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Etiology of Poliomyelitis: Cultivation of an Organism from Poliomyelitic Tissue and Transmission of the Disease in Monkeys.*

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Bacteriological cultivation experiments were attempted with Berkefeld N filtrates prepared from the nervous tissues of monkeys inoculated with poliomyelitis virus and developing typical experimental poliomyelitis. Seven strains of virus were studied in a special culture medium designated as VB medium and containing essentially minced sheep brain in a veal infusion broth free from peptone. The use of fresh living tissue and ascitic fluid such as characterize the Noguchi medium or its modifications was avoided. No attempt was made to obtain strict anerobiosis by means of the recognized methods. The reduced oxygen tension developed in the culture medium was found satisfactory. The use of brain tissue suggested itself as a suitable culture medium for a virus that is known to exhibit a marked affinity for the nervous system.

The culture medium was prepared as follows: Blood and membranes were carefully removed at autopsy from fresh sheep brain. One kilogram of tissue was added to one liter of veal infusion broth, pH 8.0, free from peptone. The mixture was coagulated thoroughly by boiling for 2 to 3 minutes and 0.2% glucose by volume was added. The cooled material was then passed 2 or 3 times through a meat chopper. The culture medium was placed in test tubes measuring 1.5 by 15 cm. to a depth of approximately 6 cm. (about 10 cc. by volume) and sterilized in the autoclave at 15 pounds pressure for 20 minutes. The final pH was adjusted (if necessary) to 7.6 Rigorous tests for sterility of the material were made before use to rule out all possible sources of contamination.

The inoculum, consisting of Berkefeld filtrates as already described, was added to a series of tubes of culture medium and incubated at 37.5 to 38.0°C. for a period of 3 to 4 weeks during which time samples were withdrawn every week or 10 days by means of a platinum loop or capillary pipette for subplants and film preparations. Two tubes in each series were left unmolested and checked after growth had occurred in the series under observation. Control

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series included Berkefeld filtrates and suspensions of poliomyelitic tissues inoculated into ordinary fluid and solid culture media; filtrates and suspensions from normal monkey brain and cord; suspensions and filtrates prepared from nervous tissues in herpes encephalitis, and neurovaccinia; and finally uninoculated VB medium by itself.

Positive cultures were obtained from poliomyelitis virus filtrates and the transfers from the original cultures have yielded successful growth in series. At the present time these organisms are in the fourteenth subplant and the original cultures are still viable after growing in the incubator for more than 18 weeks.

The organism grows in an atmosphere of reduced oxygen tension developed in the medium, stains best with Wright stain and in later subplants and older cultures it appears to be faintly or moderately Gram-positive. Under a magnification of 1500 to 2000 diameters it is a minute ovoid body ranging in size from the lowest limit of visibility to 0.05-0.1 or 0.2 M, and occurring in irregular clusters, in pairs, singly, very rarely in short chains, and in densely packed masses. The bodies are especially numerous in the particles of brain tissue found in the medium, for which they exhibit a marked preference. A sheath-like envelope appears to surround the organism, favoring the formation of zoogleal masses. Filtration experiments with the cultures during certain stages of growth suggest that ultramicroscopic forms are capable of developing into visible bodies in the culture medium.

Inoculation in monkeys of organisms obtained from cultures of poliomyelitic tissues in VB medium caused clinical symptoms and pathological effects characteristic of experimental poliomyelitis. Successful experiments have been accomplished with these cultures representing to date a dilution of about 2×10^{-27} of the original material cultivated. This was 0.1 cc. of a 5% saline Berkefeld N filtrate of poliomyelitic nervous tissue. The cultures used were in the 6th to the 13th subplants, the infectivity remained unimpaired. The subplants used for inoculation were incubated 7 to 9 days, were capable of inducing experimental poliomyelitis even when subcultured from tubes that had been stored in the incubator for 65 to 80 days. Parallel experiments in which heated cultures were used for inoculation into monkeys resulted negatively. Materials derived from culture tubes that contained no demonstrable organisms were also without effect. Control experiments included materials from normal monkeys and from the viruses of herpes, encephalitis, and neurovaccinia. All these, as well as the uninoculated culture medium by itself gave negative results.

Attempts to recultivate the typical organism from monkeys experimentally infected with the cultures and developing poliomyelitis, were successful with Berkefeld N filtrates and suspensions prepared from the nervous tissues of such animals.

The cycle of the disease has been demonstrated in monkeys inoculated with cultures. The organism has been developed from an invisible to a visible state and the typical disease induced in the experimental animal, in the brain and spinal cord of which the organism again returns to the invisible filterable stage.

It appears that the poliomyelitis virus has multiplied *in vitro*. The organisms cause pathological effects that can be augmented by successive passage of the culture material through a series of monkeys. Notable in this respect is the shortening of the incubation period.

The question of a possible life cycle and other biological considerations of the microbic agent are in the course of study. Additional inoculation experiments and investigations pertaining to morphology and virulence of cultures will be reported in the future.

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A Rapid Method for Determining Lactic Acid in Stools.

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In cultures, bacteria break down sugar into fatty acids, alcohol, lactic acid, methane, hydrogen and carbon dioxide. We have determined the amounts of the fatty acids occurring in human stools with various diets and under the conditions of constipation or catharsis.[1] Since lactic acid is produced in cultures in large amounts, this work was undertaken to answer the question whether lactic acid is present in stools in amounts comparable to those found for volatile fatty acids.

The isolation of lactic acid from stools could be carried out by extracting with ether or if a suitable precipitant were found which would free the filtrate from interfering substances, the filtrate might be directly oxidized. By means of the mercuric sulphate precipitation of West,[2] one can obtain from stool water clear filtrates prac-

[1] Grove, E. W., Olmsted, W. H., and Koenig, Karl, *J. Biol. Chem.*, 1929, **85**, 127.

[2] West, E. S., Scharles, F. H., and Peterson, V. L., *J. Biol. Chem.*, 1929, **82**, 137.