NEUTRALIZATION OF THE VIRUS OF POLIOMYELITIS BY NASAL WASHINGS.

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The occurrence of the infectious microorganism of poliomyelitis—the virus, so called—in the mucous membranes of the nasopharynx and in their secretions is now firmly established.[1,2] Not only may the virus be demonstrated by inoculation tests during the acute period[3] of the disease, but it is known to persist there in some cases for many months after convalescence[4,5] and, conversely, it has been detected in certain instances in the washings from the nasopharynx of healthy persons who have been in intimate contact with the acutely ill.[6] Finally, the fact has been determined by experiment that when the virus is introduced directly into the central nervous tissues wherein it multiplies, it also appears in the mucous membranes of the nose and throat. These facts indicate that the nasopharyngeal mucous membranes play an important part in the pathology of epidemic poliomyelitis; and the weight of opinion today is to the effect that the ingress and egress of the virus take place by way of these structures.

One of the most important questions arising out of the data presented above is that relating to the so called healthy carriers of the

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

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

[3] Flexner, S., and Clark, P. F., *J. Am. Med. Assn.*, 1911, lvii, 1685. Landsteiner, Levaditi, and Danulesco, *Compt. rend. Soc. biol.*, 1911, lxxi, 558.

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

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

[6] Flexner, S., Clark, P. F., and Fraser, F. R., *J. Am. Med. Assn.*, 1913, lx, 201. Kling, C., and Pettersson, A., *Deutsch. med. Woch.*, 1914, xl, 320.

virus, and for two main reasons. The healthy carriers may be the means of transporting the virus to other persons less resistant who may develop poliomyelitis; or the carrier, healthy when first contaminated, may subsequently develop the infection. At present the means at our disposal for studying the subject of virus carriers are so imperfect that no adequate notion of their number and distribution can be obtained. As long as the inoculation of monkeys with the washings from the nasopharynx must be relied upon to furnish this information, complete knowledge cannot be acquired.

There is, however, another fact which may prove to be significant. Assuming that during the prevalence of epidemics, many persons become contaminated with the virus, the question arises whether this condition need necessarily be either a menace to the contaminated person himself or to others. The answer to this question may lie in the reaction of the secretions of the nasopharyngeal mucous membranes to the virus present upon them. It is possible that in one person the secretions do not exercise a harmful action on the virus, while in another they do. This injurious action upon the virus may be of the nature of a protection to the individual contaminated as well as to the public in general.

It has often been observed that washings made from the nasopharynx may be ineffective when introduced into monkeys, and the lack of power to cause infection has been attributed to insufficient quantity or low infective power of the virus believed to be contained in the secretions removed. No note has been taken of the possibility that the washings are ineffective because the secretions of the mucous membrane are destructive or neutralizing to the virus of poliomyelitis. While this possible action may affect the inoculation tests in cases of acute poliomyelitis, it would be far more likely to be operative in the supposed carrier because of the small amount of virus and the probability of diminished virulence in the latter. Because of these considerations, a series of experiments was carried out to determine (1) the smallest quantity of a standard virus which can be detected in washings, and (2) the action of the washings of different persons upon the virus itself.

Reference has already been made to the fact that nasal washings, of contacts especially, have in a few instances produced polio-

myelitis when injected into monkeys. As the virus obtained directly from human beings possesses low virulence for monkeys, and is injected greatly diluted, the small number of successful inoculations is significant. Tests were made to determine the effect of concentration of washings on the activity of the virus. Amounts of virus which would certainly produce the infection if injected directly were added to a filtered washing fluid obtained from persons not having been exposed to the infection. The mixtures were separately reduced to small volume *in vacuo* at low temperatures and injected into monkeys. The results obtained were variable, for reasons which at first were not obvious, but the tests nevertheless showed that the filtered virus in certain amounts may withstand concentration in washing fluids without losing entirely its infective power.

EXPERIMENTAL.

Experiment 1.—The nasal cavities of a normal adult, H. L., were rinsed thoroughly with 50 cc. of distilled water. The collected fluids were passed through a Berkefeld filter and 0.2 cc. of a Berkefeld filtrate of a 5 per cent suspension of poliomyelitic brain was added. The mixture was reduced *in vacuo* at 37°C. to 4 cc. and injected intracranially, under ether anesthesia, into a *Macacus rhesus*. The monkey became partially paralyzed on the 10th day, completely prostrated on the 12th day, and died on the 14th day. Typical lesions of poliomyelitis were present.

Experiment 2.—The washings of a normal adult, W. T., were obtained in the manner described above. To the filtered fluid was added 0.5 cc. of a Berkefeld filtrate of active virus. The mixture was concentrated at 37°C. *in vacuo* and injected intracranially, under ether anesthesia, into a *Macacus rhesus*. The monkey remained well. As the result of a later protection test the monkey died of poliomyelitis after an appropriate injection of potent virus.

Filtration through Berkefeld or other porcelain filters is undertaken to remove the bacteria always present in the nasal and buccal secretions. But the bacteria can be either killed or their multiplication inhibited by certain antiseptic chemicals which affect to a less extent the virus of poliomyelitis. Thus 0.5 per cent carbolic acid destroys pyogenic bacteria in tissues and leaves the virus intact. Experiments also showed that ether acted more severely on the ordinary bacteria than on the virus. In order, therefore, to obviate any loss of virus which might result from its retention by the

filters, ether was employed to sterilize the washings. Preliminary tests showed that contact of ether for 20 hours with the virus contained in an emulsion of the spinal cord does not destroy it. The test made with washings indicates that while a shorter exposure may not kill all the bacteria, yet they are so greatly diminished that no ordinary infection is produced on inoculation into monkeys. And yet, as the experiments which follow show, while the filtrate contained in 0.8 per cent salt solution is active after the ether treatment, that mixed with the nasal washings is ineffective. The ineffectiveness at first believed to have been due to injury of the virus by the ether or too great dilution of the fluid inoculated, is now probably explicable in other ways.

Experiment 3.—1.8 cc. of a Berkefeld filtrate of active virus were added to 4.2 cc. of isotonic sodium chloride solution and 1 cc. of chemically pure ether. The mixture was shaken for 20 hours at room temperature. The ether was allowed to evaporate and 1 cc. of the remaining mixture, representing 0.3 cc. of virus filtrate, was injected intracranially, under ether anesthesia, into a *Macacus rhesus*. The monkey was almost prostrate on the 7th day, completely prostrate on the 8th, and etherized when moribund on the 10th day. The lesions were typical.

Experiment 4.—To 100 cc. of nasal washings from two normal adults was added 1 cc. of a Berkefeld filtrate of mixed virus, 0.1 cc. of which produced paralysis in the control monkey in 7 days. 5 cc. of chemically pure ether were added to the mixture and the whole was shaken for 20 hours at room temperature. The ether was allowed to evaporate, and 2 cc. of the mixture were injected intracerebrally and 98 cc. intraperitoneally under ether anesthesia into a *Macacus rhesus*. The monkey remained well.

Berkefeld filters withhold even very minute particles in greater amount when they are contained within a viscid or glutinous liquid. All the washings contain mucus; hence a procedure was adopted to modify the mucin so as to avoid this difficulty without at the same time injuring unduly the virus itself. The procedure consists in treating the washings with sodium bicarbonate, filtering, and then concentrating *in vacuo* at 37°C. The virus is little injured. When 0.1 cc. of a filtrate, which is on the limits of a minimum lethal dose, is used, the resulting concentrated fluid may be ineffective; when 0.2 to 0.3 cc. is employed infection results. The next protocol in which 0.3 cc. of filtrate was used is an example of the method, but identical effects were obtained with 0.2 cc.

Experiment 5.—The nasal cavity of a normal adult was thoroughly syringed with 50 cc. of sterile distilled water. 0.3 cc. of a Berkefeld filtrate of virus was added, and, after thorough mixing, 0.25 gm. of dry sodium bicarbonate was added and the fluid shaken for 20 minutes with beads. After centrifugation at high speed for 3 minutes the fluid was decanted and passed through a Berkefeld candle V. The precipitate was washed and filtered through the same candle. The mixture of the filtrates was reduced *in vacuo* at 36°C. to a volume of 2 cc., which with rinsing water was transferred to a collodion sac and dialyzed for 1 hour.

Under ether anesthesia a *Macacus rhesus* received half (3.5 cc.) of the resulting liquid into the left, and the other half into the right cerebral hemisphere. No symptoms were observed until the 6th day when the monkey became ataxic and excitable. The monkey was prostrate on the 8th day and died on the 14th day after injection. Typical microscopic lesions of poliomyelitis were present.

The treatment of the washings with sodium bicarbonate renders the effect of the inoculation certain when the use of larger quantities of the virus with washings alone fails to confer infection. The probable cause of the discrepancy has become apparent only after a more minute study of the properties of the nasal washings; but that the sodium bicarbonate acts either by allowing more virus to pass through the filter or by removing certain inhibitory influences exerted by the washings is directly indicated.

Inactivating Effects of Nasal Secretions upon the Virus.

The results of the preceding experiments, which contained obvious discrepancies, suggested a closer study of the secretions of the nose and pharynx from the standpoint of a possible inhibiting or neutralizing action on the virus of poliomyelitis. For this purpose a variety of persons was studied; some were suffering from acute poliomyelitis, and the others were apparently normal individuals.

The nasopharynx was rinsed with double distilled water and the washings were fractionally sterilized by heating to 60°C. for 3 successive days. Each person's specimen was handled separately. In earlier experiments, in order to economize animals, the washings of several persons were often mixed. It now seems not improbable that discordant results follow this procedure. The virus employed was obtained by filtering a 5 per cent suspension of glycerolated poliomyelitic monkey spinal cord. To each 30 cc. of the washing 7.5 cc. of the filtered virus were added. The mixture was then incubated at

37°C. for 24 hours. Control mixtures of virus and distilled water were subjected to the same incubation. Each cubic centimeter of the mixtures then contained 0.2 cc. of the filtrate, or at least two minimum lethal doses of the virus. The results of the first tests are given in Table I.

TABLE I.

Inactivating Effects of Nasal Secretions upon the Virus.

Date.	Virus No.	Dose of virus filtrate.	In contact with nasal washings from.	Method of sterilizing nasal washings before addition of virus.	Temperature at which virus plus nasal washings were incubated for 24 hrs.	Result.
1916		*cc.*			°C.	
Feb. 16	32	0.2	Baby C.; age 3 yrs. Acute stage of poliomyelitis.	Heated 1 hr. at 60°C. on 3 successive days.	37	Monkey died. Typical poliomyelitic lesions.
Mar. 11	32	0.2	W. T., normal adult; age 39 yrs.	"	37	Monkey remained well.
June 2	48	0.2	C. A. R. and L. M. McK. (mixed), normal adults.	"	37	"
" 2	48	0.2	"	"	4	"
" 2	48	0.2	H. E. G., normal adult.	"	37	"

The results of this experiment suggest that the nasal washings of a person suffering from acute poliomyelitis may exercise no restraining influence upon an active virus, while those from healthy persons, under identical conditions of preparation, inhibit its activity.

The next experiment comprised tests on the nasal washings of eight apparently healthy persons. The results are recorded in Table II. At first sight it appears that of the eight specimens of washings, six possessed inhibiting properties and two did not. The question arose as to whether examination by a rhinologist, who would be unaware of the experiment, would disclose any differences in the nasal mucous membranes. These examinations, consented to by the

TABLE II.

Inactivating Effects of Nasal Secretions of Adults.

Date.	Dose of Berkefeld filtrate of Virus 48.	In contact with nasal washings from.	Method of sterilizing nasal washings before addition of virus.	Temperature at which virus plus nasal washings were incubated for 24 hrs.	Result.
1916	*cc.*			°C.	
Apr. 26	0.2	M. J. P., normal adult.	Heated 1 hr. at 60°C. on 3 successive days.	37	Monkey remained well.
" 26	0.2	E. S. S., " "	"	37	"
" 26	0.2	V. H. S., " "	"	37	"
" 26	0.2	C. A. R., " "	"	37	"
" 26	0.2	L. M. McK., normal adult.	"	37	"
" 26	0.2	G. H., normal adult.	"	37	"
" 26	0.2	J. P. B., " "	"	37	Monkey died. Typical lesions.
" 26	0.2	H. E. G., " "	"	37	"
" 26	0.2	Control (sterile water).		37	"
" 26	0.2	" (isotonic salt solution).		37	Monkey remained well.

persons, were kindly undertaken by Dr. M. C. Twitchell. His report is summarized in Table III. The only comment which the examination calls for is that while the anatomical condition of the nasal and adjacent mucosas in the six persons whose secretions contained inhibiting, inactivating, or neutralizing substances were normal, those of the other two were more or less pathologic. Just what the relation of this fact is to the effects of the secretions on the virus can only be surmised; but the test demonstrates that the secretions may frequently inhibit the action of the virus in monkeys. The control tests (Table II) show that, under the conditions of the experiments, distilled water injures the filtered virus less quickly than isotonic salt solution, a fact possibly dependent upon the different osmotic conditions present in the two fluids. The inactivation of

TABLE III.

Results of Rhinoscopy of the Subjects Recorded in Table II.

Case No	Age.	Report.
	yrs.	
1 (M. J. P.)	23	Normal nasal respiration; no discharge; no history of colds. Mild hypertrophic rhinitis. Septum deflected slightly to left with large horizontal ridge.
2 (E. S. S.)	24	Normal nasal respiration; no discharge; no throat symptoms; no history of colds. Mild hypertrophic rhinitis; vocal bands red (subacute laryngitis).
3 (V. H. S.)	24	Normal nasal respiration; no discharge; no throat symptoms; no history of colds. Mild hypertrophic rhinitis. Two spurs on left side of septum.
4 (C. A. R.)	20	Normal nasal respiration; no discharge; no throat symptoms; no history of colds. Mild hypertrophic rhinitis; septum deflected, half closes right nasal cavity.
5 (L. M. McK.)	22	Normal nasal respiration; no discharge; no throat symptoms; no history of colds. Mild hypertrophic rhinitis; small ulcer on left side of septum: horizontal ridge on right side of septum.
(G. H.)	21	Normal nasal respiration; no discharge; no throat symptoms; no history of colds. Mild hypertrophic rhinitis. Acute pharyngitis of 3 days' duration.
7 (J. P. B.)*	22	Normal nasal respiration but easily obstructed when patient has a cold, especially left side of nose; secretion drops into throat on arising in the morning. Nose narrow; moderate hypertrophic rhinitis; large spur on right side of septum touches turbinate; small ulcer on left side of septum.
8 (H. E. G.)*	25	Nasal respiration interfered with, especially on right side; secretion drops into throat; frequent colds; has cold now. Septum deflected slightly to right; hypertrophic rhinitis; secretions found in right nasal cavity by anterior rhinoscopy. Acute pharyngitis and rhinitis.

* Remarks by Dr. Twitchell: "No. 7 shows the most marked chronic nasal trouble of all, and I should class it as moderate rather than severe. No. 8, at the time of examination, had an acute rhinitis and an acute pharyngitis. This to a certain extent obscures the findings in this case. Frequent colds are a marked feature in the history of chronic rhinitis. No. 8 is the only one giving this history. I should conclude that if a chronic rhinitis produces changes in the nasal secretions, No. 8 would be the one whose nasal secretions were the most changed."

the virus through dilution by the washings and incubation at 37°C. would appear to be excluded by the results of the tests with the secretions and with the controls.

Fluctuations in Inactivating Properties.

Attempts were made to ascertain whether the action described is a constant or a variable property of the secretions. For this purpose washings were made at different times, sterilized by discontinuous heating at 60°C., and tested against 0.2 cc. of the filtrate which in control tests was determined to be potent. The results of these tests are given in Table IV.

TABLE IV.

Fluctuations in Inactivating Properties.

Date.	Case.	Condition.	Dose of virus filtrate.	Result.
1916			*cc.*	
Mar. 11	W. T.	Apparently normal.	0.2	Neutralized.
June 16	"	" "	0.5	"
July 12	"	" "	0.2	Failed to neutralize.
Nov. 14	"	" "	0.2	Neutralized.
Apr. 26	H. E. G.	Chronic rhinitis.	0.2	Failed to neutralize.
June 2	"	" " (improved).	0.2	Neutralized.
July 12	"	Apparently normal.	0.2	Failed to neutralize.
Apr. 26	C. A. R.	" "	0.2	Neutralized.
June 2	"	" "	0.2	"
Apr. 26	G. H.	" "	0.2	"
Dec. 18	"	" "	0.2	"
Apr. 26	L. M. McK.	" "	0.2	"
June 2	"	" "	0.2	"
Apr. 26	E. S. S.	" "	0.2	"
July 12	"	Acute coryza.	0.2	Failed to neutralize.

Of four tests with the secretions of W. T., three neutralized the virus; of three with those of H. E. G., only one neutralized it; of two with washings from C. A. R., G. H., and L. M. McK., respectively, all neutralized it, while in the case of E. S. S., one neutralized and the other did not. The animals that did not come down were subsequently determined to be susceptible to inoculation with the virus, so that the neutralization effects could not have been simulated by an excessive resistance on their part.

In addition to the tests described, which were conducted chiefly with adults, several were made with washings from children either healthy or suffering from poliomyelitis. The results are not wholly concordant. A larger series may possibly clear up the discrepancies.

Aug. 9, 1916. The washings of C. A., an apparently healthy boy, age 14, failed to neutralize 0.2 cc. of filtrate.

Oct. 23, 1916. The washings of R. J., age 8, taken during the acute attack of poliomyelitis, but after immune serum had been administered, neutralized 0.2 cc. of filtrate. A control monkey developed fatal, typical poliomyelitis.

Nov. 14, 1916. The washings of R. C., age 8, taken on the 15th day of the attack of poliomyelitis neutralized 0.2 cc. of filtrate. This patient had not been treated with immune serum. The control animal developed typical fatal poliomyelitis.

Feb. 16, 1916. The washings of B. C., age 3, taken during the acute stage of poliomyelitis did not neutralize the filtrate. Immune serum had not been given.

While the number of observations is too small to draw definite conclusions, it is obvious that the secretions of apparently normal persons vary in the so called neutralizing power. Of the two patients with poliomyelitis whose secretions inhibited action of the filtrate, one had received immune serum, while the washings were taken from the other on the 15th day, or at a time when immunity principles are known to be present in the blood.[7] The third child with poliomyelitis yielded washings without neutralizing effect; but they were taken earlier (4th day) in the course of the infection and at a time when the immunity bodies were probably not yet abundantly present. It is possible that some relation exists between the presence of definite immunity principles in the circulating blood and the power of the nasal washings to neutralize the virus.

In each series of experiments the potency of the virus was established by control experiments, and subsequently all the monkeys not showing symptoms were tested for immunity by appropriate injections of the virus and were all found to have been susceptible to infection. Hence the lack of response was not caused by an immunity of the animals employed. The secretions of three persons out of six examined varied in their power to neutralize 0.2 cc. of the virus filtrate at different times under nearly identical conditions,

[7] Flexner, S., and Amoss, H. L., *J. Exp. Med.*, 1917, xxv, 499.

yet the only known clinical differences consisted in the presence of a rhinitis which appears to remove the inactivating power of the secretions.

Fluctuations of the Inactivating Power in Abnormal Nasal Conditions.

In April, 1916, the nasal secretions of E. S. S. neutralized 0.2 cc. of the virus filtrate, but 3 months later, during an attack of acute rhinitis, they did not. The washings from C. A. R. twice neutralized the same amount of virus at different times. Later, immediately following an acute rhinitis, no neutralizing power was observed, but the neutralizing power returned in 4 days.

The washings from H. E. G., taken when rhinoscopy revealed acute congestion of the nasal mucosa, did not possess neutralizing power, but 5 weeks later when the nasal condition had improved the washings showed the inactivating power. 6 weeks after the second test when there were no subjective symptoms of rhinitis, the washings failed to neutralize the virus. Finally, it will be recalled (Table III) that out of eight samples of nasal washings taken from apparently normal adults, only the two which were taken from subjects in which rhinoscopy revealed an acute rhinitis failed to inactivate the virus. H. E. G. is included in this list.

Effect of Fractional Sterilization and Filtration on Inactivation.

The experiments recorded indicate that the washings sterilized fractionally or passed through Berkefeld filters inactivate or neutralize virus mixed with them in the form of a filtrate of a suspension of the spinal cord of a poliomyelitic monkey. There can, therefore, be no doubt that the procedures do not themselves remove the neutralizing substances. Tests were then made to determine the comparative or quantitative effects of the procedures.

The quantity of filtrate employed for inoculation in this series of experiments was 0.4 cc., or more than four minimum lethal doses. The rinsings of the nasopharynx were made with redistilled water and they were reduced to a uniform volume of 15 cc. by concentration *in vacuo*. The fractional sterilization was carried out at 60°C. on 3 successive days. The washings and virus were left in contact 24

hours before the inoculations were made, in some instances at 37°C., in others at 4°C. The injections were intracerebral into *rhesus* monkeys under ether anesthesia. The results are given in Tables V and VI, and the comparison in Table VII.

The results lack absolute consistency. Considering the quantity of virus employed, the neutralizing action becomes more impressive. The variations in specimens from the same individual cannot now be accounted for. The existence of acute rhinitis, however, appears to diminish neutralizing power. Assuming that the process of neutralization is brought about by definite chemical bodies, they would seem to be thermolabile, since the neutralizing action of filtrates is definitely more pronounced than that of the heated specimens. Contact at 4°C. appears less effective in bringing about the neutralization than at 37°C. The prolongation of the incubation period noted in two instances is probably associated with partial but insufficient neutralization to reduce the virus below the minimum lethal dose.

Influence of Heat.

The results given above suggest that the inactivating influence is weakened or destroyed by heat. The following experiment gives more definite information concerning this fact.

Washings were taken on Nov. 16, 1916, from W. T., whose nasal secretions had on several occasions proved neutralizing. 60 cc. of sterile distilled water were used and the washings passed through a Berkefeld N candle.

To 10 cc. of washings filtrate were added 2.5 cc. of active virus filtrate and the mixture was incubated at 37°C. for 24 hours. 1 cc. of the mixture, representing 0.2 cc. of the virus filtrate, was injected intracerebrally, under ether anesthesia, into a *Macacus rhesus*. The monkey remained well.

35 cc. of the washings filtrate were reduced quickly *in vacuo* at a temperature between 60° and 70°C. to a volume of 5 cc. 1.25 cc. of active virus filtrate were added and the mixture was incubated at 37°C. for 24 hours. 1 cc. of the mixture, representing 0.2 cc. of virus filtrate, was injected intracerebrally, under ether anesthesia, into a *Macacus rhesus*. The monkey was completely paralyzed on the 7th day and died on the 8th day. Typical lesions of poliomyelitis were present.

The neutralizing substance is apparently rendered inactive by heating to 70°C., though this experiment does not exclude volatility as the reason for the disappearance of this substance. Other experiments, however, in which the concentrations were carried out *in vacuo* at 60°C. indicate that the neutralizing substances are not volatile.

TABLE V.

Neutralizing Power of Nasal Washings Heated to 60°C. for 1 Hour.

Monkey.	Dose of virus filtrate.	Fractionally sterilized nasal washings from.	Temperature at which virus plus nasal washings were incubated for 24 hrs.	Result.
	cc.		°C.	
A	0.4	W. T., normal adult.	37	Died in 33 days.
B	0.4	" " "	4	" " 31 "
C	0.4	G. H., " "	37	Remained well.
D	0.4	" " "	4	Died in 11 days.
E	0.4	C. A. R. (acute rhinitis).	37	" " 8 "
F	0.4	" " "	4	" " 8 "
G	0.4	Control (distilled water).	37	" " 14 "

TABLE VI.

Neutralizing Power of Nasal Washings Passed through a Berkefeld Filter.

Monkey.	Dose of filtrate of virus.	Berkefeld filtered nasal washings from.	Temperature at which virus plus nasal washings were incubated for 24 hrs.	Result.
	cc.		°C.	
H	0.4	W. T., normal adult.	37	Remained well.
I	0.4	" " "	4	Died in 16 days.
J	0.4	G. H., " "	37	" " 19 "
K	0.4	" " "	4	Remained well.
L	0.4	C. A. R. (4 days after acute rhinitis).	37	" "
M	0.4	" (4 " " " ").	4	" "

TABLE VII.

Effect of Berkefeld Filtration and Heat on the Neutralizing Power of Nasal Washings

Nasal washings from.	Condition of person from whom washings were obtained.	Result of neutralizing test against 0.4 cc. of Berkefeld filtrate of virus.			
		Filtered (Berkefeld) washings plus virus allowed to remain for 24 hrs.		Fractionally sterilized washings (60° C. on 3 days) plus virus allowed to remain for 24 hrs.	
		37°C.	4°C.	37°C.	4°C.
W. T., adult.	Normal.	+*	−	±†	±†
C. A. R., adult.	Acute rhinitis.			−	−
" "	4 days after acute rhinitis.	+	+		
G. H., "	Normal.	−	+	+	−
Control (distilled water).				−	

* The sign + indicates neutralization; ±, marked prolongation of the incubation period preceding paralysis.

† Incubation period greatly prolonged. Monkeys developed no symptoms until 33 and 31 days, respectively, after inoculation.

DISCUSSION.

The power of the secretions of the nasopharynx of certain but not all individuals to bring about the inactivation or neutralization of the active virus of poliomyelitis has been demonstrated. The term active is employed to indicate that the samples of virus were obtained from strains adapted to the monkey, and could be relied upon to cause infection in the doses employed, almost without exception.

The inactivating property of the secretions mentioned is the more surprising in view of the resistance displayed by the poliomyelitic virus to such chemical antiseptics as glycerol and phenol.

In their manner of action, the neutralizing substances resemble more the specific immunity bodies contained within the blood serum of persons and monkeys who have suffered an attack of poliomyelitis. Like them, they appear to be thermolabile. And yet the experiments here recorded do not actually identify the two classes of substances.

It is known that the blood serum of certain adults who apparently

have never suffered from poliomyelitis is capable of neutralizing[8] the filtered poliomyelitic virus.[9] But in the few instances in which this property has been discovered, the adults yielding the serum had been in contact with acute cases of poliomyelitis, and artificial immunization cannot be excluded.

On the other hand, it seems not improbable that the inactivating or neutralizing power of the nasal secretions may play a part in protection against poliomyelitic infection, and even may represent an external system of defense against invasion of the virus by way of the nasopharyngeal mucosa.

If this view is supported by further studies, we should find that the secretions of children are less frequently neutralizing than those of adults, although many tests will be necessary to establish this distinction. In that case, we may find that the secretions of persons attacked by poliomyelitis at the period of onset of the disease lack neutralizing power, although later, when the immunization reactions have been aroused, inactivation may result, as has been shown to happen in particular instances in our series (page 516).

It appears, however, that the power of a given secretion to inactivate or neutralize the virus is not wholly a fixed one. Fluctuations in the property have been detected and described. Common and slight inflammatory conditions, *e.g.*, as in acute and even chronic rhinitis, apparently tend to remove or diminish the neutralizing power of the secretions. If this observation should be supported by further experiment, knowledge concerning one of the conditions favoring persistent contamination of the nasopharynx with the virus may be obtained. It does not follow, however, that this contamination need necessarily lead to infection, for the accomplishment of which disturbance of still other defensive mechanisms may be necessary. However, the production of healthy carriers of the poliomyelitic virus may rest upon the power or lack of power in the secretions to inactivate the virus. Should this be the case, then of many persons exposed only a fraction would become carriers, because the greater

[8] Flexner and Lewis, *J. Am. Med. Assn.*, 1910, liv, 1780.

[9] Peabody, F. W., Draper, G., and Dochez, A. R., A Clinical Study of Acute Poliomyelitis, Monograph of The Rockefeller Institute for Medical Research, No. 4, 1912.

part would possess secretions capable of neutralizing and hence destroying the virus.

The variation in inactivating power does not depend alone upon inflammatory changes. Irregularities have been noted which cannot now be explained. They may be merely apparent and depend upon the experimental method to which we are at present limited. Inoculation experiments in single series are not wholly trustworthy. Filtration through porcelain is also open to errors of experiment, since the blocking of the porous spaces may easily exclude essential constituents of the washings. Fractional sterilization is also not a wholly reliable means of preventing bacterial development and yet of retaining unimpaired labile organic constituents. In view of all this, some degree of irregularity is to be looked for.

If this property of the secretions to inactivate or neutralize the virus of poliomyelitis is established, comparative tests should be made on large groups of persons at different seasons of the year in order to determine whether it bears any relation to the seasonal prevalence of poliomyelitis. We are engaged now in collecting observations covering this point; but reference to the tables will show that most of the tests were made during the spring, summer, and autumn. Moreover, they embraced few children of the most susceptible ages.

SUMMARY.

1. The results of 56 experiments have shown that washings of the nasal and pharyngeal mucosas possess definite power to inactivate or neutralize the active virus of poliomyelitis.

2. This power is not absolutely fixed, but is subject to fluctuation in a given person. Apparently inflammatory conditions of the upper air passages tend to remove or diminish the power of neutralization. But irregularities have been noted, even in the absence of these conditions.

3. Too few tests have been made thus far to ascertain whether adults and children differ with respect to the existence of this neutralizing property in the nasal secretions. While the inactivating property was absent from the secretions of one child during the first days of poliomyelitis, it was present in another to whom immune

serum was administered, and in still another on the 15th day of illness when convalescence was established.

4. The neutralizing substance is water-soluble and appears not to be inorganic; it appears to be more or less thermolabile, and its action does not depend upon the presence of mucin as such.

5. It is suggested that the production of healthy carriers through contamination with the virus of poliomyelitis may be determined by the presence or absence of this inactivating or neutralizing property in the secretions. Whether this effect operates to prevent actual invasion of the virus and production of infection can only be conjectured. Probably the property is merely accessory and not the essential element on which defense against infection rests. It is more probable that other factors exist which help to determine the issue of the delicate adjustment between contamination and infection.