THE EFFECTS OF NASALLY INSTILLED VIRUS OF POLIOMYELITIS ON THE CEREBROSPINAL FLUID AND THE BLOOD OF MONKEYS

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While much has been written on the ultimate effects of neurotropic poliomyelitis virus dropped into the nose of *Macacus* monkeys and made to run over the olfactory nervous areas, little has been published on the immediate, remote influence of the presence of the virus on the nasal membranes.[1] The subject is one of real importance, if for no other reason than because of the commonly held belief that spontaneous mass immunization to poliomyelitis among children results from the chance presence in the upper respiratory tract of the widely disseminated virus, not only during the prevalence of epidemics, but even during interepidemic periods. That the nasal portal of entry of the virus is effective is shown by the frequency with which the paralytic disease arises among nasally instilled monkeys in the absence of

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[1] Jungeblut and Hazen (1) subjected four *rhesus* monkeys to "extensive" spraying of the nose and throat with a 10 per cent suspension of poliomyelitis virus during a period of 2½ months. The spraying was done twice a week, each animal receiving 20 sprays of about 1 cc. Three animals survived the test period, and were bled 1 month after the last treatment. The serum was tested for antivirus, and the monkeys were inoculated cerebrally with virus. All three monkeys developed paralytic poliomyelitis, and the sera exerted no detected neutralizing action. The doses of virus (1 cc. of a 10 per cent suspension), and the virus (0.5 cc. of a 1 per cent suspension) and serum (0.5 cc.) mixtures, indicate either a strain of low virulence, or the use of overwhelming quantities in the tests. However, the results seem unequivocal, inasmuch as Flexner (2), employing more measured amounts of virus, also reported that monkeys refractory to nasal instillation of virus were devoid of serum antiviral activity and exhibited average susceptibility to the intracerebral injection of potent virus.

all demonstrable lesions in the infected membranes; and the pathogenic effects of the virus so inoculated are little, if at all, surpassed by its direct cerebral inoculation.

It is not possible to detect the immediate effect of virus introduced directly into the brain, because of the injury of the tissues attending the injection. It is, however, common knowledge that monkeys which resist such inoculations, remaining free from obvious, detectable symptoms of disease, are as receptive to a subsequent injection of more potent virus as are control animals. Indeed, monkeys may resist more than one cerebral inoculation and yet respond to a later injection of virus in a manner indistinguishable from that exhibited by previously uninoculated animals. Since the injection of virus, once or repeatedly, into or beneath the skin in monkeys induces active immunity, the failure of a corresponding state to develop in intracerebrally inoculated animals is worthy of emphasis.

While it is, as stated, impossible, because of associated effects, to determine the immediate action of the virus when introduced cerebrally, no such barrier exists to the detection of effects when the virus is brought into contact with the olfactory area in the nasal membranes. If, for example, the cerebrospinal fluid is withdrawn by cisterna puncture and the cells present in it be counted, the striking fact will emerge that the presence of the virus invariably produces changes in the cell content, irrespective of whether symptoms of disease do or do not appear (3).[2]

This delicately adjusted response of the cerebrospinal fluid to the presence of the virus of poliomyelitis on the nasal membranes has engaged our attention for some time. We have followed the phenomenon under a variety of conditions and circumstances, which it will be the purpose of this paper and succeeding ones to describe. In studying the reaction we have had in mind its relation, if any, to the immunity which may be induced in monkeys by means of successive inoculations of the virus; and collaterally, the immunity which arises in children who are exposed to the virus and respond to its presence with mild attacks of illness—in their nature poliomyelitic—or who, through an unperceived or undetected series of events, become, in the

[2] It should be stated at this point that the instillation of sterile salt solution into the nares of *Macacus rhesus* on 6 successive days does not affect the number of cells in the cerebrospinal fluid withdrawn by cisterna puncture.

common phrase, specifically spontaneously immunized and protected against such infection.

This first paper will deal with the manner in which normal monkeys respond to one or multiple instillations of virus with changes in the cerebrospinal fluid, in comparison with discoverable alterations, of an immunity character, in the animals treated.

Methods

Instillation.—1 cc. of a lightly centrifuged (300 revolutions per minute, for 1 minute) 10 per cent suspension of glycerolated monkey medulla and spinal cord, washed in two changes of salt solution, is instilled with a medicine dropper into each nostril of the animal.

For the second and for each subsequent nasal instillation, a new suspension is prepared, preferably from a glycerolated specimen other than that used previously. More consistent results can be obtained when suspensions are prepared from specimens which have been kept in glycerol less than 10 weeks.

The monkey is held by an assistant (no etherization is necessary for this procedure) in an upright position, with head bent backward. By attaching an ordinary rubber urethral tip to the dropper containing the virus, this device can be placed tightly against the nostrils and more force can be exerted, thus allowing the suspension to distribute itself to all parts of the upper nasal respiratory tract.

Cisterna Puncture.—The position of holding the monkey is important. After the animal has been thoroughly etherized, the back of the neck shaved and sterilized, an assistant places it on its abdomen, letting the head drop forward over the edge of the table; the assistant holds the head firmly with both hands.

A *sharp* hypodermic needle (1 inch cannula, No. 20 gauge) is inserted *vertically* almost its entire length until it reaches the cisterna magna region, which can be readily determined in the fingers by the sudden release of resistance. The stylet is then removed, and clear fluid is allowed to flow. The fluid is quickly taken off by a capillary pipette and placed in small Wassermann tubes.

Occasionally the first few drops of fluid may be tinged with blood, in which case pipetting is continued until a clear specimen is obtained.

Cells.—After gentle shaking, the cerebrospinal fluid is conveyed by a capillary pipette to the white cell counting chamber. No dye is added to the fluid. 4 square millimeters, or one large square in each corner of the usual 9 square millimeter cell counter, are counted each time.

If red cells are present, these can be eliminated by adding 3 per cent of an acetic acid solution, using equal parts of this solution and spinal fluid. A long pipette with a total capacity of 0.1 cc., divided into 100ths, is sufficiently accurate. The acetic fluid solution is drawn up to the 5th mark, after which the spinal fluid is brought up to the 10th or final mark. The entire contents are then expelled into a small, conical shaped test tube, allowing the mixture to remain in the tube for several minutes before counting.

Globulin Test.—The Noguchi butyric acid test brings out a positive reaction much earlier than do other methods.

(1) 0.5 cc. of a 10 per cent butyric acid solution, made up in salt solution, is added to 0.1 or 0.2 cc. of clear spinal fluid. The test tube is placed in boiling water and allowed to remain for 2 minutes in the boiling water bath.

(2) Then 0.1 cc. of N/10 sodium hydroxide is added and the tube is again placed in the boiling water for 1 or 2 minutes. Almost immediately, or in a few minutes, a precipitate forms and settles to the bottom in strongly positive reactions. If only a small amount of globulin is present, it may take up to an hour to form a precipitate.

Temperatures.—Rectal temperatures are taken daily, following the first instillation of virus and every day thereafter, usually in the morning, between 9:00 and 11:00 a.m. The thermometer is allowed to remain in the rectum for 1 minute. The Fahrenheit scale is used.

PROTOCOLS

TABLE I

Macacus rhesus Responding Symptomatically to One Course of Instillations. Temperatures, Cell Counts, Globulin, and Symptoms

No. of monkey	Virus strain, dosage, dates of instillation	Dates	Temperatures	Cells	Globulin	Symptoms
I	Philadelphia, 1932,			36 (normal)		
	4 doses, 3/27,	3/29	101.8	73		
	3/28, 4/5, 4/6/33	3/31	103	96	+	
		4/3	103	108	±	
	*Accelerating doses	4/5	102.6	193	±	
		4/6	102			
		4/7	104.6	158	+	
		4/8	104.6	166	+	Tremor, ataxia; right deltoid paralyzed
		4/10	104.4	290	+	No change
		4/11	105.4			" "
		4/12	103	188	+?	
		4/13	101	64	±?	More active. Recovered without residual paralysis

* The accelerating instillations affected temperatures, cell counts, and globulin content. They did not affect the paralytic symptoms.

TABLE I—*Concluded*

No. of monkey	Virus strain, dosage, dates of instillation	Dates	Temperatures	Cells	Globulin	Symptoms
II	Mixed, 2 doses, 6/14, 6/15/33			29 (normal)		
		6/16	102.2	65		
		6/19	104.2	165		
		6/20	104.6	320		Tremor
		6/21	104.6	405		
		6/22	103.6	570	+	Ataxia, followed by paralysis, arms and legs
III	Havana, 1934, 6 doses, 1/2–1/8/35			22 (normal)		
		1/9	103	36		
		1/11	104	148		
		1/14	105.6	496		Deltoid paralysis, followed by prostration
IV	Mixed, 2 doses, 1/8, 1/10/35			36 (normal)		
		1/12	103	140		
		1/14	105.2	504	+	Tremor, ataxia
		1/16	103.4	580		Arms paralyzed
		1/17				Prostrate
V	Mixed, 3 doses, 1/8, 1/10, 1/12/35		103.6	29 (normal)		
		1/14	103.6	490		Ataxia
		1/16	106	610	++	Arms paralyzed
		1/17				Prostrate
VI	Philadelphia, 1932, 2 doses, 1/24, 1/26/35			30 (normal)		
		1/26	101.2	35		
		1/28	105.2	374		
		1/30	103.2	580	+	Tremor, ataxia, arm paralysis
VII	Cooperstown, 1928, 3 doses, 2/18–2/23/35			27 (normal)		
		2/23	102.6	33		
		2/25	103.6	145		
		2/27	104.4	455		
	Accelerating dose	3/1	104.6	220		Tremor
		3/4	104	210		Deltoid paralysis
		3/6	103	365		
		3/8	104	390		Arms, legs paralyzed
		3/12	102.8	405		

TABLE II

Macacus rhesus Responding Asymptomatically, with Cell Changes. Temperatures, Cell Counts, Globulin, and Symptoms

No. of monkey	Virus strain, dosage, dates of instillation	Dates	Temperatures	Cells	Globulin	Symptoms
VIII	New York, 1933, 2 doses, 12/26, 12/27/33			48 (normal)		
		12/29	103.4	97		No symptoms
		12/31	103.2	120		
		1/2/34	104.4	410		
		1/5	102	640		
IX	Mixed, 1 dose, 2/13/34			42 (normal)		
		2/14	103.4	60		
		2/15	103	220		
		2/16	103	465		
		2/17	103.2	520		
		2/19	104.4	405	0	No symptoms
		2/20	104	260		
		2/21	102.4	125		
		2/24	102.8	102		
		3/5	101.8	44		
X	Mixed, 1 dose, 2/13/34			55 (normal)		
		2/14	103.4	60		
		2/15	102.2	110		(Sacrificed for histology)*
XI	Mixed, 1 dose, 2/13/34			35 (normal)		
		2/14	101.8	55		
		2/15	104	514	++	(Sacrificed for histology)
XII	Philadelphia, 1932, 2 doses, 2/5, 2/7/35			14 (normal)		
		2/7	103	45		
		2/9	103.2	614		No symptoms
		2/11	103.4	710		
		2/13	103.6	320		
		2/16	102.4	190		
XIII	Cooperstown, 1928, 4 doses, 2/18–2/23/35			27 (normal)		
		2/23	102.6	33		No symptoms
		2/25	103.6	145		
		2/27	104.4	155		
		3/1	104.6	220		

* Two *Macacus rhesus* were injected as follows: one cerebrally and peritoneally with a suspension of the mixed medulla, pons and cervical cord; the other in the same manner, with a suspension of the intervertebral ganglia. No symptoms resulted in either animal.

TABLE III

Macacus rhesus Given Two or More Courses of Instillations, the Earlier Nonsymptomatic, the Later Paralytic.

Temperatures, Cell Counts, Globulin, and Symptoms

No. of monkey	Virus strain, dosage, dates of instillation	Dates	Temperatures	Cells	Globulin	Symptoms
XIV	New York, 1933, 2 doses, 12/26, 12/27/33	12/26	102.6	48 (normal)		
		12/29	103.4	97		
		12/31	103.2	120		Fleeting symptoms, including excitement and partial ptosis
		1/2/34	104.4	410		
		1/5	102	640		
		1/8	101.4			
	New York, 1933, 6 doses, 2/5–2/10/34	2/7	103	105		
		2/9	103.4	62		
		2/13	102.6	100		No symptoms
		2/15	102.8	45		
		2/16	101.6			
	Philadelphia, 1932, 3 doses, 3/7, 3/9, 3/13/35			26 (normal)		
		3/9	102.8	24		
		3/11	103	65		No symptoms
		3/13	101.8	34		
		3/15	103.6	64		
		3/18	101.6	43		
	Havana, 1934, 3 doses, 5/15, 5/17, 5/21/35			31 (normal)		
		5/18	103	33		
		5/21	103.2	37		
		5/22	105.6	265		
		5/24	106	580	+	Tremor, ataxia, legs paralyzed
		5/29				Prostrate
XV	New York, 1933, 2 doses, 12/26, 12/27/33	12/26	103	44 (normal)		
		12/29	103	58		
		12/31	102.6	230		No symptoms
		1/2/34	103.6	260		
		1/5	103	365		
		1/8	102.4			

* The 1933 New York and the 1932 Philadelphia strains acted alike; the 1934 Havana strain acted differently. In keeping is the neutralization of 0.2 cc. Philadelphia virus by 0.8 cc. of serum taken after the Philadelphia virus instillations.

TABLE III—*Concluded*

No. of monkey	Virus strain, dosage, dates of instillation	Dates	Temperatures	Cells	Globulin	Symptoms
XV—*cont'd*	New York, 1933, 6 doses, 2/5–2/10/34			50 (normal)		
		2/7	102	64		
		2/9	102.4	125		No symptoms
		2/13	103.2	37		
		2/15	102	42		
		2/16	103			
	Havana, 1934, 3 doses, 5/15, 5/17, 5/21/35			24 (normal)		
		5/18	101.8	27		
		5/21	103.6	30		
		5/22	105.8	215		
		5/23	105.2			
		5/24	105.8	670		
		5/25	104.2			Tremor, ataxia, legs paralyzed
		5/28				Prostrate
XVI	New York, 1933, 5 doses, 12/27/33–1/2/34	1/2	103	65		No symptoms
		1/4	103.2	80		
		1/6	103	130		
		1/8	103	335		
	New York, 1933, 5 doses, 2/5–2/10/34	2/5		67 (normal)		
		2/7	103	205		
		2/9	102.8	535		
		2/13	105.2	620		
		2/15	103	212		
		2/17	104	420		Ataxia, weak arms
		2/19	104	450		Prostrate
XVII†	Mixed, 1 dose, 2/13/34	2/13		42 (normal)		
		2/14	103.4	60		
		2/15	103	220		No symptoms
		2/16	103	465		
		2/17	103.2	520		
		2/19	104.4	405		
		2/21	102.4	125		
		3/5	101.8	44		
	Mixed, 2 doses, 2/19, 2/21/35	2/19		23 (normal)		
		2/21	102.4	36		
		2/22	106			
		2/23	105.2	534	+	
		2/25	105.8			Tremor, ataxia
		2/26	104			Arm paralyzed
		2/27				Prostrate

† The same animal as recorded in Table II (No. IX). Bled Oct. 30, 1934, serum tested Nov. 3, 1934; 0.8 cc. serum did not neutralize 0.2 cc. Mixed Virus filtrate. The control monkey was prostrate on the 11th day.

TABLE IV

Macacus rhesus Resisting Multiple Instillations; Tested for Antivirus. Temperatures, Cell Counts, Globulin, and Symptoms

No. of monkey	Virus strain, dosage, dates of instillation	Dates	Temperatures	Cells	Globulin	Symptoms
XVIII	Mixed, 4 doses, March, 1933			No counts*		No symptoms
	Mixed, 4 doses, 1/10–1/13/34	1/13		65 (normal)		
		1/15	102.4	135		
		1/17	102.6	110		
		1/19	101.8	180		
		1/22	102	285		
		1/24	103	265		
		1/26	102.2	212		
		1/29	102.6	125†		
	Cooperstown, 1928, + New York, 1933, 6 doses, 1/29–2/3/34	1/29		125‡		
		1/31	101.8	105		
		2/2	102.2	320		No symptoms
		2/5	102.4	210		
		2/7	103	410		
		2/9	102	385		
		2/13	103	65		

* Bleeding of July 1, 1932, did not neutralize Mixed Virus in mixture of 0.7 cc. serum and 0.3 cc. virus filtrate.

† Neutralization conducted on June 19, 1935, of serum of Jan. 29, 1934, did not neutralize Mixed Virus in mixture of 0.8 cc. serum and 0.2 cc. filtrate.

‡ Cell count still high from previous instillations.

TABLE V

Macacus cynomolgus Instilled Repeatedly and Tested for Antivirus

Monkey	Course	Virus strain, dosage, dates of instillation	Symptoms
Cynomolgus A	1st	Mixed, 6 doses, 11/2–11/7/31	6 days after the last instillation, deltoid became weak; no progression of paralysis. Complete recovery
(Previously fed by stomach tube, without effect)	2nd	Mixed, 6 doses, 1/4–1/9/32	No symptoms
	3rd	Mixed, 3 doses (accelerating), 1/16–1/18/32	No symptoms
	4th	Mixed, 3 doses, 2/1–2/3/32	No symptoms
*Bled for serum, 3/10/32	5th	Mixed, 3 doses, 3/11–3/17/32	No symptoms

Neutralization Test

* Serum taken Mar. 10, 1932, after 4th course of instillation, mixed with virus in proportion of 0.9 cc. serum and 0.1 cc. filtrate of Mixed Virus; neutralized. Two injections given to 2 *rhesus* controls, Mar. 15 and 26, 1932 (acceleration); animals became paralyzed on 7th and 10th day respectively from same dose of virus filtrate.

Intracerebral Test

Apr. 22, 1932, 0.1 cc. Mixed Virus filtrate failed to induce symptoms. Two controls—one *cynomolgus* and one *rhesus*—became paralyzed on the 3rd and 8th day respectively from same dose of filtrate.

TABLE VI

Macacus cynomolgus Instilled Repeatedly and Tested for Antivirus

Monkey	Course	Virus strain, dosage, dates of instillation	Symptoms
Cynomolgus B	1st	Mixed, 6 doses, 11/2–11/7/31	No symptoms
	2nd	Mixed, 6 doses, 1/4–1/9/32	No symptoms
	3rd	Mixed, 3 doses (accelerating), 1/16–1/18/32	No symptoms
	4th	Mixed, 3 doses, 2/1–2/3/32	No symptoms
*Bled for serum, 3/10/32	5th	Mixed, 6 doses, 3/11–3/17/32	No symptoms

Neutralization Test

* Serum taken Mar. 10, 1932, after 4th course of instillations, mixed with Mixed Virus in proportion of 0.9 cc. serum and 0.1 cc. Mixed Virus filtrate, introduced into *Macacus rhesus*; no neutralization. Two injections of serum-virus mixture given: one, Mar. 15, 1932; the other (accelerating) on Mar. 26. Paralysis ensued on 10th day after the accelerating dose.

Intracerebral Test

Apr. 22, 1932, 0.1 cc. Mixed Virus filtrate injected into *Cynomolgus* B. Paralysis on 6th day. Two controls also became paralyzed.

TABLE VII

Macacus cynomolgus and Macacus rhesus Instilled Repeatedly and Tested for Antivirus

Monkey	Course	Virus strain, dosage, dates of instillation	Symptoms
Cynomolgus C	1st	Mixed, 6 doses, 11/2–11/7/31	No symptoms
(Mate to tube-fed *Cynomolgus* A)	2nd	Mixed, 6 doses, 1/4–1/9/32	Paralysis, 6th day
Rhesus D	1st	Mixed, 6 doses, 1/4–1/9/32	No symptoms
	2nd	Mixed, 3 doses (accelerating), 1/16–1/18/32	No symptoms
	3rd	Mixed, 3 doses, 2/1–2/3/32	No symptoms
*Bled for serum, 3/10/32	4th	Mixed, 3 doses, 3/11–3/17/32	No symptoms

Neutralization Test

* Mar. 15 and 26, 1932, (acceleration), 0.9 cc. serum and 0.1 cc. Mixed Virus filtrate into *Macacus rhesus*; no neutralization; paralysis 10th day after accelerating dose.

Intracerebral Test

Apr. 22, 1932, 0.1 cc. filtrate of Mixed Virus; paralysis 7th day.

In summing up the series of four monkeys (Tables V, VI, and VII) submitted to multiple courses of the instillation of virus, we find that, first, *Cynomolgus* A developed abortive symptoms of poliomyelitis from the first course, recovered, and proved durably immune, as shown by tests for antivirus and resistance to the cerebral injection of virus; second, that *Cynomolgus* B resisted five courses of instillation without producing detectable antivirus and without becoming in any degree refractory to a cerebral inoculation; and third, that *Rhesus* D behaved precisely as did *Cynomolgus* B, while *Cynomolgus* C resisted a first course of instillations merely to respond with paralysis to a second course given 2 months later.

Although these tests were carried out before the examination of the cerebrospinal fluid became a regular practice, there is every reason to believe that the succession of cell changes regularly occurred during the course of each series of instillations, as was always found to occur when examinations were made.

This series of tables completes the protocols of the main experiments. Tables VIII and IX which follow deal with complementary matters only. They are inserted to show: first, that the pleocytic cerebrospinal fluid contains no detectable virus at the height of the cell increases and even after acceleration inoculations; and, second, that repeated cisterna punctures in nonvirus-instilled animals do not change the average normal cell counts in the fluid.

TABLE VIII

Macacus rhesus Injected Cerebrally with Pleocytic Cerebrospinal Fluid from Nasally Instilled Monkeys

Monkeys	Virus strain, dosage, dates of instillation	Cells	Intracerebral inoculation	Symptoms
A	Mixed, 2 doses, 6/14, 6/15/33	29 (normal) 6/16–6/19, 65–165 Fluid withdrawn each day and pooled	6/20/33, 1.6 cc., 1st pooled fluid	No symptoms
		6/20–6/22/33, 320–570 Fluid withdrawn each day and pooled	6/28/33, 1.5 cc., 2nd pooled (accelerating) fluid	No symptoms
B	Philadelphia, 1932, 2 doses, 6/14, 6/15/33	19 (normal) 6/16–6/19/33, 37–118 Fluid withdrawn each day and pooled	6/20/33, 1.75 cc., 1st pooled fluid	No symptoms
		6/20–6/22/33, 470–515 Fluid withdrawn each day and pooled	6/28/33, 2 cc., 2nd pooled fluid	No symptoms

TABLE IX

Normal Macacus rhesus Controls, Cisterna Puncture, with Cell Counts

Monkeys	June 21, 1933	June 23, 1933	June 26, 1933	June 28, 1933	June 30, 1933
C	22	26	20	35	25
D	32	27	21	24	27

DISCUSSION

The protocols presented establish several important points. In the first place, they show unmistakably that the bringing of the virus into contact with the nasal membranes is never an indifferent process in monkeys. The response to the presence of active virus is prompt and invariable; and this response takes place irrespective of whether obvious clinical signs of disease do or do not arise, and always in advance of any such symptoms as may arise.

Most monkeys do not resist the initial course of instillations; the response has, perhaps, little relation to the number of instillations in monkeys of average susceptibility. Certain monkeys, however, while not markedly refractory, possess a degree of resistance above the average; they respond to the larger number of instillations, and there is inconclusive evidence that the spacing of the instillations makes a difference. Occasionally, monkeys in which the cells in the cerebrospinal fluid, while increased, remain at a low level, will develop higher cell counts if the animals are reinstilled on the 8th to the 10th day, a procedure to which in instances of intracerebral reinoculation we have given the name "acceleration." Instilled monkeys which pass through the usual course of incubation, showing beginning and then severe symptoms of poliomyelitis, have rising cell counts, coinciding with rising temperatures, while those which escape obvious clinical signs of infection tend to have lower cell counts and a correspondingly lower temperature range. Globulin as an index of inflammatory changes in the cerebrospinal fluid is irregularly demonstrable and bears an inconstant relation to high cell count.

Among the refractory monkeys which have resisted the initial course of instillations (although exhibiting changes in the cerebrospinal fluid) are some which, after a rest period, come down characteristically in response to a second course of instillations of the same virus as that employed in the first course. The reason for this disparity is not known. There remains a small residue of monkeys which, having resisted two such courses, now seems capable of resisting multiple courses of instillation without developing obvious symptoms of infection. None of these exceptionally refractory monkeys is indifferent to the virus instillations, for all react with cell changes in the cerebro-

spinal fluid. The refractory state, therefore, resides apparently in the nerve cells, the principal seat of usual virus attack—not in the nervous tissues as a whole.

Certain monkeys develop transient, slight, so called "abortive" symptoms of poliomyelitis as a result of the instillations. These animals have acquired increased resistance to virus injected into the brain, and their blood serum has become antiviral; and yet, reinstillation arouses cell changes in the cerebrospinal fluid qualitatively identical, quantitatively less marked, perhaps, than in monkeys failing to present obvious clinical signs of infection.

The series of reactions in the cerebrospinal fluid seems, therefore, independent of specific immune properties in the instilled monkeys. That there is no direct relationship between specific immunity and the changes induced in the cerebrospinal fluid is further shown by the important fact that, with present methods, active immunity invariably fails to develop in a detectable way, even in monkeys which have passed asymptomatically through multiple courses of instillation. These animals which are highly refractory to nasal virus (although always, probably, exhibiting cerebrospinal fluid alteration), never develop humoral antiviral properties, and are as susceptible to the cerebral injection of virus as are the normal controls.

The last observation brings us back to a consideration of the relationship which may exist between the monkeys nasally instilled which develop no symptoms of illness, and the many instances of general immunity arising spontaneously in human populations from the chance entrance of virus into the nasal passages. All that can be stated at present is that the two species behave in diametrically opposite ways. While there is indubitable evidence that unperceived immunization is taking place widely among human populations, there is also evidence that monkeys, while subject to the direct inoculation of poliomyelitis, are strongly refractory to the virus in highly dilute condition, such as occurs in ordinary contact exposures; and this refractory state is bound up with the complete inability of the monkey to initiate the requisite physiological changes which attend and lead to active immunity, independent of symptomatic response, when the virus is introduced in a way to reach the central nervous organs directly.

There is, therefore, a close correlation discernible between the unsuccessful cerebral and the asymptomatic nasal inoculation of virus. Probably the directly injected virus which does not lead to symptoms, produces changes corresponding to those readily detectable in the cerebrospinal fluid of nasally instilled animals; and as such asymptomatic, cerebrally injected monkeys have not been rendered immune, so those failing to respond with symptoms to nasal inoculation, similarly acquire no immunity. From this it would appear that in their fundamental physiological reactions to the virus of poliomyelitis, man and the monkey are widely divergent.

The virus passing by way of the olfactory area of the nasal membrane to the brain acts on tissues directly, and not through intermediation of the cerebrospinal fluid. This fluid remains constantly free of detectable virus. Even at the earlier stages (Table VIII) no virus can be demonstrated in the fluid; and in the course of active disease, when nerve and supporting tissue cells are severely injured, virus appears never to escape in ascertainable quantities into the fluid, either in man or the monkey. The virus displays strong avidity for cells, in this respect exceeding in cellular affinity other viruses which attack the tissue structures of the nervous system.

It would not be without interest to ascertain whether, in the process of unperceived mass immunization of children, cell changes occur in the cerebrospinal fluid. We know already that such changes attend mild illnesses believed to be poliomyelitis; opportunity to determine this point will arise in connection with outbreaks in institutions. And we may learn that during epidemic prevalences of poliomyelitis, cases which hitherto have been diagnosed mild poliomyelitis, merely because pleocytosis has been discovered in the cerebrospinal fluid of anxious and nervous individuals, may be the objective index of an otherwise unperceived process of active immunization taking place within them.

CONCLUSIONS

Macacus rhesus and *Macacus cynomolgus* exhibit a striking sensitivity to the presence of the virus of poliomyelitis on the nasal mucous membranes.

Irrespective of whether detectable symptoms of clinical poliomyelitis do or do not arise in the nasally instilled animals, the cere-

brospinal fluid changes quickly in response to virus placed in the nasal passages.

Two sets of changes occur in the cerebrospinal fluid: the constant and most pronounced change consists of increase in the content of white cells, chiefly of the lymphocytic type; the inconstant and less profound one consists of detectable amounts of globulin in the fluid (free from red corpuscles) withdrawn by cisterna puncture.

As early as 48 hours after instillation of the virus, a marked increase in cells is already detectable in the fluid; the increase grows from day to day, reaching a maximum sometimes in another day or two, sometimes not until 4, 5, or 6 more days elapse. In many instances a rise of temperature follows or coincides with the rising tide of cells; and the onset of clinical symptoms of disease bears also a relation to the cell count.

The number of cells in the cerebrospinal fluid and the temperatures tend to be higher in monkeys which develop paralytic symptoms; occasionally exceptions to this rule occur, in which instilled monkeys remaining asymptomatic exhibit high cell counts; very rarely do the latter show the higher temperatures.

Monkeys once instilled which fail to become symptomatically affected again react by cerebrospinal fluid changes to later courses of instillation. A second or still later course may induce paralysis; or highly exceptional or refractory animals may go through several courses of instillation without developing clinical symptoms, although never failing to respond with changes in the cerebrospinal fluid.

The virus instillations of *Macacus* monkeys do not lead to active immunization unless clinical symptoms of infection have resulted from the inoculations and attended the cerebrospinal fluid changes. In the complete absence of clinical symptoms the instilled animals fail to develop blood antiviral properties, and they are as susceptible to the cerebral injection of virus as are the control monkeys.

On the other hand, instilled monkeys which have shown even mild and fleeting (abortive) clinical symptoms of infection, resist cerebral inoculation and exhibit blood antiviral or neutralizing properties.

Monkeys which have developed clinical symptoms of disease and have recovered are, as stated, actively immunized; they remain, however, sensitive to the presence of virus on the nasal membrane, reacting

with cerebrospinal fluid changes, differing only in degree from the nonimmune animals.

Detectable virus does not appear in the pleocytic cerebrospinal fluid at any stage of the pathological processes.

The current belief is that mass immunization is proceeding in an unperceived manner through the chance entrance of virus into the nasal passages of children. It is not known whether, apart from all symptoms of disease, cerebrospinal fluid changes occur in the course of this unexpressed, spontaneous process. A large gap seems to exist between man and the monkey in the capacity of the former to become immunized by way of the nasal membrane, and the inability of the latter to do so. It is common knowledge that monkeys do not become immune through unsuccessful cerebral inoculations of virus, and the same seems to be true of the nasal channel of virus penetration into the central nervous organs.

BIBLIOGRAPHY

1. Jungeblut, C. W., and Hazen, E. L., *Proc. Soc. Exp. Biol. and Med.*, 1930–31, **28**, 1004.
2. Flexner, S., *J. Am. Med. Assn.*, 1932, **99**, 1244.
3. Flexner, S., *Science*, 1933, **77**, 413; **78**, 129.