RESPIRATORY VERSUS GASTRO-INTESTINAL INFECTION IN POLIOMYELITIS

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The belief is general among investigators, although not unanimous, that poliomyelitis is a particular kind of upper respiratory virus infection, in which the atrium of penetration of the virus into the nervous system is the olfactory area of the nasal mucous membrane. Indeed, evidence exists indicating strongly that the virus passes both to and from the nervous organs along this path, using the axons as lines of communication in both directions.

Wickman (1), in his now classical monograph, regarded the probable portals of entry of the then hypothetical infective agent of the disease to be both the respiratory and the digestive tracts. Pathological conditions of the former structures were slight and inconstant, while the small intestine, of children especially and the lower ileum in particular, frequently showed swelling of the lymphoid apparatus, with which was associated swelling of the mesenteric nodes.

Today we are better equipped to deal conclusively with the important problem of the portal of entry of the infecting agent, because we know it to be a virus and that *Macacus* monkeys are subject to inoculation with it. We possess, therefore, an effective method of investigating the efficiency of the two channels of infection, and coincidentally, we have means of determining the presence of virus in human cases in one or the other location, as well as the capacity of the virus to survive when experimentally introduced into the monkey.

While the respiratory source of infection is generally accepted, the gastro-intestinal source is much disputed. There is, indeed, no difference of opinion on the greater ease and frequency with which infection can be experimentally induced *via* the nasal passages, or virus detected in secretions from those passages. The real question

at issue is, whether under rigid experimental conditions, infection is ever secured by way of the stomach and intestines, and whether virus occurs in these viscera at all except by accident, and is capable of surviving in them beyond the time needed for its mechanical passage through these viscera.

We may largely disregard the early experimental tests of the adequacy of the gastro-intestinal route of infection, on the grounds that they were carried out at a time when understanding of the pathogenesis of poliomyelitis was still in a rudimentary state. The case for this mode of infection has been made anew, and strongly, by Levaditi, Kling, and their coworkers (2), in a series of papers extending from 1929 to 1933; and during the past two years in a series of reports by Toomey in this country. There is no real correspondence between the experiments and views of Kling and Levaditi and those of Toomey; but they agree in the essential point of their contention that the virus does penetrate to the central nervous system by way of the digestive tract.

The experimental work on which their deductions are founded is simple in the case of Levaditi and his associates. They believe that they have discovered in *Macacus cynomolgus* a species more subject to poliomyelitis than its close relative, *Macacus rhesus*, which hitherto has been chiefly employed for experimental inoculation. While *Macacus rhesus* has not served them for feeding tests with virus, *Macacus cynomolgus* has been held to do so. The experiments themselves are performed very simply. Virus is suspended in milk or other diluent, and introduced by tube into the stomach; or it is mixed with banana and fed in the ordinary way. And in order to circumvent the stomach and the buccal and nasal cavities, it is injected directly into the intestine of laparotomized animals.

The actual number of experiments carried out on *Macacus cynomolgus* was not large, but the high proportion of successful tests makes up for the limited number of feedings. Of seven *cynomolgi* fed by tube or otherwise, six developed paralytic poliomyelitis. Two of this species received direct, syringe injections into the small intestine, from which one did and the other did not become paralyzed. And, finally, of three *Macacus rhesus* inoculated intracerebrally with mesenteric nodes removed from three of the fed paralyzed monkeys, two responded with typical poliomyelitis.

The conclusions drawn by them from these tests are sufficiently obvious: *Macacus cynomolgus* is highly susceptible to fed virus and also to virus injected directly into the intestine; from the intestine the virus penetrates to the central nervous system along two channels—nerves and lymph (ultimately to the blood). No explanation is offered as to the way the virus reaches nerve channels through the intact mucous membrane. A single experiment made many years ago by Landsteiner and Levaditi, in which virus was injected directly into the mesenteric vein, is cited to show that it is possible for lymph or blood to carry virus to the central nervous organs under circumstances leading to the paralytic disease.

The theoretical and practical significance of these experiments is so large that they were promptly repeated by Clark and his associates (3), who failed to confirm them, and by Dr. C. P. Rhoads, whose results also were negative. Rhoads went a step further and subjected the previously fed monkeys to nasal instillations of virus; the animals responded with typical symptoms of paralytic poliomyelitis.[1] This test did away with the argument of individual lack of susceptibility advanced by Levaditi to explain the failure of certain feeding tests.

Recently Toomey (4) has attacked anew the question of the gastro-intestinal portal of entry of the virus, and has devised experiments which give characteristic positive and negative infection results. He has, however, departed widely from the methods previously employed which sought to do the minimum degree of damage to the intestinal structures and to maintain them, at the time of inoculation, in a state of physiological function. Toomey's manner of approach may, therefore, be called drastic. He clamped off sections of the small intestine and filled them to ballooning with suspensions of virus; infection sometimes followed. Or he injected virus into the subserosa; here, again, paralysis sometimes occurred. And, finally, he combined virus with a toxic filtrate of colon and other intestinal bacteria, and found such mixtures even more effective in producing paralysis in the overdistended, constricted gut or the inoculated serosa, than virus alone.

Toomey's experiments are founded on the long known fact that whenever virus comes into close relationship with nerve fibrils, infection may occur. Now, unmyelinated fibers, the most readily entered by the virus, are embedded in the intestinal wall. It is apparent that the particular techniques Toomey employed favor the making of the necessary contact. It matters little whether the path to the nerve fibers is supplied by mechanical means or by the use of a chemical agent, such as the "enteric toxin," which is not an indifferent substance, as shown by the "diarrhea and anorexia" it provokes. Such bacterial extracts have long been known to produce focal necrosis and hemorrhage in the intestine, and they in turn provide the breaks in continuity favoring the passage of virus into the submucosa, where unmyelinated fibers abound.

It may, therefore, be questioned whether Toomey's experiments do more than bring new evidence to support the impenetrability of the intact mucosa of the intestine to the virus. It is probably true, as he holds, that virus injected directly into the gut, after laparotomy, is swept onward; and yet, success has not been wanting to this class of experiments, as shown by the early ones of Leiner and von Wiesner, and the recent experiments of Kling and Levaditi. The explanation of the success is to be sought probably in the contamination, in the course of the needle puncture, of the wall of the intestine containing nerve fibers. That fed virus is not swept rapidly through the gut is revealed by extraction tests made with the feces; adequate time is afforded for the contact of vast amounts of virus with the mucous membrane, and yet when precaution succeeds in keeping virus away from the buccal and nasal cavities, symptoms of infection fail to arise.

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[1] These experiments, although carried out in 1929, have not been previously reported.

EXPERIMENTAL

Feeding Tests on Cynomolgi.—Rhoads' feeding experiments departed in detail from those of Kling and Levaditi, and wholly in that he later nasalized with the same strain of virus the monkeys which had failed to react to the fed material. The table which follows summarizes the tests.

TABLE I

Rhoads' Feeding Tests in Macacus cynomolgus, 1929

Monkey	No. and date of feedings	Amount of feeding	Mixed Virus suspensions	Interval	Preliminary treatment	Result
	1929	*cc.*	*per cent*	*wks.*		
Cynomolgus						
1	9, Nov. 1	25	20	1	$NaHCO_3$ + opium	Died of intercurrent disease
2	9, " 1	25	20	1	" " "	No symptoms
3	2, Dec. 12	12	20	2	" " "	" "
4	2, " 12	12	20	2	" " "	" "

Of these four monkeys, one died of intercurrent disease; the others remained free from all symptoms for an indefinite period of time. After a rest period of 4 or more weeks, the survivors were given nasal instillations of 10 per cent glycerolated virus; they all showed symptoms on the 8th to the 12th day after the first instillation. The symptoms were abortive in one monkey, and they progressed to paralysis and death in the other two animals. Leg paralysis first manifested itself in one, and face and eyelid paralysis (double ptosis) in the other. These results speak for themselves.

The severe epidemic of poliomyelitis of 1931 led us to reexamine the question of the gastro-intestinal portal of entry of the virus. We proceeded in a somewhat more comprehensive manner, studying the effects of tube feedings in ways simulating those employed by Kling and Levaditi, and searching anew for the virus in the intestine and the mesenteric nodes of human victims succumbing to the acute disease. As will be observed in Table II, three *cynomolgi* were tube fed, and three dropper fed. The latter animals were held in a semirecumbent position, and the suspension was dropped slowly into the mouth, time being allowed for swallowing. The three abundantly tube fed

monkeys all remained well. It is noteworthy that the only animal developing symptoms was dropper fed. The chances of buccal and nasal contamination are obviously greater in dropper than in tube feedings.

TABLE II

Feeding Tests in Macacus cynomolgus, 1931

Monkey	No., date, and manner of feedings	Amount of feeding	Mixed Virus suspensions	Interval	Results
	1931	*cc.*	*per cent*		
Cynomolgus					
1	7, Sept. 28, stomach tube	180	10	Daily	No symptoms
2	7, " 28, " "	180	10	"	" "
3	7, " 28, " "	410	10	"	" "
4	7, " 28, dropper	180	10	"	" "
5	7, " 28, "	210	10	"	" "
6	7, " 28, "	180	10	"	*Left leg flaccid; right leg partially paralyzed. Sacrificed for histology

* The lesions, typical of poliomyelitis, were widespread (5).

The surviving, symptomless *cynomolgi*, 1 to 3 months after the feedings, were instilled on 6 successive days with suspensions of the same strain of virus used in the feedings. 4 to 7 days after the last instillations, four animals manifested symptoms; three developed widespread paralysis; the fourth passed through an abortive attack of poliomyelitis. It succumbed to a second course of instillations in a typical manner. One monkey escaped infection.

Four control monkeys were used in the nasal test—two *cynomolgi* and two *rhesi*. One of each pair became paralyzed. The previously fed *cynomolgi* proved, therefore, at least as subject to infection by the nasal route as the control animals.

To these two sets of essentially negative feeding tests made with *Macacus cynomolgus* should be added a third carried out at a later date. This experiment was made in order to examine the mesenteric nodes for virus, since Kling and Levaditi had been successful in producing paralytic infection with nodes taken from fed animals. The mesenteric nodes in *Cynomolgus* A and B were removed surgically. After recovery and a proper interval, the nasal instillations were given.

TABLE III

Feeding Tests in Macacus cynomolgus, 1934–1935

Monkey	Dates of tube feedings	Amount of feeding	Virus suspensions	Results
		cc.		
Cynomolgus A	Oct. 15, 1934	5	25 per cent Mixed Virus	No symptoms
	" 20, 1934	5	25 " " " "	" "
	" 25, 1934	10	25 " " " "	" "
	Feb. 16, 1935	25	25 " " Philadelphia, 1932, virus	" "
Cynomolgus B	Oct. 15, 1934	10	25 per cent Mixed Virus	" "
	Feb. 16, 1935	25	25 " " Philadelphia, 1932, virus	" "

TABLE IV

Nasal Tests in Macacus cynomolgus, 1935

Three instillations of Philadelphia, 1932, virus given on Mar. 19, 21, 25, 1935

Monkey	Cells in cerebrospinal fluid		Results
	Dates	Cells	
	1935		
Cynomolgus A	Mar. 19	16	Temp. 103–103.4°F.
	" 21	24	
	" 25	27	
	" 27	40	
	" 29	420	Excited; globulin ++; Temp. 104.8°
	" 30	—	Paralysis; Temp. 105.8°
	Apr. 1	—	Prostrate; " 103.4°
Cynomolgus B	Mar. 19	21	Temp. 102.6–103°F.
	" 21	20	
	" 25	29	
	" 27	185	Temp. 104°
	" 29	460	" 107°. Tremor; ataxia
	" 30	—	Paralysis
	Apr. 2	—	Prostrate

Comments.—The feeding tests carried out by Rhoads and ourselves from 1929 onwards confirm those of Clark and his associates; and they stand in sharp contrast to those of Kling, Levaditi, and their coworkers. They are perhaps more convincing because they are more complete than any similar tests previously made, by reason of the comparisons made on the fed animals with respect to nasal susceptibility to the same strain of virus. The contrast is very impressive and brings into sharp relief the fact of the impenetrability of the normal intestinal mucous membrane to virus present in enormous amounts, at the same time that the fed monkeys are capable of taking up readily, by way of the olfactory area of the nasal mucous membrane, far smaller quantities of virus, which induce characteristic experimental poliomyelitis.

We may now summarize the results as given by Kling and Levaditi and obtained by ourselves. The former fed seven *cynomolgi*, of which six became paralyzed; we fed eleven, of which ten remained symptomless. Of ten of the monkeys of the latter series, subjected later to nasal instillation, nine responded with symptoms (and lesions) of the disease. We believe, therefore, that when paralysis arises in the course of the feeding experiments, contamination of the buccal and nasal membranes with virus may be considered to have taken place.

Direct Intestinal Injection of Virus

Leiner and von Wiesner (6) found in 1910 that laparotomized *Macacus rhesus*, although resisting direct feedings, would respond with paralysis to virus directly (needle) injected into the small intestine. This particular experiment has been repeated from time to time with contradictory results. Kling and Levaditi performed it on *cynomolgi*, in keeping with and in support of their belief that this species of *Macacus* is more subject to infection by the intestinal route. Of two *cynomolgi* injected by them (on both the needle puncture was seared with cautery), one remained well and one became paralyzed. Clark and his associates injected four laparotomized *cynomolgi*: two died of intercurrent disease; the other two remained well. The results obtained by us in seven tests, in which direct intestinal injection of virus was made, are summarized in Table V.

Comments.—In the series of experiments summarized in Table V

TABLE V

Direct Intestinal Injection of Virus, 1934–1935

Monkey	Date of inoculation	Weight	Virus suspensions	Results
	1934	*gm.*		
Cynomolgus				
1	Nov. 19	1600	1 cc. 25 per cent Mixed Virus	No symptoms
	1935			
2	June 27	1675	25 " 25 " " Philadelphia	" "
3	" 27	1360	5 " 25 " " "	" "
4	Mar. 21	1885	25 " 25 " " "	Mar. 28, Temp. 102.4°F. Mar. 29, 10 a.m., Temp. 104.6°. 5:30 p.m., 105.4°, ptosis, tremor Mar. 30, dead. Typical lesions; puncture wound closed; hemorrhage into intestinal wall
5	" 21	1700	25 " 25 " " "	Apr. 8, Temp. 105.4°, 524 cells in cerebrospinal fluid Apr. 9, ptosis; tremor Apr. 10, death, respiratory failure. Typical lesions
Rhesus				
6	May 3	1650	25 " 25 " " "	No symptoms
7	" 3	1580	5 " 25 " " "	" "

attention is directed to Monkeys 3 and 7, which were chosen because they were smaller and younger than those usually employed for experiment. Of the two *cynomolgi* becoming paralyzed, Monkey 4 proved informing. Microscopical examination of the injection site, which to gross inspection was healed, showed hemorrhage into the intestinal wall, a finding which supported our belief that in the making of such injections of virus, the latter may readily be introduced into the tissues, as well as into the intestinal lumen. It may be regarded

as the more remarkable that these drastic inoculations do not lead more often to symptomatic effects. An illustration of the capriciousness of this general class of experiments is to be found in Monkey 1. On February 16, 1935, this *cynomolgus* was given by tube 25 cc. of a 25 per cent salt solution suspension of Philadelphia virus. On March 7 (19 days after the feeding) the animal was tremulous and ataxic, and showed deltoid paralysis. The cerebrospinal fluid contained 434 cells and the globulin was +. Eventual recovery with residual arm paralysis ensued.

Mesenteric Nodes

Kling and Levaditi, in their support of the invasion of the virus from the digestive tract, revived the question of the infectivity of mesenteric nodes. In two instances—one after feeding, the other after injection of virus into the intestinal lumen in *cynomolgi*—they induced paralysis by inoculating the nodes cerebrally into *Macacus rhesus* monkeys. This result led them to postulate a lymphatic and eventual blood carriage of virus to the central nervous system, in addition to the recognized, more direct, neural route. They offer two experiences in support of this point of view, one being the successful inoculation by Flexner and Lewis (7) of mesenteric nodes removed at human autopsy, and the other the injection of virus by Landsteiner and Levaditi (8) into the mesenteric vein of *Macacus* monkeys.

These early tests scarcely suffice today, in view of the greater stringency of the experimental technique. The node removed at autopsy in 1910 was taken in the course of the ordinary procedure, without the use of sterile instruments; in making the intravenous injection, the danger of perivascular nerve contamination was not regarded.

We have, therefore, restudied this subject, employing for inoculation mesenteric nodes removed aseptically at autopsies performed on acutely fatal human cases, before the brain and spinal cord were exposed, and from fed *Macacus rhesus* and *cynomolgus* monkeys. We have gone beyond the mere testing of the nodes and have tested also the intestinal mucosa of the lower ileum, in which the lymph nodes are hypertrophied. The 1931 epidemic of poliomyelitis provided us with nodes from three autopsies; the inoculations were made with the fresh nodes and with glycerolated specimens for acceleration. Eight *rhesi* were injected cerebrally with this material; symptoms did not arise in any instance. In three of the eight animals, nodes from single human cases, and in five the pooled nodes from all three, were employed as inocula. During the recurring outbreaks of poliomyelitis

in New York from 1911 to 1913, nodes from five acutely fatal cases were injected cerebrally, peritoneally, and into the sciatic nerve; in no instance was disease produced.

The mucosa of the ileum containing the enlarged nodules was scraped off, suspended in salt solution, after being ground with sand, shaken, centrifuged, and filtered through Berkefeld candles, the filtrate being concentrated *in vacuo* by Clark's method and injected cerebrally and peritoneally into three *rhesi*. No symptoms arose. These negative tests confirm a larger series made in 1911 to 1913 with intestinal mucosae rendered bacteria-free with 0.5 per cent phenol.

Two recent sets of experiments designed to show whether virus introduced into the stomach by tube, or into the intestine by needle injection, passes in detectable quantities into the mesenteric nodes, are summarized in the following protocols.[2]

Protocol I. Macacus rhesus.—(1) 50 cc. of a 20 per cent suspension of virus tube fed; after 3 hours, mesenteric nodes removed surgically;[3] 5 days later additional nodes excised. Suspensions of each set of nodes were injected cerebrally and peritoneally into a *Macacus rhesus*. No symptoms developed.

(2) 100 cc. of a 20 per cent suspension of virus tube fed; second similar feeding 4 hours later. 7 hours after first feeding, nodes removed surgically and injected cerebrally and peritoneally into a *Macacus rhesus*. No symptoms followed. At the time of the second removal of nodes, rectal washings made and the fluid passed through a Berkefeld candle; filtrate injected cerebrally and peritoneally into two *rhesi*. Both became paralyzed; spinal cord lesions typical.

(3) Two *rhesi* were fasted, laparotomized, and each given 80 cc. of a 20 per cent suspension of virus into the ileum. 6 hours after operation, nodes were excised surgically and injected cerebrally and peritoneally into two healthy *rhesi*. No symptoms arose either in the monkeys given mesenteric node injections or the injections into the gut.

Protocol II. Macacus cynomolgus.—(1) Three tube feedings of 5, 5, and 10 cc. of a 25 per cent suspension of virus given a *cynomolgus*. 11 days after the first feeding no symptoms had arisen. Nodes were removed and injected cerebrally; 7 days later an accelerating injection given. No symptoms appeared in fed or injected animals.

(2) *Cynomolgus* laparotomized and given heavy suspension of virus by direct, needle, injection into ileum. 11 days later nodes were excised and injected cerebrally into a *rhesus*; accelerating injection given 7 days later. No symptoms arose in either animal.

(3) 10 cc. of heavy suspension of virus tube fed. After 11 days, removed

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[2] Ether anesthesia was employed in all operative procedures.

[3] I wish to thank Dr. Harold L. Amoss for carrying out the operative procedures.

nodes injected cerebrally into a *rhesus*; accelerating dose 7 days later. No symptoms appeared in either animal.

(4) 25 cc. of a 25 per cent suspension of virus injected into ileum of laparotomized *cynomolgus*. Symptoms of poliomyelitis appeared on the 19th day, death ensued on 21st day. The excised nodes were injected twice: immediately at the time of autopsy, and 7 days later. No symptoms arose.

Comments.—The larger series of negative tests given with mesenteric nodes and intestinal mucosa from acutely fatal human cases, and from the tube fed and needle injected monkeys—both *Macacus rhesus* and *cynomolgus*—carry their own interpretation. They stand in sharp contrast to the early, successful inoculation of human mesenteric nodes reported by Flexner and Lewis, and the recent ones of Kling and Levaditi. We believe that the original experiment of Flexner and Lewis was faulty in technique; we have no explanation to offer for the positive inoculations reported by Kling and Levaditi. There is one unequivocal report among a number of dubious ones, of the finding of the virus in the intestinal washings of a fatal human case. This case, reported by Kling, Pettersson, and Wernstedt (9), takes on additional interest because the tracheal washings were also infectious. It is open to conjecture whether in this exceptional instance, virus from the nasopharynx was not both swallowed and aspirated in considerable amounts during the last hours of life.

Intravenous Inoculations

Kling and Levaditi cited an earlier successful injection of virus into a mesenteric vein as indicating the occurrence of a lymph-blood conveyance of the infectious agent to the central nervous system in the monkey. In this way the significance of the demonstration of virus in the mesenteric nodes of fed monkeys was emphasized. The comprehensive series of tests made by Flexner and Amoss (10) would seem to negative this point of view; and the failure by us to induce poliomyelitis by the injection of mesenteric nodes of man and the monkey, and of the mucosa of the ileum of man, contrasts with the two successful experiments of Kling and Levaditi.

In referring to the instance in which Landsteiner and Levaditi induced paralysis by virus injected into the mesenteric vein, we stated that no account had been taken of the perivascular nerves which, if contaminated, would convey the virus to the spinal cord. We have

tested this point in two ways, of which one incidentally again brings the vascular route under examination. In one experiment four *Macacus rhesus* monkeys were injected intravenously on 7 successive days with 5 cc. of a 5 per cent suspension of virus, by way of the saphenous vein. Three control *rhesi* were given nasal instillations an equal number of times. The controls became paralyzed on the 10th, 11th, and 17th day respectively. Three of the four intravenously inoculated monkeys remained well, the fourth becoming partially paralyzed as described in the following protocol. In the other experiment, virus was injected directly into the vascular wall.

Protocol. Macacus rhesus.—June 10 to 16, 1931, seven daily intravenous injections of 5 cc. of 5 per cent glycerolated suspension of Mixed Virus. June 25, tremor; ataxia; right deltoid paralyzed; legs very weak. June 26, right arm paralyzed; legs almost paralyzed; sits up. July 1, improved. Oct. 2, complete recovery.

This experiment is virtually a confirmation of the earlier results obtained by Flexner and Amoss. It is, however, desirable to account for the occurrence of paralysis in the one monkey of the four. The injection by syringe of the virus into the lumen of the vein cannot be carried through in all instances without some degree of contamination of the vascular wall and contained nerve fibers. Hence two monkeys received injections of virus directly into the coats of the exposed saphenous vein, where it would come into close relation with nerve fibrils.

There is still another point to be considered. Toomey (11) has emphasized the unmyelinated nerve fibrils in the skin in order to account for the occasional paralysis arising during active immunization of virus intradermally. The like behavoir of the unmyelinated fibers in the intestinal wall and the skin is brought together by him in this way.

Since it is not feasible to make successive inoculations into the submucosa, it was decided to study also the cerebrospinal fluid in monkeys receiving multiple intradermal injections of virus. A deduction could be made of the ease with which the nerve fibrils in these two locations carry virus to the center as compared with the olfactory areas in the nasal membrane.

Protocols. Saphenous Vein Experiment.—Two *Macacus rhesus* monkeys were operated under ether anesthesia and aseptic technique. The long saphenous vein

in the right side was exposed for 3 or 4 inches. 5 per cent suspension of glycerolated Mixed Virus was injected at several points into the perivascular tissues and muscularis; blebs were raised in the latter. The cells in the cerebrospinal fluid were counted and temperatures taken.

Monkey A			Monkey B		
Dates	Temperatures	Cells	Dates	Temperatures	Cells
1935	°F.		*1935*	°F.	
Oct. 11		19 (normal)	Oct. 11		9 (normal)
" 12	103.4	25	" 12	102.8	14
" 14	103	57	" 14	103.2	18
" 16	104.2	25	" 16	103.8	45
" 18	104	12	" 18	104.2	115
				Monkey excited	
" 21	102.8	14	" 21	103	52
				Less excited	

Comment.—Both Monkeys A and B responded to the virus injected into the vascular coats. The effect on the cerebrospinal fluid of Monkey A was rapid, but transitory, and no clinical symptoms appeared; while the effect on the cerebrospinal fluid of Monkey B was more slowly developed, but more pronounced. This animal had what was probably a mild abortive infection. Hence the virus actually passed from the coats of the vessels along nerve fibers to the center, as had been surmised in the monkey (page 220) responding to the repeated intravenous inoculations.

Skin Experiment.—Two *rhesus* monkeys were given ten intradermal injections of 2 cc. each of 5 per cent Mixed Virus at 3 day intervals. Temperature readings and cell counts in the cerebrospinal fluid were made.

Monkey C			Monkey D		
Dates	Temperatures	Cells	Dates	Temperatures	Cells
1935	°F.		*1935*	°F.	
Sept. 14		2? (normal)	Sept. 14		24 (normal)
" 16	102.8	17	" 16	102.4	25
" 19	102.4	22	" 19	102	29
" 21	103.2	27	" 21	103.8	23
" 26	104	24	" 27	103	19
" 30	103.2	45	" 30	104	70
Oct. 3	103	37	Oct. 3	103	18
" 10	102.8	31	" 10	103	22

Comment.—The reactions in the cerebrospinal fluid from successive intradermal injections of virus were negligible. A transient increase in the cell count occurred in Monkey D; it was unattended by clinical symptoms. The nerve fibrils in the skin are obviously much poorer conductors of virus to the center than are those of the olfactory areas in the nasal membranes. This fact could be inferred from the great differences in clinical symptomatic response from the two kinds of inoculations. Not improbably the unmyelinated fibers in the wall of the intestine are also relatively poor conductors, as compared with the olfactory nerves.

Nerve Conduction

That the virus of poliomyelitis has an especial affinity for nerve structures and tends to travel along the axons is generally believed. No other virus is known to be so strictly neurophilic. Although a more sensitive indicator than the monkey might reveal it in the general tissues and humors of the body, at present we are ignorant of its affinity for these tissues or its capacity to survive in blood or lymph. Its absence from the cerebrospinal fluid under circumstances in which it is abundantly present in the adjacent, damaged cells of the spinal cord and brain, is noteworthy (12). One set of conditions is known to exist in which at least survival occurs in organs remote from the central nervous system. Flexner and Amoss (13) injected large amounts of virus intravenously into a *rhesus* monkey; no symptoms followed, and when the animal was etherized 17 days later, a suspension of the spleen proved infective when inoculated cerebrally into another monkey. At the same time the spinal cord and medulla were without effect.

On the other hand, virus introduced into and sealed in the brain passes into the nasal mucous membrane and its secretions, as far as known, only along the olfactory nerve filaments; it also passes, as Flexner, Clark, and Amoss (14) showed, along nerve channels to the abdominal sympathetic ganglia. The virus of poliomyelitis, therefore, shares with the viruses of rabies and Borna disease, the property of wandering long distances along nerve fibers. Tests for virus in the abdominal sympathetic ganglia were carried out again in 1933. As the protocols which follow show, the presence there was again demonstrated.

Protocol. Experiment A.—Dec. 8, 1933, *Macacus rhesus* given double (intracerebral and intraperitoneal) injections of suspension of abdominal sympathetic ganglia taken from two *rhesi* acutely paralyzed after cerebral inoculation of virus. Dec. 18, tremor, left facial and leg paralysis. Dec. 20, dead.

Experiment B.—Dec. 20, 1933, sympathetic ganglia from Monkey A doubly injected into a *Macacus rhesus*. On the 8th day a second (acceleration) inoculation of the ganglia from an acutely paralyzed monkey. Slight, evanescent symptoms lasting 2 days followed the accelerating inoculation. Result doubtful.

Elimination of Virus

Intestinal.—The manner of elimination of the virus from the digestive tract has a bearing on the disputed question of the gastro-intestinal portal of infection. Levaditi, Kling, and Lépine investigated this subject, with results so irregular that interpretation is made difficult or impossible. Their methods of procedure were not such as to make success probable, as they employed for inoculation unconcentrated filtered extracts of the fecal discharges of fed monkeys or of artificial mixtures of feces and virus. Clark and his associates applied the effective concentration and dialysis method they devised to a search for virus in the dejecta from the intestine. Their positive results may be regarded as conclusive, and the slight irregularity in them such as would be expected to arise from the complex materials subjected to extraction and the small number of monkeys inoculated with the concentrates. In brief, their findings show that after direct intestinal injection of virus, infective doses may be recovered from the feces; that, similarly, the feeding of virus suspensions or filtrates for several days, is followed by the elimination of virus in a viable and infective state; and also that the mere feeding of large quantities of filtrate for one day, suffices to give to the dejecta effective properties.

Dr. Henry W. Scherp has investigated this subject, in a somewhat more extensive manner, using the Clark concentration method of preparing the extracts. The results obtained are given in the protocols that follow.

Protocol I. Macacus rhesus.—Two monkeys were fed by stomach tube 30 cc. of a 10 per cent milk suspension of Mixed Virus on 2 successive days; feces collected from 6th to the 30th hour after second feeding. The extracted filtrates, concentrated and dialyzed, were inoculated into two *Macacus rhesus* by intracerebral and intraperitoneal injections. Both inoculated monkeys became prostrate on the 7th day. The fed animals remained well.

Protocol II. Macacus rhesus.—Two monkeys received 30 cc. of a 10 per cent milk suspension of Mixed Virus by stomach tube on 2 successive days. Feces were collected from each at 24 and 96 hour intervals and 5 days after second feeding. The concentrate from each was injected intracerebrally and intraperitoneally into *Macacus rhesus*. The 24 hour fecal specimens only yielded infective filtrates; the animals receiving the concentrates prepared with the 96 and 120 hour specimens remained well. The fed animals showed no symptoms.

Protocol III. Macacus cynomolgus.—Fed by stomach tube on 4 successive days with 30 cc. of 10 per cent suspension of virus in milk. Feces collected 24 hours and 5 days after last feeding. Concentrates prepared from each specimen, and inoculated intracerebrally and intraperitoneally into *Macacus rhesus* monkeys. The animal receiving the 24 hour specimen became paralyzed; the animal receiving the 5 day specimen remained well. The fed animal remained well.

Although the tests represented in the protocols are few in number, they are consistent in showing that when large feedings are given and the virus reaches the intestine in an active state, it fails to attach itself to the mucous membrane in a way leading to prolonged elimination or increase in amount. The gut is not a favorable locus for the virus. Under the circumstances it is not, therefore, among other things probable, as Kling and Levaditi would have it, that virus escaping from the intestine may contaminate potable water supplies, be thus widely distributed, and result in water-borne epidemics of poliomyelitis (15).

Nasal and Buccal Membranes

The presence of virus in the secretions or in the substance of the nasal and buccal mucous membranes in man and the monkey has been established beyond all doubt (16). The detection of virus in these locations is difficult, because of inadequacy of methods of securing it in sufficient concentration for favorable inoculation. Scherp tested the Clark concentration method on the excised mucous membranes of paralyzed monkeys. Three tests were made. In one the virus had been injected cerebrally; in two, nasal instillations had been given. Two of the three tests were successful, in that virus in infective quantities was recovered—one from the cerebrally inoculated, and one from nasally instilled monkeys. The extracts contained a troublesome amount of mucus which made filtration difficult. The results are given in the protocols, since they may have interest in leading to a more effective way of finding virus in the upper respiratory membranes.

Protocol I. Macacus rhesus.—Given two double (cerebral and peritoneal) injections of concentrate prepared from two *rhesi* cerebrally injected with Mixed

Virus, paralyzed on the 7th and 8th day respectively. The first inoculations were made on Dec. 24, 1931, and the second (accelerating) on Jan. 1, 1932. 7 days after the second injections there were tremor, ataxia, and right arm paralysis. 2 days later the monkey was moribund. The lesions were typical of poliomyelitis.

Protocol II. Macacus rhesus.—Doubly injected with concentrate prepared from two monkeys which became paralyzed following six daily nasal instillations of Mixed Virus, one on the 10th and the other on the 14th day after the first instillation. The first injections of concentrate were given on Dec. 15, and the second (accelerating) on Dec. 23, 1931. 7 days after the second inoculation there were present tremor and leg paralysis. The lesions in the spinal cord were characteristic.

SUMMARY AND CONCLUSION

The debated problem of gastro-intestinal *versus* respiratory mode of infection in poliomyelitis has been restudied by several investigators recently, with conflicting findings. Kling and Levaditi in Europe carried out experiments from 1929 to 1933, which led them to the conclusion that the digestive tract affords a ready entrance of the virus of the disease into the body. They believe that the substitution of *Macacus cynomolgus* for *Macacus rhesus* as the animal of choice for the tests supports this point of view. Toomey in the United States has arrived at a similar conclusion, not by employing a particular species of monkey for experiment, but by the use of drastic measures of inoculation, which insure that the virus makes contact with the unmyelinated nerve fibers embedded in the intestinal wall. Toomey's methods are so severe and artificial that his results cannot be regarded as simulating a natural mode of infection.

We have repeated the tests of Kling and Levaditi, but in a far more comprehensive manner than was followed by them, and, like Clark and his associates who early repeated them, we have failed to confirm them. Indeed, we do not find *Macacus cynomolgus* and *rhesus* to differ in any essential way in their response to the presence of the virus of poliomyelitis in the body. *Cynomolgi* do not respond to virus introduced into the stomach when contamination of the buccal and nasal cavities is avoided; they respond, as do *rhesi*, to virus directly injected into the intestine when virus passes into the intestinal wall and makes the necessary nerve fiber contact. Both *Macacus cynomolgus* and *Macacus rhesus* which have resisted feedings of virus are subject to nasal instillations of the same strains of virus and in the same degree.

On the basis of the experiments reported in this paper we can reaffirm the conclusion previously arrived at by ourselves, and confirmed

independently by investigators in Europe and America, namely that the only established portal of entry of the virus of poliomyelitis into the central nervous system of man is the nasal membrane, and especially the olfactory nervous areas in that membrane.

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