

REVIVED ACTIVITY OF THE VIRUS OF POLIOMYELITIS.

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In course of the experimental studies of poliomyelitis carried out during the past 10 years, the varieties and limits of activity of the virus itself have been given little more than passing attention. Although it was early found that not all specimens of the central nervous tissues taken post mortem from acute cases of poliomyelitis equally induced the experimental disease in monkeys, the distinctions in activity have never been accurately defined. The statement that about half the specimens are inoculable upon monkeys is merely a rough approximation, since by increasing the number of monkeys injected, the percentage of successful transmissions is made to rise. There is, however, agreement on the fundamental points, namely that certain specimens are more readily inoculable than others and certain individuals among the susceptible simian species are more easily infected than others.

There is agreement on a third fundamental point. When the human virus of poliomyelitis is first implanted on the monkey, it is less active or virulent than it becomes after several successive implantations or transfers. At the outset the proportion of inoculated monkeys escaping infection, and, having become infected, surviving the disease, is higher than later when, as we say, the virus has become adapted to the new species.

It is obvious that under strictly natural conditions, the monkey is far less susceptible to infection with the virus of poliomyelitis than is man. No instance is known of accidental or as we say "spontaneous" communication of poliomyelitis to the monkey. The recently inoculated and infected animals can be kept in closest contact with healthy and uninoculated companions, without the latter ever acquiring the infection. The protective or defensive mechanisms which the monkey possesses must be circumvented or overcome, as by direct artificial inoculation, in order that infection may ensue.

And yet the experimental disease in the monkey, once it is induced, is more fatal than the original disease in man. An adapted virus commonly infects all the inoculated animals, and of the infected, few if any tend to survive. Histological examination discloses, as a rule, extensive infiltrative lesions of the floor of the fourth ventricle, to which the death of the animal is readily and perhaps properly ascribable.

This remarkably high mortality rate can to a small extent be mitigated by nursing. It is of course difficult to care for severely paralyzed monkeys as severely affected human beings are cared for. However, when great care and patience are exercised with feeding, bedding, etc., occasionally a seriously paralyzed animal may be brought safely through the acute stages of the disease and started on the course of convalescence and recovery. This recovery is rarely complete; although remarkable restorations of function occur in the affected muscles, residual paralyses practically always remain. In this respect there is further analogy between the epidemic disease in man and the experimental disease in monkeys.

Certain human strains of the virus, as indicated, either do not induce the experimental disease at all or quickly lose the power of infection and thus fail to become adapted to the monkey. This loss in power when it occurs tends to be complete. That is, a weak strain tends not to provoke a mild infection; it produces no infection at all. This point is determinable by reinoculation or by neutralization tests. Animals which have recovered from paralyses are immune and their blood serum is neutralizing to the most potent virus, while those receiving the weak strains without exhibiting symptoms react to the powerful strain as do previously uninoculated monkeys.

No readily applicable method is known for cultivating the virus of poliomyelitis outside the body so that its properties may be studied in detail. It is, however, possible to cultivate the virus artificially *in vivo* in the monkey and thus to determine certain of its effects. As has been stated, such successive transfers establish the virus at a high level of activity. Hence the question arises whether this high level will be maintained over a long period and through an indefinite series of animal passages.

The view has been held that certain epidemic diseases in man attain malignancy through successive direct, rapid passages of the inciting microbes from individual to individual. In this way it has been sought to account for the sinister character of institutional outbreaks of streptococcus and pneumococcus pneumonia and the general outbreaks of epidemic influenza. Since no intermediate means are known of the transport of the virus of poliomyelitis from person to person, it is this successive direct mode of communication which has been charged with the initiation of destructive epidemics of the disease.

Epidemics are characterized by movements in two opposite directions: they not only tend to rise from a mean and reach a peak of prevalence or incidence, but they tend also to fall from this peak to or below the mean when the epidemic wave of incidence has spent itself. This is the case no matter whether subsequent rises and falls do or do not take place. If, therefore, the successive passage from individual to individual serves to elevate the activity of the inciting microbe, a point should ultimately be reached when this same process serves to depress the activity in order to account for the phenomena observed.

It is not our purpose to pursue this theme in detail. Our object in this paper is merely to describe a specimen of the virus of poliomyelitis which has been observed to pass through three distinct phases of activity or virulence. The correlation of the facts with the views currently held on the epidemiology of poliomyelitis will not be undertaken at this time.

The specimen of virus to be described was obtained in 1909. It is denominated M. A. It consisted originally of central nervous tissues taken from a fatal case of epidemic poliomyelitis in a child.¹ The implantation on *Macacus rhesus* monkeys offered no special difficulty; the activity at first was not high, but rose quickly on successive transfer. At the outset occasionally a paralyzed animal recovered; later, recovery almost never occurred. The effective infecting dose of the virus fell with the enhancement of virulence. At the period of maximal activity it was 0.01 to 0.001 cc. of a 5 per cent emulsion centrifuged and passed through a Berkefeld filter, given by intracerebral injection.

¹ Flexner, S., and Lewis, P. A., *J. Exp. Med.*, 1910, xii, 227.

Maximal virulence of the strain was maintained for a period of about 3 years, that is from the autumn of 1909 until the winter of 1912, during which it was passed through many monkeys. In the winter of 1912 a change became apparent, consisting in failure at times of a given dose to induce infection. Along with this change another was noted; now and then an animal developing paralysis would recover. The deterioration of the virus continued until it was reestablished at about the level of strength which was exhibited at the original passages made in 1909.²

Efforts to augment the virus by more rapid animal passages were unsuccessful; the lower level of activity appeared to be fixed. In this important respect the deteriorated monkey strain of virus differed from the original human strain from which it was derived. Moreover, the debasement did not affect one sample only of the virus, but affected all samples, the offspring of the original material.²

The sample or strain of M. A. virus we are considering has now undergone another alteration. It has entered on a new phase of enhanced activity; that is, its virulence has been restored and its high power of inducing infection revived.

In order to follow these new events it is necessary to recall our custom to place in 50 per cent neutral glycerol and to preserve at 4°C. samples of the spinal cord and brain of all animals succumbing to poliomyelitis. In this way a complete series of glycerolated specimens is collected. It had been our belief, based on previous observation, that glycerolation does not modify the virus, but rather preserves it over several years essentially unchanged in activity. In accordance with the custom, samples of the earlier potent and of the later deteriorated M. A. virus were thus set aside. As was stated in the earlier publication describing the deterioration, the question was raised "whether after a resting period a second enhancement can be accomplished by a new series of passages."² This question can now be answered in the affirmative.

The second rise in virulence of the M. A. strain was noted in 1918. Between 1912, at which time the reversion took place, and 1918, when high activity was again noticed, the strain was passed through an

² Flexner, S., Clark, P. F., and Amoss, H. L., *J. Exp. Med.*, 1914, xix, 195.

occasional *Macacus rhesus* monkey. The method was to inject intracerebrally an emulsion of the virus, in order to secure infection and by that means recent material for glycerolation. When the infection seemed to follow more readily than ordinarily, a Berkefeld filtrate was prepared and injected in the same manner, in order to determine whether any rise in potency had occurred.

No appreciable difference in the readiness with which infection could be induced or in the severity of the disease was observed until February of the year 1918, when it was found that not only was the emulsion effective, but also the Berkefeld filtrate prepared from it. The next step was to employ diminishing quantities of the filtrate in order to ascertain the degree of activity of the virus. In the tests which follow, this degree has been determined up to the point of establishing a large increase in potency.

EXPERIMENTAL.

Monkey 1.—Jan. 28, 1918. *Macacus rhesus* given intracerebrally 2 cc. of a Berkefeld filtrate of a 5 per cent glycerolated suspension of spinal cord and brain.³ Feb. 4. Ataxia; movements slow; facial asymmetry (paralysis). Feb. 5. Died. The autopsy disclosed well marked lesions visible to the naked eye in the medulla and the spinal cord. The microscope revealed the typical conditions of experimental poliomyelitis in the midbrain, spinal cord, and intervertebral ganglia.

Monkey 2.—Apr. 3, 1918. *Macacus rhesus* given intracerebrally 0.5 cc. of a Berkefeld filtrate of a 5 per cent suspension of the glycerolated spinal cord and brain of the previous animal. Apr. 10. Ataxia, tremor of head; paralysis of arms, back, and neck. Apr. 11. Prostrate. Apr. 15. Died. The midbrain, spinal cord, and intervertebral ganglia showed typical lesions of experimental poliomyelitis.

This particular strain of the M. A. virus was now passed from time to time through other monkeys, and the restored activity was found to endure. After a further resting period, suspensions of glycerolated materials from Monkeys 1 and 2 were passed through two monkeys in September and October, 1921. The animals developed typical paralytic effects and were etherized on the 10th and 12th days respectively after the intracerebral inoculations.

With the active brain and cord material derived from these animals, a new series was inoculated into Monkeys 5, 6, 7, and 8.

Monkey 5.—Nov. 3, 1921. *Macacus rhesus* received 0.2 cc. of a 5 per cent glycerolated suspension intracerebrally. Nov. 8. Excitement. Nov. 9. Excitement increased; tremor of head. Nov. 11. Ataxia; left facial paralysis; deltoids weak. Nov. 13. Legs weak; convulsions. This animal was nursed to recovery; a residue of paralysis remained.

³The inoculations were made on etherized animals.

Monkey 6.—Nov. 10, 1921. *Macacus rhesus* given 0.2 cc. of a Berkefeld filtrate intracerebrally. Nov. 16. Excitement. Nov. 17. Tremor of head, and paralysis of face and both arms. Nov. 18. Left leg paralyzed. Etherized. The macro- and microscopic lesions of the central nervous organs are typical of experimental poliomyelitis.

Monkey 7.—Nov. 18, 1921. *Macacus rhesus*, 0.02 cc. of a Berkefeld filtrate of a 5 per cent suspension injected intracerebrally. Nov. 24. Excitement. Nov. 26. Tremor of head, ataxia, and paralysis of arms and legs. This animal was nursed to recovery. The left arm remained paralyzed; the other effects disappeared.

Monkey 8.—Nov. 28, 1921. *Macacus rhesus*, 0.02 cc. of a Berkefeld filtrate of 5 per cent suspension. Dec. 5. Excitement; head tremor; convulsions. Dec. 6. All extremities and back paralyzed; almost continuous convulsions. Etherized. The lesions present in the central nervous organs were typical.

The foregoing protocols show conclusively that the M. A. strain of virus which had become greatly reduced in potency in 1912, had regained a large part if not all of the lost power by 1918, and the revived virus was still potent in 1921.

In 1922 another test was made of the restored virus of 1918.

A portion of the glycerolated brain and cord tissue of Monkey 2 was emulsified and suspended in salt solution in strength of 5 per cent. On Feb. 1, a *Macacus rhesus* was injected intracerebrally with 0.5 cc. of this suspension. By Feb. 8, widespread paralysis had developed and the animal was etherized. A Berkefeld filtrate was prepared from a 5 per cent suspension of the brain and spinal cord of this animal, of which 0.2 cc. was injected intracerebrally into another *Macacus rhesus* on Feb. 9. Complete paralysis of the extremities and back and a left facial palsy were present on the 16th. Although an effort was made to save the animal with nursing, it was unsuccessful. Death occurred on Feb. 18. Typical lesions of experimental poliomyelitis were present in the central nervous organs.

This experiment showed, therefore, that the restored virus of 1918 was still highly active after a resting period of about 4 years in glycerol.

SUMMARY.

A strain of the virus of poliomyelitis has been described which has passed through several stages of virulence as tested upon monkeys.

The first stage consisted of the adaptation of the original human virus to the monkey. In this process high virulence was readily achieved.

The adapted, virulent strain of virus was passed regularly through monkeys and maintained its activity for about 3 years, when diminution became apparent. The loss of power of the virus was such that it may be said to have returned approximately to the level of the original human virus. This change constituted the second stage.

The third stage is represented by recovery of the high virulence. This revival occurred, it seems, during the sojourn of the virus in glycerol and required several years for its consummation. It was first noticed nearly 6 years after the low level of the second stage became established. The potent virus of the third stage has been found to remain active over a period of at least 4 years while preserved in glycerol.

What constitutes at least a superficial resemblance between the wave-like rises and falls of the incidence of epidemic poliomyelitis and the phenomena of increase and decrease in virulence of the specimen of virus has been alluded to. The two processes differ, however, essentially in respect to the time factor, since the fluctuations of the epidemic wave occupy small and those of the virulence occupy large increments of time.