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Isolation of Poliomyelitic Virus from Human Stools.*

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In 1912 a report from Sweden[1] described experiments in which the virus of poliomyelitis was recovered from human colonic washings obtained during the disease, and in convalescence. Sawyer[2] also reported in 1915, its recovery from the rectal washings of a convalescent case. More recently no one seems to have been able to repeat these experiments until Harmon[3] made brief mention of 5 successful attempts in which the virus was obtained from the rectal washings of 4 out of 20 patients tested; and Kramer[4] has stated that he has succeeded with similar material.

The present note also records instances in which the virus has been recovered from the stools, and is a preliminary report of the technical procedures employed in 3 of our successful experiments.

The circumstances under which the virus was isolated may be briefly described as follows: The patient was an infant (Daniel Sk., aged 18 months) who, on October 18th, 1937, had contracted a mild, febrile illness (abortive poliomyelitis) which lasted but a few days. His older brother, aged 6 years, had contracted severe, paralytic poliomyelitis 8 days previously. On the day after the onset of illness a stool was obtained from Daniel and an extract of it was subsequently inoculated intracerebrally and intraäbdominally into one monkey (8-33) which developed severe and extensive experimental poliomyelitis after a period of incubation of 7 days. Definite microscopic lesions characteristic of the experimental disease were found in the spinal cord when this monkey was sacrificed and a successful passage to monkey 8-48 was made. These results fulfill the criteria which we believe are necessary to warrant the acceptance of the result as positive. Neutralization (or reinoculation) tests, with established or fresh strains of poliomyelitic virus, have not been

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1 Kling, C., Pettersson, A., and Wernstedt, W., *Communications Inst. méd. État Stockholm*, 1912, **3**, 5.

2 Sawyer, W. A., *Am. J. Trop. Dis. and Prev. Med.*, 1915, **3**, 164.

3 Harmon, P. H., *J. Am. Med. Assn.*, 1937, **109**, 1061.

4 Kramer, S. D., personal communication.

performed because the adequacy of this procedure as a method for identifying poliomyelitic virus has been recently disputed. For the fact that different strains of poliomyelitic virus fall into different immunologic groups, renders it questionable whether these methods can at present be satisfactorily used as diagnostic tests.[5]

The technic of preparing the first stool specimen for inoculation, which was in some respects exploratory, was as follows: The stool was collected on October 19th in a pint fruit jar and was allowed to stand overnight in the refrigerator. The next morning 120 cc. of distilled water were added and the diluted stool was agitated and allowed to stand for 2 hours; 90 cc. of the supernatant fluid was then pipetted into a rubber-stoppered flask and, as a bactericidal agent, 13.5 cc. of anesthetic ether was added to make an approximate 15% suspension. This suspension was well shaken and then again placed in the refrigerator and allowed to stand overnight. On the next morning it was centrifuged at 2000 r.p.m. for one hour and the supernatant fluid below the ether-layer was then removed. (Aerobic bacterial cultures on blood-agar plates, taken from the specimen at this time, showed no growth.) To 60 cc. of this material, 1.5 cc. of normal monkey serum was added preparatory to concentration by freezing and evaporation at low temperatures in the Flosdorf-Mudd apparatus.[6, 7] The evaporating process was allowed to run for 6 hours, the original volume of 61.5 cc. being brought to 12 cc. The concentrate was then left in the refrigerator overnight, and on October 22nd half of it was inoculated into one monkey, the doses used being 1 cc. intracerebrally and 5 cc. intra-abdominally. This monkey (8-33) developed paralysis on October 29th. It had shown relatively little fever prior to the onset of paralysis. It was sacrificed on October 30th. The histological lesions were characteristic and extensive. Passage to monkey 8-48 (by cerebral and peritoneal routes) was satisfactorily accomplished.

This result has been successfully repeated on at least 2 subsequent occasions with other stool specimens obtained from this same child. The second time the virus was recovered, it was from a stool

[5] Burnet, F. M., and MacNamara, J., *Brit. J. Exp. Path.*, 1931, **12**, 57; Weyer, E. R., PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 289; Flexner, S., *J. Am. Med. Assn.*, 1932, **99**, 1244; Paul, J. R., and Trask, J. D., *J. Exp. Med.*, 1933, **58**, 513; *Ibid.*, 1935, **61**, 447; Kramer, S. D., *Arch. Neurol. and Psychiat.*, 1935, **33**, 1371; Kessel, J. F., Van Wart, R., Fisk, R. T., and Stimpert, F. D., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 326; Howitt, B. F., *Science*, 1937, **85**, 268; Trask, J. D., Paul, J. R., Beebe, A. R., and German, W. J., *J. Exp. Med.*, 1937, **65**, 687.

[6] Flosdorf, E. W., and Mudd, S., *J. Immunol.*, 1935, **29**, 389.

[7] Paul, J. R., *J. Bacteriol.*, to be published.

obtained on the 14th day from onset (monkey 8-49, period of incubation 6 days). This strain was successfully passed to monkey 8-96 by cerebral and peritoneal routes. The third time the virus was recovered it was from a stool obtained on the 25th day. Here a modification of the earlier technic was followed, based on the fact that in later experiments we had often found stool material, prepared in the way described, to be lethal for the monkey, particularly if inoculated intracerebrally in amounts of from 0.5 to 1.0 cc. Of 8 monkeys thus inoculated intracerebrally, 5 of them died within 3 days of the inoculation. Localized necrosis of the brain was demonstrable in 3 of these. This fact subsequently led us to limit our inoculation to the intraäbdominal route using either concentrated or unconcentrated stool extracts. In the omission of the intracerebral inoculation we were guided by the fact that we have recently found it unnecessary to use the intracerebral route invariably, in attempts to infect monkeys with human material (spinal cord) containing poliomyelitic virus.[8] So far, of 11 monkeys inoculated intraäbdominally, but one has died of peritonitis and bacteremia. The third time on which the virus of poliomyelitis was recovered from our patient Daniel Sk., the monkey (8-69) had been inoculated intraabdominally with 30 cc. of a stool extract in which the preparation of the material was approximately the same as that used with the first and second specimens, except that the extract was not concentrated. This monkey developed fever after a period of incubation of 10 days, paralyses appeared 4 days later. Extensive lesions of experimental poliomyelitis were found in the cord when the animal was sacrificed. This strain was also successfully passed by cerebral and peritoneal routes to a second monkey (8-93). Stool specimens obtained from the same child on the 43rd and 64th days from onset failed to yield the virus.

Summary. A virus fulfiling the clinical and pathologic criteria for its identification as poliomyelitic virus has been recovered from the stools from a child with abortive poliomyelitis on the second, fourteenth and twenty-fifth days after onset. This was accomplished twice after intracerebral and intraäbdominal monkey inoculation, and once after intraäbdominal inoculation alone.

[8] Trask, J. D., and Paul, J. R., *Science*, 1938, **87**, 44.