained from a fatal case of poliomyelitis in man and thus to confirm Flexner and Lewis' experiments on the monkey were Landsteiner, Levaditi, and Pastia.[14] Flexner and Clark[15] about the same time reported several similar successful inoculations. The latter authors drew attention to the fact that when they injected filtrates of the tonsillar and nasal tissues infection failed; but when the same materials were rendered bacteria-free with 0.5 per cent phenol and inoculated as suspensions infection followed.

Thus far all the successful inoculations of the virus of poliomyelitis noted upon monkeys have been secured with materials obtained from recent typical cases of the disease in man or the experimental reproduction of that disease in those animals. An experimental answer to the important question of the period of the survival of the virus in the nasal and buccal mucosa was first successfully attempted by Lucas and Osgood,[16] who were still able to determine its presence in the experimentally infected monkey at the expiration of nearly 6 months.

Wickman's important studies[2] pointed unmistakably to the part played by so called abortive cases of poliomyelitis and healthy carriers of the microbic cause in disseminating poliomyelitis. The discovery of the experimental disease in monkeys led to the search for the virus in the nasopharynx of those two classes of persons. By far the most extensive inoculation tests published are those of Kling, Pettersson, and Wernstedt.[1] They even go beyond the phases of the subject indicated and include an investigation of the period of survival of the virus in the nasopharynx. Unfortunately this highly important study is marred by the fact that the criteria of the experimental disease in monkeys which the authors came to adopt do not conform to those which all experimenters accept as distinctive of poliomyelitis in the monkey. The point is an essential one. A close review of their protocols has led us to doubt the validity of their conclusions.

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[14] Landsteiner, K., Levaditi, C., and Pastia, C., *Semaine méd.*, 1911, xxxi, 296.

[15] Flexner, S., and Clark, P. F., *J. Am. Med. Assn.*, 1911, lvii, 1685.

[16] Lucas, W. P., and Osgood, R. B., *J. Am. Med. Assn.*, 1913, lx, 1611.

The inoculation experiments which form the basis of this paper deal with this aspect of the subject.

Meanwhile, it has been shown through inoculation in one instance by Flexner, Clark, and Fraser[17] that healthy persons may harbor the virus in their nasal and buccal secretions. The instance in which the demonstration was made related to the parents of a young child acutely ill with poliomyelitis. The contact, therefore, between the carriers and the case was an intimate one. This unquestioned observation was followed by a similarly conclusive one by Kling and Pettersson,[18] who, it may be remarked in passing, attribute failure to incite typical clinical and anatomical effects in their earlier experiments to the injections of insufficient amounts of the virus. Finally, Taylor and Amoss[19] have recorded two instances—one an abortive or non-paralytic case of poliomyelitis and the other a healthy child who subsequently developed frank paralysis—in which the concentrated nasopharyngeal washings inoculated into monkeys induced the typical experimental disease.

Nature of Materials and Interpretation of Experiments.

The experiments which we are reporting were made with human materials of two sorts; namely, tonsils and adenoids removed during life and tonsils and pharyngeal mucosa excised after death. The object in all the experiments was to ascertain the presence or absence of the virus as far as this could be determined by the inoculation test. In this way it was hoped to throw light on the persistence of the virus in the nasal and buccal membranes and their discharges.

The outcome of the tests is not so consistent as we would wish. But the inconsistency which will appear is instructive, for not only does it point the limitations of the inoculation test, but it helps clear up, at the same time, the more indefinite clinical effects which sometimes follow the injections of supposedly virus-containing materials. It may be mentioned here that we did not confirm the experiments of Kling, Pettersson, and Wernstedt respecting an atypical variety of poliomyelitis in the monkey which they finally came to attribute to the inoculation of too little virus-containing washings. Our tests, as will appear, were carried out with tissues and with quantities that should have sufficed for infection had the virus been present in amounts to be expected and in an active state.

[17] Flexner, S., Clark, P. F., and Fraser, F. R., *J. Am. Med. Assn.*, 1913, lx, 201.

[18] Kling, C., and Pettersson, A., *Deutsch. med. Woch.*, 1914, xl, 320.

[19] Taylor, E., and Amoss, H. L., *J. Exp. Med.*, 1917, xxvi, 745.

The last considerations are pertinent because of the striking contrast in the results obtained with human tissues removed either by operation or post mortem. The obvious deduction is to the effect that the upper respiratory mucosa carries far more active virus in the latter instance. All the postmortem tissues inoculated by us were derived from acute cases. Analogy with other acute infections leads to the assumption that in the fatal instances there is more active multiplication of the virus than in cases which go on to recovery. This is perhaps the simplest interpretation to put upon our tests.

The discrepancy seems to illuminate the question as to the time of maximum infectivity in epidemic poliomyelitis. As far as observations made during the great epidemic, which prevailed in New York State and elsewhere in 1916, can be construed, the implication is to the effect that this period is relatively brief and is greatest early in the disease. It cannot be said, however, that this point is definitely established. As a matter of fact, the literature contains records which directly controvert this idea. The matter is perhaps one not to be settled absolutely but rather to be placed on a relative basis. What we need first to know are the conditions under which the virus of poliomyelitis can be definitely determined to be present in the nasopharynx. This question we have endeavored to answer by a study of tissues removed post mortem and of hypertrophied tonsils and adenoids extirpated during life from persons who had suffered a typical attack of poliomyelitis some weeks or months earlier. In comparing the two classes of tissue, attention must be given the fact that the former, that is the materials removed post mortem, come from cases earlier in the course of the disease.

The results of the inoculation of the tonsillar and adenoid tissues removed during life have also an important bearing on the question of the chronic carriage of the virus upon the nasopharynx. The test of Lucas and Osgood[16] showed that the virus may be demonstrable on the nasopharyngeal mucous membrane of the monkey as long as 5 months after infection had been experimentally induced. Flexner and Clark[20] demonstrated it in the nasopharyngeal mucosa of a monkey surviving the paralysis more than 4 weeks and failed, in the

[20] Flexner, S., and Clark, P. F., *J. Am. Med. Assn.*, 1911, lvi, 585.

same animal, to detect it in the spinal cord. The large series of tests with washings from human cases made by Kling, Pettersson, and Wernstedt[1] on the basis of which they believe they have shown chronic carriage of the virus to be common, have never been subjected to a searching control with human tissues. Our experiments with surgically removed tonsils and adenoids may be regarded as covering this point. It happens, indeed, that our results are quite opposed to those of the Swedish observers and teach rather that the chronic carriage is, at least, exceptional. On the whole the epidemiological data are in conformity with our experimental results.

The Swedish authors studied nine convalescents over a maximum period of 7 months on the basis of which they formulated the following deductions: "that the secretion from the mucous membranes of the mouth and intestine of persons who have recovered (from poliomyelitis) has had the power of infecting monkeys still several months, in one case 204 days (nearly 7 months), after the onset of the illness, giving rise to an experimental poliomyelitis with fatal issue . . . . It was only in one case that we did not succeed in demonstrating the presence of the virus after the comparatively short time of 30 days."[21]

In interpreting this statement and in considering the discrepancy with our studies, it is necessary to take into account the immediately succeeding paragraphs:

"During the time occupied by the investigations, the virus had changed its character, so that it no longer caused inflammations with cellular exudations. Instead of this the degeneration of the nerve cells, the changes of the glia cells and the neurophagocytosis caused by the enlarged glia cells have been the characteristic changes. They have thus been of the same type as those appearing in the monkeys injected with secretions from abortive cases, and virus carriers, changes which . . . . we consider ourselves justified in assuming to be due to a less virulent virus. *The experiment also shows, that the microbe rather quickly—already after 8–14 days—loses its power of causing inflammatory exudations in the inoculated animals. This fact is of very great importance from a practical point of view since it perhaps gives us the right to assume that the virus, possibly rather soon after the termination of the acute stage, gets weaker.*"[22] (Author's italics.)

We propose now to present our results in the form of tabulations with such discussion as seems called for. The postmortem tissues

[21] Kling, Pettersson, and Wernstedt,[1] p. 159.

[22] Kling, Pettersson, and Wernstedt,[1] pp. 159–160.

employed for inoculation were obtained very soon after the death of the patient, when they were either inoculated after a short interval, or placed in the preserving fluids, which consisted of 50 per cent sterile glycerol or 0.5 per cent phenol. The tissues removed surgically were put immediately into the preservative fluids. The inoculations were made at leisure. The glycerolated or phenolized specimens were suspended and injected partly intracerebrally and partly intraperitoneally. Previous experiments had shown that the weak phenol destroyed associated bacteria in the tissues without acting appreciably upon the virus of poliomyelitis.[15] In this respect the phenol was superior to the glycerol, which also was without observed injurious action on the virus, but which removed the bacteria much more slowly.

Histological studies were made with the nervous organs of all monkeys succumbing to or after the inoculations. All the animals which developed typically clinical poliomyelitis showed characteristic histological lesions in the spinal cord and medulla and intervertebral ganglia. In none of the animals in which the clinical symptoms were dubious did we find histological lesions either resembling those of typical poliomyelitis or corresponding with the degenerative and peculiar neurophagocytic ones which Kling, Pettersson, and Wernstedt[1] describe and attribute to the action of specifically weakened poliomyelitic virus.

Examination of Table I brings out the fact that both the tonsils and nasal mucosa of fatal early cases of poliomyelitis are infectious for monkeys. No real distinction can be drawn between the two sets of materials as regards their infectivity. The fact that now one tissue from an individual succeeds while the other fails is probably as ascribable to differences in the susceptibility of the individual monkeys as to irregularity in distribution of the virus. The inferiority of filtrates to emulsions or suspensions of the tissues is manifest. It is clear also that phenol is a favorable medium for preparation of the tissues for inoculation, as it tends quickly to destroy bacteria associated with the virus without materially injuring the virus itself. Probably the 0.5 per cent phenol is only relatively innocuous for the virus, since in the instance of H. K., in which two *rhesus* monkeys were inoculated,

TABLE I.

Human Tissues Obtained post Mortem.

Case.	Day of disease.	Monkeys inoculated.	Material injected.	Symptoms.	Histology.	Remarks.
J. A. R.	6	2 *M. rhesus.*	Berkefeld filtrate of tonsils and pharynx mucosa.	None.		Filtrate of spinal cord negative; emulsion positive.
R. P.	7	2 *M.* "	(1) Emulsion of tonsils.	Tonsils none.	Typical lesions from nasal mucosa.	Glycerol preservation. Nasal mucosa positive. Tonsils negative.
			(2) Emulsion of nasal mucosa.	Nasal mucosa typical.		
M. K.	6	2 *M.* "	Heim filtrate of tonsils and nasal mucosa separately.	None.		Glycerol preservation.
J. C.	3	1 *M.* " 1 *M. cynomolgus.*	Heim filtrate of tonsils and nasal mucosa separately.	Nasal mucosa none. Tonsils typical.	Typical lesions from tonsils.	Glycerol preservation. Tonsils positive in *M. cynomolgus.* Nasal mucosa negative.
B. T.	6	1 *M. rhesus.*	Emulsion of tonsils.	Typical.	Typical lesions.	Phenol preservation. Reinoculation of spinal cord positive.
J. G.	7	1 *M.* "	" " "	"	" "	Phenol preservation.
S. A.	9	2 *M.* "	" " " and pharynx mucosa separately.	None.		" "

H. K.	6	2 *M. rhesus*	Emulsion of tonsils.	Typical, with recovery.		Phenol preservation in successive quantities to remove resistant bacteria.
G. G.	6	3 *M.* "	" " "	Typical.	Typical lesions.	Phenol preservation.
A. K.	8	1 *M.* "	" " "	None.		
J. W.	4	1 *M.* "	" " " and pharynx mucosa.	"		
G. C.	?	1 *M.* "	Heim filtrate of tonsils.	Indefinite.	Tuberculosis.	
B. H.	10	1 *M.* "	Heim filtrate of tonsils.	None.		
A. S.	3	1 *M.* "	Emulsion of tonsils intrasciatic and intraperitoneal.	"		Glycerol preservation.

TABLE II.

Human Tissues Removed Surgically.

Case.	Day of disease.	Monkeys inoculated.	Material injected.	Symptoms.	Histology.	Remarks.
H. G.	8	1 *M. rhesus.*	Berkefeld filtrate of tonsils and adenoids.	None.		
I. P.	13	1 *M.* "	Emulsion of tonsils and adenoids.	Gradual emaciation with diarrhea. Died 15th day.	No lesions in nervous organs.	Phenol preservation.
A. S.	13	1 *M.* "	Heim filtrate of tonsils and adenoids.	Indefinite. Recovered.		
J. P.	13	1 *M.* "	Emulsion of tonsils and adenoids.	None.		Glycerol and phenol preservation.
H. B.	14	1 *M.* "	Emulsion of tonsils and adenoids.	Emaciation. Died 15th day.	Tuberculosis.	Phenol preservation.
C. M.	17	1 *M.* "	Filtrate of tonsils and adenoids.	None.		" "
H. N.	18	2 *M.* "	Emulsion of tonsils and adenoids.	"		
C. U.	19	2 *M.* "	Berkefeld filtrate of tonsils and adenoids.	"		
J. A.	19	2 *M.* "	Emulsion of tonsils and adenoids	Indefinite.	Tubercles in spinal cord.	Glycerol preservation. Succumbed to tuberculosis.
F. S.	22	1 *M.* "	Berkefeld filtrate of tonsils and adenoids.	None.		

J. W.	25	2 *M. rhesus.*	Emulsion of tonsils and adenoids.	None.		Glycerol preservation.
H. F.	25	2 *M.* "	Emulsion of tonsils and adenoids.	(1) None. (2) Pressure. Symptoms from which recovered. Then indefinite. Death on 12th day.	No lesions suggestive of poliomyelitis. No degeneration of nerve cells in medulla, cord, or intervertebral ganglia, and no cellular infiltration.	Glycerol preservation. Small sterile cyst at point of inoculation.
F. S.	27	2 *M.* "	Emulsion of tonsils and adenoids.	None.		Glycerol preservation.
L. F.	27	2 *M.* "	Emulsion of tonsils and adenoids.	"		Glycerol preservation.
A. L.	27	1 *M. cynomolgus.*	Emulsion of tonsils and adenoids.	"		Phenol preservation.
C. J.	28	1 *M. rhesus.*	Filtrate of tonsils and adenoids.	"		
H. F. S.	28	2 *M.* "	Berkefeld filtrate of tonsils and adenoids.	"		
A. S.	35	1 *M.* "	Berkefeld filtrate of tonsils.	"		
G. C.	35	1 *M.* "	Emulsion of tonsils and adenoids.	"		Phenol preservation.
T. P.	39	1 *M.* "	Filtrate of tonsils and adenoids.	Indefinite. Recovered.		" "

TABLE II—*Concluded.*

Case.	Day of disease.	Monkeys inoculated.	Material injected.	Symptoms.	Histology.	Remarks.
J. O'B.	49	2 *M. rhesus.*	Emulsion of tonsils and adenoids.	(1) Died suddenly 8th day. (2) Gradually lost strength. Died 15th day.	(1) No lesions in nervous organs. (2) No lesions in nervous organs.	Glycerol preservation.
D. M.	50	2 *M.* "	Emulsion of tonsils and adenoids.	(1) None. (2) Indefinite.	(2) No lesions in nervous organs.	Glycerol preservation 5 days. Cyst in cerebrum at point of inoculation.
F. McK.	50	2 *M.* "	Emulsion of tonsils and adenoids.	(1) Typical. Died 41st day. (2) None.	(1) No lesions in nervous organs.	Phenol preservation.
T. M.	90	1 *M.* "	Emulsion of tonsils and adenoids.	None.		Phenol preservation. Reinoculation with emulsion.
J. W.	90	1 *M.* "	Emulsion of tonsils and adenoids.	Died suddenly $2\frac{1}{2}$ mos. after 1st and 19 days after 2nd inoculation.	No lesions in central nervous organs.	Phenol preservation. Reinoculation with emulsion.
M. K.	120	1 *M.* "	Emulsion of tonsils and adenoids.	Indefinite after 2nd inoculation. Recovered.		Phenol preservation. Reinoculation with emulsion.
E. S.	150	1 *M.* "	Emulsion of tonsils and adenoids.	Indefinite after 2nd inoculation. Recovered.		Phenol preservation. Reinoculation with emulsion.

and in which the tissues employed were put through three successive solutions of the phenol to render them approximately bacteria-free, a milder form of poliomyelitis developed. The number of tests made is insufficient to account for certain obvious discrepancies as, for example, the failure of tissues derived from cases dying on the 3rd and 4th day of the disease to infect. Doubtless the fact that only one monkey was used in each instance for inoculation had something to do with the failures. But at times the supply of monkeys was too small and precarious to permit of more being tested. Experience with inoculations of the spinal cord and medulla from human cases has shown us that the failure to incite infection in a single *rhesus* monkey does not indicate lack of power to infect still other individual animals of the same species.

The deduction from the tests summarized in the table is to the effect that the nasal and pharyngeal mucosæ of persons succumbing to poliomyelitis during the 1st week or 10 days of the disease probably regularly contain the poliomyelitic virus.

A glance at Table II shows at once a fundamental difference in the results of inoculating the specimens removed surgically and those obtained at autopsy. The extent of the distinction is not greatly lessened by the probability that the tests carried out with the surgical tissues err on the side of negativity. That certain of the surgical specimens contained virus in some amount we think most probable. But the essential fact remains that under the conditions of the experiments they regularly failed to incite infection and paralysis in the monkeys.

The two sets of tissues, those removed post mortem and those removed during life, differed especially in one respect; namely, that the former came from cases of poliomyelitis in the 1st week and the latter later in the course of the disease. We are inclined to attribute to this circumstance, with which may be associated the tendency for microorganisms to multiply more freely in the last hours of life, the great differences in effects observed.

In order to favor the induction of infection with the specimens removed relatively late after recovery from the acute symptoms of poliomyelitis, the reinoculation method of Flexner, Noguchi, and

Amoss[23] was also employed. By this method it is possible to convert a subminimal infective dose of the virus into an effective dose. But still no success was achieved with the surgical tissues.

DISCUSSION.

The epidemiology of poliomyelitis has still to be worked out in detail, as many factors governing the spread of the disease remain to be discovered. That the virus or microbic cause is communicated by personal contact is now generally admitted. That the virus occurs in the nasopharynx, which constitutes the chief locus of ingress and egress to and from the body is also conceded. The fact that the virus has been, if rarely, detected in healthy persons who have been in intimate contact with early cases of poliomyelitis, and even in certain individuals who have recovered from the acute effects of the disease, has led to the generalization that like some other diseases of bacterial origin, and notably epidemic meningitis, healthy and chronic carriers of the virus are frequent. This view has received its main support from Kling, Pettersson, and Wernstedt, whose studies we have discussed. A critical analysis of the basis of their contention fails, however, to carry conviction, and the doubt which has arisen as to the true interpretation of their results is deepened, we think, by our more searching tests.

The results of the experiments reported in this paper conform closely with clinical experience in the United States, at least, and especially with the observations made by epidemiologists in the course of the wide epidemic in New York State and elsewhere during the summer and autumn of 1916. The conclusion reached at that time was to the effect that the communicability of the disease was a phenomenon chiefly of the early stages, while the frankly paralyzed person and the convalescent were to be feared much less. In our experiments infection was secured with tissues obtained during the 1st week, approximately, of the disease but not at the later periods.

CONCLUSIONS.

The virus of poliomyelitis occurs in the nasopharynx of man and monkeys.

[23] Flexner, S., Noguchi, H., and Amoss, H. L., *J. Exp. Med.*, 1915, xxi, 91.

In man it has been detected by the inoculation test in washings from acute cases, rarely in similar washings from healthy contacts, in the nasopharyngeal tissues obtained from fatal cases in the 1st week of infection, but rarely, if ever, from nasopharyngeal tissues removed surgically at later periods in the course of the disease.

In monkeys, also, the virus has been detected in the secretions from acute experimental infections, in the nasopharyngeal tissues derived from early cases, and rarely from cases several weeks or months after recovery from the acute symptoms.

The inoculation of tonsils and adenoids obtained from cases of undoubted poliomyelitis either yielded definite results in the form of typical paralysis and histological lesions in the central nervous organs of the monkeys injected, or no symptoms or lesions which could be confounded with poliomyelitis. The indefinite symptoms and atypical lesions described in a certain class of inoculated animals by Kling, Pettersson, and Wernstedt were not encountered in our experiments.

The deduction from the experiments reported is to the effect that the virus is regularly present in the nasopharynx in cases of poliomyelitis in the first days of illness, and especially in fatal cases; that it diminishes relatively quickly as the disease progresses, except in rare instances; and that it is unusual for a carrier state to be developed. Hence the period of greatest infectivity of patients would appear to be early in the disease, which is probably the time at which communication of the virus from person to person takes place.

Available evidence proves that healthy carriers of the virus occur. We do not, however, possess data which indicate the frequency with which carriage arises. The fact that even after a severe and wide epidemic, such as occurred in the United States in 1916, the disease may virtually disappear within 2 or 3 years, points to the probability that enduring carriers of the active virus, whether healthy or chronic, are of exceptional occurrence.

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