DIFFUSION AND SURVIVAL OF THE POLIOMYELITIC VIRUS.*

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In earlier publications[1] we have presented data bearing on the view that the minute microörganism cultivated from poliomyelitic tissues and described by Flexner and Noguchi,[2] constitutes the cause of epidemic poliomyelitis. In this paper we propose to deal with the power of diffusion and the period of survival of the virus of poliomyelitis contained in the central nervous organs, since these factors affect the evidence submitted concerning the specific activities of the microörganism.

We have alluded in a previous paper to the factor of the survival of the virus at thermostatic temperature,[3] but this has never been accurately determined and could only be inferred from experiments made to determine other qualities of the virus. The question of the power of diffusion of the virus from a fragment of infected nervous tissue into the surrounding medium has never been directly considered.

The capacity for diffusion and survival of the virus bears directly upon the evidences that have been brought forward to support the specific nature of the microörganism under consideration. We now know that certain cultural strains after a number of removals from the nervous tissues from which they were derived, and great dilution, may still set up poliomyelitis in monkeys upon inoculation. Although the experiments demonstrating this fact are convincing, it is nevertheless desirable to ascertain by direct experiment what the capacity for diffusion and survival of the ordinary virus of

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[1] Flexner, S., and Noguchi, H., *Jour. Exper. Med.*, 1913, xviii, 461. Flexner, S., Noguchi, H., and Amoss, H. L., *ibid.*, 1915, xxi, 91.

[2] Flexner and Noguchi, *loc. cit.*

[3] Flexner, Noguchi, and Amoss, *loc. cit.*

poliomyelitis is, in order to exclude the remote possibility of the persistence within the cultures of an active agent of invisible character.

EXPERIMENTAL.

The method employed to study the diffusion and survival of the virus is simple. Fragments of brain tissue containing the virus are placed in tubes of ascitic fluid and kidney fragment overlaid with paraffin oil, the medium employed for actual cultivation tests. The tubes are not enclosed in anaerobic jars, and are kept at 37° C. From time to time a tube is removed from the thermostat, and a quantity of the ascitic fluid in proximity to the brain tissue is pipetted off, after which the brain fragment is removed. Both fluid and fragment are used for inoculating monkeys by intracerebral injection. Since the tubes have not been enclosed in an atmosphere of hydrogen, multiplication of the minute organism will probably not take place. Whether or not it does take place can be determined by suitable tests.

It is essential to employ a strain of the virus of high virulence. This was done in the experiments by selecting a monkey from which to take the brain fragments, which had succumbed to an inoculation of a highly active sample of the ordinary virus of poliomyelitis. The virus is capable of producing paralysis when injected intracerebrally in quantities of 0.1 to 0.3 of a cubic centimeter of a Berkefeld filtrate of a 5 per cent. emulsion of the spinal cord. Before the brain tissue submitted to incubation is employed for inoculation, an emulsion is prepared containing 0.2 of a gram of the tissue which inoculated intracerebrally into a control animal causes paralysis in a period varying from five to eight days. Subsequently it is necessary merely to remove the inoculated tissue at stated intervals from the thermostat and to inoculate the corresponding quantity of 0.2 of a gram of brain fragment, in order to determine the period during which the virus survives in an active state under the conditions of the experiment.

The tests on the diffusion of the virus were conducted with quantities of 0.2 of a cubic centimeter of the ascitic fluid adjacent to the brain fragment which had been pipetted off at intervals after

incubation. The quantity of 0.2 of a cubic centimeter was chosen because it represents the average quantity of the fluid medium which is transferred from the original to subsequent tubes of ascitic fluid medium in the course of the cultivation of the minute micro-organism from infected brain tissue. Should the virus diffuse freely from the brain tissue into the surrounding medium, the ascitic fluid should then become infectious. No experiments have ever been conducted to ascertain whether diffusion takes place at all, or the extent to which it does occur. Should the diffusion be so slight that 0.2 of a cubic centimeter of the ascitic fluid contains less than a minimal dose capable of producing poliomyelitis in the monkey, the transfer of this quantity of the fluid medium from one tube to another could not result in the carrying over of an effective amount of the virus.

INOCULATION OF BRAIN TISSUE.

Experiment 1.—Control. Jan. 7. Injected intracerebrally into a *Macacus rhesus* an emulsion containing 0.2 gm. of brain removed from a recently paralyzed monkey. Jan. 10. Excitable. Jan. 11, A.M. Tremor, ataxia. P.M. Arms and legs weak; later prostrate. Jan. 12. Died. Typical poliomyelitis.

Experiment 2.—Brain tissue incubated for 10 days. Jan. 18. Injected intracerebrally into a *Macacus rhesus* 0.2 gm. of brain tissue, in emulsion, derived from the same animal as in experiment 1, which had been incubated in ascitic fluid kidney medium for 10 days. Jan. 31, A.M. Arms paralyzed, paralysis of right side of face. P.M. Prostrate. Feb. 1. Etherized. Typical poliomyelitis.

Experiment 3.—Brain tissue incubated for 20 days. Jan. 28. Same procedure as in experiment 2. Feb. 4. Excitable, ataxic, left arm paralyzed. Feb. 6. Prostrate. Feb. 8. Died. Typical poliomyelitis.

Experiment 4.—Brain tissue incubated for 30 days. Feb. 9. Same procedure as in experiment 2. No effects produced.

Experiment 5.—Brain tissue incubated for 40 days. Mar. 1. Same procedure as in experiment 2. No effects produced.

INOCULATION OF ASCITIC FLUID.

Experiment 6.—Ascitic fluid incubated for 10 days. Jan. 18. 0.2 c.c. of ascitic fluid adjacent to brain tissue employed in experiment 2, removed by pipette after 10 days' incubation, inoculated intracerebrally into a *Macacus rhesus*. No effects produced.

Experiment 7.—Ascitic fluid incubated for 20 days. Feb. 1. Same procedure as in experiment 1. No effects produced.

Experiment 8.—Ascitic fluid incubated for 30 days. Feb. 9. Same procedure as in experiment 1. No effects produced.

DISCUSSION.

The experiments described indicate that, with a highly active virus, 0.2 of a gram of the brain tissue taken from a recently paralyzed monkey will cause certain paralysis in a *Macacus rhesus* when injected by the intracerebral route. When, however, specimens are taken from the same portions of the brain from which the test fragment was removed and incubated in the ascitic fluid kidney medium, they still cause paralysis when similarly inoculated into a *Macacus rhesus* on the tenth or the twentieth day of incubation, but not on the thirtieth or the fortieth day. But the ascitic fluid adjacent to the brain fragments fails to set up paralysis in a *Macacus rhesus* when injected intracerebrally in quantities of 0.2 of a cubic centimeter after an incubation of ten, twenty, or thirty days.

The meaning of the experiments is clear. At the temperature of the thermostat, the ordinary virus of poliomyelitis survives in the autolyzing brain fragment containing it for a period of about twenty days, while it seems incapable of diffusing from the fragment into the surrounding medium in considerable quantity during the period of its active survival in the tissues.

The period of survival of the virus at 37° C. indicated by the experiments is almost exactly paralleled by the tests made by Levaditi[4] with fragments of infected intervertebral ganglia which he submitted to cultivation *in vitro*. Levaditi observed that the longest period at which the virus could still be detected in an effective state was twenty-one days, which period agrees exactly with our observations. Levaditi also noted that only the intervertebral ganglionic tissue, and not the surrounding plasma, was infectious. From this he concluded that the virus which he believed to have increased, multiplied only in association or symbiosis with nerve cells; but the observation is readily explained on the supposition that failure to detect the virus in the plasma was due to lack of its diffusion from the ganglionic tissue into the surrounding medium.

Hence the experiments performed for the express purpose of determining the limits of survival and degree of diffusion of the

[4] Levaditi, C., *Compt. rend. Soc. de biol.*, 1913, lxxiv, 1179; 1913, lxxv, 202.

virus at 37° C. have yielded results which coincide almost exactly with the results attributed by Levaditi to multiplication of the virus *in vitro* in association with proliferating tissue cells. It is highly probable that in Levaditi's experiments no multiplication of the virus whatever took place; and, moreover, that the discrepancy in respect to infectiousness observed between the ganglionic tissue and the plasmatic medium is explained, not by the proliferation of the virus within the former, but by the failure of diffusion into the latter.

Our experimental results are supported by observations recorded by Flexner and Noguchi,[5] which indicated that when no demonstrable growth of the microörganism has taken place in the ascitic fluid medium, the original tube, as well as the fluid in the early transfers, is wholly without power to incite poliomyelitis upon inoculation into monkeys.

The cultures, it will be recalled, are developed in the ascitic fluid kidney medium from fragments of infected brain tissue. From the original cultures obtained, subcultures are made by transferring after an inoculation of one or two weeks quantities of the ascitic fluid varying in amount from 0.2 to 0.5 of a cubic centimeter. The possibility of transferring in this way from one tube to another a certain quantity of the original virus of poliomyelitis contained within the brain fragment has always been considered. On the other hand, it has been pointed out that with each successive transfer, so rapid a dilution of any original virus present would doubtless take place that the fluid would soon lose infectious power from this source.[6] It now, however, appears that the quantity of virus which diffuses from the brain fragment into the ascitic fluid is so small that it may be disregarded from the outset.

Similar considerations relate to the survival of the virus in the brain tissue itself employed for the culture medium. Since the period of this survival is only about twenty days, cultures which have been incubated for weeks or months, and which are still active, cannot owe their infective power to the original virus, but must owe it to the multiplied microörganism. Moreover, when the

[5] Flexner and Noguchi, *loc. cit.*

[6] Flexner, Noguchi, and Amoss, *loc. cit.*

additional fact is taken into account that the successfully inoculated cultures were often many generations removed from the brain fragment from which they were derived, and in one instance had remained in the thermostat for a period exceeding one year, further consideration need hardly be devoted to the possibility that the experimental poliomyelitic infection is attributable in part to the coöperation of unchanged virus and culture of the minute microorganism.

CONCLUSIONS.

The ordinary virus of poliomyelitis present in aseptically removed brain tissue of paralyzed monkeys survives in an ascitic fluid kidney medium at the temperature of 37° C. for a period of at least twenty, but not of thirty days.

Under the conditions of moderate anaerobiosis, the minute microorganism cultivated from poliomyelitic tissues tends not to develop in cultures from the brain tissue; hence its presence does not complicate the survival test.

The diffusion of the ordinary poliomyelitic virus from a noncomminuted fragment of brain tissue into a surrounding medium of ascitic fluid is so slight as not to be detectable by inoculation experiments conducted with usual quantities of the fluid.

The specific effects of the microörganism cultivated from poliomyelitic tissues are not caused by an admixture in the cultures of the ordinary virus of poliomyelitis; hence they must be caused by the pathogenic action of the microörganism itself.

The minute microörganism is therefore to be regarded as the specific microbic cause of epidemic poliomyelitis.