REINFECTION (SECOND ATTACK) IN EXPERIMENTAL POLIOMYELITIS

By SIMON FLEXNER, M.D.

(From the Laboratories of The Rockefeller Institute for Medical Research)

(Received for publication, January 11, 1937)

The phenomena of immunity in poliomyelitis have been the subject of increased interest and experimental inquiry in the last few years. To a certain extent the renewed interest owes its origin to the efforts made recently to immunize children in mass against the disease (1). The undertaking, now discontinued, was based on two observations made early in the experimental study of the malady, (*a*) that monkeys which have recovered from an attack tended to resist reinoculation with the virus and (*b*) that animals given a number of subinfective doses developed humoral neutralizing antibodies (2). The existence of the immunity and the presence of the antibodies were so correlated as to make it appear that the one condition depended on the other.

As experience has grown it has become apparent that the two immune states, one based on recovery from an attack of the disease and the other a symptomless reaction to virus injections, are not strictly identical. They do agree in that under both sets of conditions humoral antibodies usually appear; they differ in that symptomless immunization is less protective against reinoculation than is the state of resistance which develops upon an active or symptomatic infection.

On the other hand, that reinfection, called second attack, does occur after recovery from frank poliomyelitis is established for children and for monkeys (3). The phenomenon of reinfection has been given little attention and so far has been dealt with only in connection with the epidemic disease in man concerning whom some dozen cases have been reported in the literature. The problem of reinfection can be more thoroughly studied in the monkey, and this paper will be devoted to the presentation of the results of such a study which has been carried on during the past several years as material for it became available.

It is well known that experimental poliomyelitis is far more severe

and fatal in the monkey than the natural disease in man. Although the monkey is not a natural host, it can be infected readily with the virus by means which closely simulate the mode of infection now recognized as occurring ordinarily in man. When the paralysis in monkeys is extensive, death usually results; but every so often one of these animals may be nursed back to recovery which, as in man, is sometimes complete and sometimes attended by residues of permanent paralysis.

Moreover the monkey at times develops milder forms of the infection in which the paralysis is wanting or is of limited extent, in which cases recovery is the rule. In these latter instances a few days may witness the onset, extension, and disappearance of the paralysis. This class of infections has been compared with the abortive cases of the disease in man. Both in man and the monkey humoral antibodies tend to accompany these milder, as they do the more severe, forms of infection.

The recognized portal of entry of the virus in man is the olfactory nasal membrane, and the instillation of virus into the nares of monkeys is an effective way of producing infection. In the study of reinfection in monkeys which follows, the virus has been inoculated intranasally. This form of instillation avoids all injury of tissue and by employing the olfactory nervous tract for the introduction of the virus into the brain and spinal cord, places it in that peculiar relation to the ordinary defensive mechanisms of the body which we assume to occur in the course of the human infection.

The inoculation of monkeys is made with quantities of virus which are much greater than operate to produce infection in man. The limits of activity of the virus instilled nasally are wide, but they are less wide than with other neurotropic viruses. There is no appreciable difference in the infective effects between a virus containing 1 and 10 per cent of the spinal cord of previously infected monkeys, and there is little difference between the effects produced by one or two and six or seven daily instillations of the virus suspension. But the virus itself displays puzzling and capricious fluctuations of activity which make consistent experiment difficult. The nature of the causes which are responsible for the changes in activity is not known. The several kinds of virus employed in the experiments reported were obtained

from human cases of poliomyelitis occurring in 1909 and the succeeding epidemic years to about 1920, and later in 1928, 1931, 1933, 1934, and 1935.

The specimen now called mixed virus was first successfully passaged in monkeys in 1909, and as its activity fluctuated, strengthened by additions of active specimens as they were secured up to 1920 or thereabouts. Mixed virus is therefore a polyvalent specimen, a fact which is not to be lost sight of in considering the protocols to follow. The mixed virus contains no admixture of specimens secured in the epidemic years in New York of 1928, 1931, 1933, and 1935, the epidemic year in Philadelphia of 1932, and the Cuban epidemic year of 1934. Although it will appear that certain of the recovered monkeys were originally inoculated with specimens of 1928, 1931, 1933, and 1935, it will also appear that the specimens of these dates were rarely used for reinoculation for the reason that they have fallen below the standard of activity or virulence demanded for these tests.

The results of the nasal instillations of the virus into monkeys differ in an essential way from the results of the chance effective entrance of the virus into the nose in man. The incidence of frank poliomyelitis in children even in epidemic years is low; the production of frank symptoms by the inoculations in monkeys is high—up to 80 to 90 per cent of the monkeys instilled. While this difference is a great advantage to the experimenter, it is desirable to keep in mind that there is something essentially artificial in the experiments on reinfection which are to be presented. Despite these considerations we are of the belief that the experiments have an important bearing on present day discussions of immunity in poliomyelitis.

The forms of the disease as observed in man and produced experimentally in monkeys are brought into closer harmony by the studies made on the cerebrospinal fluid of inoculated animals. Changes commonly occur in the fluid consisting first of mononuclear cell increases and second, and less frequently, of the appearance of globulin. The increase in cells arises quickly and tends to precede the onset of clinical symptoms and may occur without any other sign of infection being detected. Interesting as is this phenomenon in normal or previously uninoculated monkeys (4), it is even more informing as it occurs in animals which have already passed through one or even two attacks of paralytic poliomyelitis.

The employment of recovered or convalescent monkeys for reinoculation has been turned to another account, namely, the consideration of the occurrence of distinct immunologic strains of virus. The protocols which follow are arranged so as to show whether reinfection was induced by a corresponding (homologous) or differing (heterologous) virus.

EXPERIMENTAL

Primary Attack.—It is immaterial to the problem of reinfection how the first, or primary, attack of poliomyelitis was induced. The virus is capable of using any nerve route for ascent to the central nervous organisms (5). For the most part the original attack followed from intracerebral inoculation, but it also followed nasal instillation, subcutaneous or even intravenous injection of the virus.

Clinical Records.—The effects of the instillations were determined by daily examinations consisting of direct observation, rectal temperature readings taken at the same hour each day, and systematic examination of the cerebrospinal fluid withdrawn by cisternal puncture. The inoculated monkeys were released one at a time into a wire enclosure large enough for the attendant to enter. Any awkwardness in running and climbing could be quickly determined by the trained observer and the voice, state of the hair, and other peculiarities accurately noted. Prior to the virus instillations the cells in the cerebrospinal fluid were counted once or oftener and the countings were repeated at 48 hour intervals after the inoculations. Experience had taught us that cisternal punctures alone and the nasal instillation of inactive substances, such as physiological salt solution or suspension of virus of low virulence, do not affect materially the cell count. For the detection of globulin, Noguchi's butyric acid reagent was used.

Virus Suspensions.—The suspensions, varying in concentration from 1 to 10 per cent, were prepared from glycerolated monkey spinal cord and medulla previously washed in two changes of salt solution. The crude suspensions were centrifuged lightly (300 revolutions per minute) for 1 minute, and a new suspension was made for each instillation. The glycerolated specimens were less than 10 weeks old. The non-etherized monkey is held in the upright position with head bent backward. An ordinary rubber urethral tip is attached to the dropper and placed tightly against the nostril, when 1 cc. of the suspension is forced into the nose. The fluid distributes itself over the nasal membrane, the excess, which is small, escaping into the nasopharynx. The indications are that the swallowed or even aspirated suspension is without pathogenic effect.

Cisternal Puncture.—The monkey is thoroughly etherized and the back of the neck shaved and cleaned (surgical sterilization). An assistant places it on its abdomen with the head dropping over the edge of the table, the head being firmly held with both hands. The hypodermic needle (1 inch cannula, No. 20 gauge) is inserted vertically almost its entire length until it reaches the cisterna

TABLE I

Second Attacks, Homologous Reinfections

Experiment	1st attack	2nd attack	Neutralization	Comment
I 11/9/12	Intracerebral injection of M.A. virus. Arms and legs paralyzed. Recovery complete	11 mos. rest period. Reinforcement subcutaneous injections of M.A. virus, 440 cc. 5% suspension, from 7/23 to 11/11/13. Sudden development of paralysis on 11/15, and death	None	The dose of virus was increased in last 4 wks. followed by the reinfection
II 10/13/13	Intracerebral injection of M.A. virus. Partial paralysis of arms and legs and facial paralysis. Recovery with residue	Subcutaneous injection of M.A. virus, 5 cc. 5% suspension, 12/16 and 12/19/21. Paralytic symptoms and death	"	Possibly an instance of induced relapse rather than of 2nd attack*
III 12/23/33 *Macacus cynomolgus*	Intracerebral injection of mixed virus, 12/31/33 to 1/2/34. Progressive symptoms. Recovery complete	2 nasal doses of mixed virus, 2/19 and 2/21/34. 2/24 beginning symptoms; increase, prostration, and death. Cells rose from 34 and 30 to 320	"	Typical reinfection
IV 12/27/33 to 1/2/34	5 nasal doses of 1933 virus. Cells rose to 335. No clinical symptoms	6 nasal doses of 1933 virus, 2/5 to 2/10/34. Cells rose from 67 to 620. High cell count and fever (105°) coincided. Paralysis and death	"	Example of symptomless involvement of cerebrospinal fluid followed by typical attack

* Cases of relapse in man and monkey are collected in Poliomyelitis, International Committee for the Study of Infantile Paralysis, Baltimore, 1932, 93 and 187. Lovett's case cited as an instance of second attack by Amoss, in Rivers' Filterable viruses, Baltimore, 1928, 179, is probably another instance of relapse. The quiet period between attacks of 2 years adopted by Still for distinguishing between relapses and reinfections is a useful arbitrary device.

TABLE II

Second Attacks, Heterologous Reinfections

Experiment	1st attack	2nd attack	Neutralization	Comment
V 11/6/33	Intracerebral injection of 1933 virus, 11/6 and 11/15 (accelerating dose). Progressive symptoms with deltoid paralysis. Recovery	2 nasal doses of Philadelphia virus, 3/7 and 3/9/35. Cells rose from 21 to 760. Globulin +. Paralysis and death	None	Typical reinfection
VI 12/1/33	Intracerebral injection of 1933 virus with acceleration. Excitement, tremor, ruffled hair. No paralysis	2 nasal doses of Philadelphia virus, 1/24 and 1/26/35. Cells rose to 320. Paralysis and death	After recovery from 1st attack, Philadelphia virus not neutralized	2nd, paralytic, attack following abortive attack
VII 12/23/33	Intracerebral injection of mixed virus. Excitement and tremor. No frank paralysis	2 nasal doses of Philadelphia virus, 1/24 and 1/26/35. Cells rose from 30 to 580. Paralysis and death	None	" "
VIII 12/26/33	2 nasal doses of 1933 virus. Excitement, tremor, double ptosis. Cells rose from 48 to 640. Recovery. 2 subsequent nasal instillations: (*a*) 1933 virus (homologous); cells rose from 82 to 105; (*b*) Philadelphia virus; cells rose from 26 to 64	3 nasal doses of Havana virus, 5/15 to 5/21/35. Cells rose from 33 to 580. Globulin +. Paralysis and death	3 separate bleedings. Philadelphia virus neutralized; mixed virus not neutralized	2nd paralytic attack following previous mild paralytic one
IX 12/26/33	2 sets of nasal doses of 1933 virus, 12/33 and 2/34. Cells in first series rose from 44 to 465; in second series from 50 to 125. No clinical symptoms	3 nasal doses of Havana virus, 5/15 to 5/17/35. Cells rose from 27 to 670. Paralysis and death	None	Symptomless changes in cerebrospinal fluid followed by typical paralytic attack. Havana virus highly virulent specimen

X 3/9/34	Intravenous inoculation of 1933 virus after normal horse serum intraspinally. Paralysis, prostration, recovery	2 nasal doses of Philadelphia virus, 6/24 and 6/26/35. Cells rose to 690. Temperature 106.6°. No paralysis	Serum taken after 1st attack did not neutralize Philadelphia virus	2nd attack abortive
XI 6/26/34	Intracerebral injection of 1931 virus, 6/26/34; acceleration, 7/3. Paralysis and recovery. 3 nasal doses of Philadelphia virus, 3/7 to 3/13/35. No cell rise or clinical symptoms. 4 nasal doses of Havana virus, 5/15 to 5/24/35. No cell rise or clinical symptoms	2 nasal doses of mixed virus, 11/6 and 11/8/35. Cells rose from 16 to 930. Paralysis and death	Serum taken after recovery from 1st attack neutralized Philadelphia, and did not neutralize mixed virus	Typical reinfection
XII 10/10/34	Intracerebral injection of 1928 virus. Paralysis with recovery. 2 nasal tests: (*a*) Philadelphia virus, 3/7 to 3/9/35; no effects; and (*b*) Havana virus, 5/15 to 5/24/35; temperature 105°. No clinical symptoms	2 nasal doses of mixed virus, 11/6 and 11/8/35. Cells rose from 32 to 834 with coincident temperature of 106.8° followed by paralysis and death	Serum taken after 1st attack did not neutralize Philadelphia virus; and serum taken after first 2 unsuccessful instillations did not neutralize mixed virus	" "
XIII 11/13/35	Intracerebral injection of 1935 virus with acceleration. Paralysis with recovery	2 nasal doses of mixed virus, 3/17 and 3/19/36. Cells rose from 9 to 540. Paralysis followed by recovery. 2 nasal doses of Philadelphia virus, 6/17 and 6/19/36. No cell rise or symptoms. 6 nasal doses of mixed virus, 12/1 to 12/3/36. Cells rose from 22 to 104. 2 accelerating doses, 12/10 and 12/11/36, during fall in cells. No effect and no other clinical signs. 6 nasal doses of Philadelphia virus, 1/13 to 1/15/37. Cells rose from 24 to 360. No fever	Serum taken after 1st attack did not, after 2nd attack did neutralize mixed virus. 2nd serum neutralized Philadelphia virus	" "

TABLE II—*Concluded*

Experiment	1st attack	2nd attack	Neutralization	Comment
XIV 4/6/36	Intracerebral injection of mixed virus. Paralysis and recovery	2 nasal doses of Philadelphia virus, 6/17/36. Cells rose to 660. Paralysis and recovery. 4 nasal doses of mixed virus, 11/4 to 11/5/36. Cells rose to 135. No clinical symptoms. 4 nasal doses Philadelphia virus, 12/8 to 12/9/36; accelerating dose, 12/19/36. Cells rose to 245 and temperature to 105°. No other symptoms	Serum taken after 1st attack did not neutralize Philadelphia virus; serum taken after 2nd attack did not neutralize mixed virus	Typical reinfection
XV 10/20/33	Intradermal injections of 32 cc. human 1933 virus, 10/20, 12/13/33. 6 nasal doses of 1933 passage virus, 1/31 to 2/5/34. Cells rose to 1150. Globulin +. Paralysis. Recovery with residues	3 nasal doses of mixed virus, 1/8 to 1/12/35. Cells rose to 880. Globulin +. Paralysis followed by recovery. Second set of residues. Nasal instillations as follows: Philadelphia, 6/24 to 6/29/35, no cell rise; Philadelphia, 12/9/35, cells rose to 127; Havana, 1/14/36, cells rose to 145; no other symptoms in any test; intracerebral injection of mixed virus, 6/2/36, no symptoms. 6 nasal doses of mixed virus, 12/17 to 12/19/36. Cells rose from 18 to 55. No clinical symptoms. 6 nasal doses of Philadelphia virus, 1/26 to 1/28/37. Cells rose from 28 to 102. No fever	After 1933 intradermal injections serum did not neutralize mixed, 1928, 1932, or 1933 virus. After recovery from 2nd (mixed virus) paralysis, serum neutralized mixed virus	" "

TABLE III

Reinforced Monkeys Responding to Reinoculation. Heterologous

Experiment	1st attack	2nd attack	Neutralization	Comment
XVI 3/5/35	Intracerebral injection of Havana virus. Abortive attack. Temperature 106°. Recovery. Subcutaneous injections of 50 cc. 5% suspension Havana virus, 4/8 to 5/10/35	2 nasal doses of Philadelphia virus, 6/24 and 6/26/35. Cells rose from 28 to 240. Paralysis and death	None	Typical reinfection
XVII 3/5/35	Intracerebral injection of Havana virus. Abortive attack. Recovery. Subcutaneous injections of 50 cc. 5% suspension Havana virus, 4/8 to 5/10/35. 6 nasal doses of Havana virus, 1/14 to 1/25/36. Cells rose from 30 to 835. No fever or other symptoms. Cells 74 to 83% polymorphonuclears. No bacteria	2 nasal doses of mixed virus, 3/17 and 3/19/36. Mononuclear cells rose from 35 to 785. Arm and leg paralysis. Recovery. Later died of tuberculosis	"	" "
XVIII 3/4/31	Subcutaneous injection of aluminum hydroxide and mixed virus, 4/20 to 4/26/32. 7 nasal doses of mixed virus. Paralysis. Recovery with residues. Subcutaneous injections of 1120 cc. 5% suspension mixed virus, 10/2/31 to 2/14/33. No symptoms	4 nasal doses of Philadelphia virus, 3/27 to 4/10/33. Paralysis with recovery. 4 nasal doses of 1933 virus, 1/10 to 1/13/34. Cells rose to 460 and temperature to 104.2°. Later died of tuberculosis	Serum after reinforcement neutralized mixed virus; did not neutralize Philadelphia virus. Serum taken after 2nd attack did neutralize Philadelphia virus	Typical reinfection in hyperimmune monkey

TABLE IV

Reinforced Monkeys Resisting Reinoculation. Homologous

Experiment	1st attack	Nasal instillation	Neutralization	Comment
XIX 6/30/31	Cisternal injection of mixed virus. Paralysis with recovery. Subcutaneous injections of 1120 cc. 5% suspension mixed virus, 10/2/31 to 2/11/33. No symptoms	4 nasal doses of mixed virus, 3/27 to 4/10/33. No cell count. No clinical symptoms. Test not repeated	Reinforced serum neutralized mixed virus	The failure to count cells and repeat the test with other specimens of virus leaves experiment inconclusive
XX 5/7/31	Cisternal injection of mixed virus. Paralysis and recovery. Subcutaneous injections of 970 cc. 5% suspension mixed virus, 10/2/31 to 12/2/32. No symptoms	6 nasal doses of mixed virus, 12/27/32 to 1/3/33. No cell count. No symptoms. Test not repeated	" "	" "
XXI 12/8/32	Intracerebral injection of Philadelphia virus. Paralysis with recovery. Reinforced with 255 cc. 5% suspension of Philadelphia virus	4 nasal doses of Philadelphia virus, 3/27 to 4/10/33. No cell count. No symptoms. Test not repeated	Reinforced serum neutralized Philadelphia virus	" "

TABLE V

Reinforced Monkeys Resisting Reinoculation. Heterologous

Experiment	1st attack	Nasal instillation	Neutralization	Comment
XXII 2/9/31	Intracerebral injection of mixed virus. Marked non-paralytic infection. Recovery. Reinforced with 1120 cc. 5% suspension mixed virus. Companion to Experiment XVIII	4 nasal doses of Philadelphia virus, 3/27 to 4/10/33. No cell counts. No symptoms. Test not repeated	Serum taken prior to nasal test neutralized Philadelphia virus	The failure to count cells and to repeat nasal test leaves experiment inconclusive
XXIII and XXIV 1/7/32	Intracerebral injections of 1931 virus. Both monkeys developed paralysis and both recovered. Reinforced with 180 cc. 5% suspension 1931 virus given subcutaneously	4 nasal doses of Philadelphia virus, 3/27 to 4/10/33. No cell counts. No symptoms. Test not repeated	Serum taken before reinforcement did not neutralize mixed virus; serum taken after reinforcement neutralized Philadelphia virus	" "
XXV 10/7/32	7 nasal doses of Philadelphia virus. Paralysis and recovery. Reinforced with 255 cc. 5% suspension Philadelphia virus given subcutaneously	4 nasal doses of mixed virus, 3/27 to 4/10/33. No cell counts. No symptoms. Test not repeated	Reinforced serum neutralized Philadelphia virus	" "

TABLE VI

Originally Resistant Monkeys

Experiment	Preparation	Testing	Neutralization	Comment
XXVI and XXVII 5/20/32	2 *Macacus rhesus* of same weight given subcutaneously 1250 gm. of dry acetone precipitate mixed virus	Monkey XXVII given 6 nasal doses of mixed virus, 12/27/32 to 1/3/33. Paralytic poliomyelitis, death. Monkey XXVI inoculated as follows: (*a*) Nasal doses of mixed virus, 3/27 to 3/28/33; accelerated, 4/8 to 4/10. No effect. (*b*) 4 nasal doses of mixed virus, 1/10 to 1/13/34. Cells rose to 285. No clinical symptoms. (*c*) 4 nasal doses of mixed 1928 and 1933 virus, 1/29/34. Cells rose to 125. Evanescent weak arms. Accelerating nasal doses, 2/2 and 2/3. Cells rose to 385. No clinical symptoms	None Serum taken on 4 occasions from monkey XXVI failed to neutralize mixed virus	In all series of experiments with *Macacus* monkeys occasionally highly resistant animals are encountered. The acetone precipitated virus produced no immunity. One monkey was of average, the other of greater natural resistance. The sole evidence of latter's response to the inoculations was revealed by the cell counts

magna region. Sudden release of pressure indicates the penetration; the stylet is removed and the fluid allowed to escape, collected by means of a capillary pipette, and placed in small Wassermann tubes. When blood-tinged fluid appears, pipetting is carried on until the specimen becomes clear before the collection.

DISCUSSION

The tabulations constitute the experimental data on the reinfections. The abbreviated protocols leave no doubt that second attacks of poliomyelitis can be induced in monkeys by using the nasal mode of inoculation. And the occurrence is not exceptional, although it is also not invariable. The proportion of convalescent monkeys which responds under favorable conditions of experiment is high. Certain convalescent monkeys respond to the first instillations, others only after successive instillations. We have already recorded (4) that monkeys not previously instilled will resist one and respond to another instillation sometimes, so that it is not surprising that the identical set of reactions should occur in the convalescent monkeys.

Fortunately, the tests were numerous enough to cover the experimental disease in its main manifestations: mild, almost undetectable cases; typically abortive cases; and cases as severe as are ever encountered. It cannot, therefore, be argued that only the light convalescents are subject to reinfection.

It must, however, be kept in mind that the test used is a severe one. The amount of virus instilled is huge compared to anything likely to happen in man. On the other hand, the monkey, compared to man, is relatively an insusceptible species. Contact, cage infections in monkeys practically do not occur. The very rare such instances which have been reported among the thousands of inoculated monkeys serve merely to emphasize the strong natural resistance they display. On the other hand, the disease in monkeys is severer, as a rule, than the human malady. The defensive mechanism, once broken down in the monkey, is less competent than that of man.

Compared to second infections so far recognized in man, the tests with monkeys would suggest that they are the more susceptible species. No one knows, of course, what might happen in man if convalescents received such vast doses of virus intranasally. The two sets of conditions cannot, therefore, be compared numerically. What the experiments in monkeys can be made to do is to arouse greater

interest in cases of second attack in man and possibly reveal the mechanism involved.

The cases of second attack in man may safely be assumed to express themselves in other ways than in frank paralysis as they do in monkeys. All the examples of reinfection in man so far reported are of the paralytic variety. The study of the reinoculated convalescent monkeys has shown conclusively that milder effects arise which are detectable only through the changes occurring in the cerebrospinal fluid accompanied by fever temperatures. It is a curious fact that the cerebrospinal fluid which never harbors the virus should register so readily the presence of virus on the nasal membrane. Incidentally, the fact should be emphasized that the height of this cell reaction in the fluid often coincides with fever temperatures either preceding the onset of obvious clinical symptoms or occurring in the absence of those symptoms.

That the presence of humoral antibodies is an insufficient bar to the penetration of virus from the nose to the brain, many recent studies on actively immunized monkeys have shown (6). This finding, highly important in relation to efforts made to produce effective vaccines, is confirmed in an interesting manner by the further finding that convalescent monkeys in which the titre of humoral antibodies has been increased by reinforcement or hyperimmunization respond to nasal instillations with typical paralytic second attacks. The tables contain brief protocols of two kinds of reinforced monkeys—those which did and those which did not respond with reinfection symptoms. It will be noted that the failures to respond consist of animals nasally instilled only once and in which the cerebrospinal fluid was not examined. The reason for this disparity is that these monkeys were among the early convalescents studied and before the technical methods later employed had been put into use.

There is probably nothing strictly accidental in the failure of nasal instillation at one time and its effectiveness at another. One specimen or strain of virus may well be better suited to induce infection in a given convalescent animal than is another, but the degree of virulence irrespective of specimen or strain undoubtedly plays an essential part in the result. Note should be taken of the fact that in the long run only two specimens of virus proved strictly reliable—mixed virus and

Philadelphia virus, to which for a short time the Havana strain could be added. But not all passages of any of these viruses were uniformly effective. Fluctuations already referred to still occurred to disturb the expected results. The 1933 virus was highly virulent for a time, only to fluctuate widely and to sink to so low a level that it was never possible to re-establish it.

Can no more than two attacks of paralytic poliomyelitis be induced in monkeys? Still (3) in his collected cases includes one of a third attack in a child. It is still too early to make confident statements about third or further attacks in the monkey. So far we have had available three monkeys which, having passed successfully through two paralytic attacks, have been subjected to additional inoculations. None developed a third paralytic attack, but all responded with increases in cells in the cerebrospinal fluid. The monkey in Experiment XIII, after a rest period of $6\frac{1}{2}$ months, reacted to mixed virus; the monkey in Experiment XIV, after a similar rest of $4\frac{1}{2}$ months, reacted successively to mixed and Philadelphia virus; and the monkey in Experiment XV, after a rest of $6\frac{1}{2}$ months, reacted mildly to mixed virus. In these three instances the virus specimens used corresponded with those which had previously produced paralysis. After a further rest interval the three monkeys will be instilled again with a heterogeneous specimen. Knowledge is still too small to permit of generalizations concerning an induced nervous tissue immunity in opposition or in addition to a humoral immunity (7). We do know that the virus has a wide distribution in these tissues even in monkeys with localized paralysis. Certain animals possess naturally high nervous tissue immunity. One such instance has been recorded (Experiment XXVII). The problem of local immunity of the nervous tissues, natural or induced, is one to be left to future study.

So long as knowledge of the variety of the virus is so small, we should speak of particular or specific strains with caution. There are reasons to believe that passage viruses differ somewhat from each other immunologically and that a given specimen of human virus changes in the course of monkey passages (8). To designate the specimens derived from epidemics in different years or places as strains is to employ a rough classification only. In the experiments reported, the mixed virus and the Philadelphia virus have exhibited contrasting powers of

infection to which, for convenience, the terms homologous and heterologous have been tentatively applied. When we pass from the infective properties of these specimens to the more subtle immunological properties the difference still holds in spite of occasional overlapping as revealed by the neutralization test.

Finally, the protocols show that reinfection is capable of being induced by the same (homologous) specimen with which the original infection was produced, as well as with alien (heterologous) specimens of different origin. The position to be assigned the Havana virus is still undetermined. Its effectiveness in the few instances in which other virus specimens proved powerless may be due more to the degree of virulence than to true heterology.

CONCLUSIONS

Monkeys which have recovered from an attack of experimental poliomyelitis are subject to reinfection by the nasal route.

Second attacks of the disease result from inoculation with the specimen of virus used to produce the first attack and with specimens of different origin.

Reinfection takes place in monkeys which have recovered from mild and from severe attacks and in convalescent animals which have been subjected to hyperimmunization.

The 2 year quiet period proposed by Still to separate relapses from second attacks, judging from the monkey, is probably excessive. Until greater attention is given the reinfections of varying intensities in man, conclusions on this point must be wholly tentative.

I wish to acknowledge with thanks the very valuable technical assistance given me by Mr. Peter Haselbauer.

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