EXPERIMENTAL POLIOMYELITIS INDUCED BY INTRACUTANEOUS INOCULATION[1]

A STRAIN OF THE VIRUS APPARENTLY PECULIARLY INFECTIVE WHEN INJECTED BY THIS ROUTE

JAMES D. TRASK AND JOHN R. PAUL

From the Departments of Pediatrics and Medicine, Yale University School of Medicine, New Haven, Connecticut

Received for publication, December 30, 1935

The inoculation of the virus of poliomyelitis by the intracerebral route has generally been acknowledged as the most constantly effective method of inducing experimental poliomyelitis in the monkey. The nasal passages represent another route effective with some strains of the virus. Earlier procedures also included the intraperitoneal and the intraneural routes; in the latter case the virus was usually inoculated into the sheath of the sciatic nerve. Some difficulty seems to have been encountered in inducing the disease by "extraneural" routes (International Committee, 1932, pp. 80–87; 130–144). It may suffice to say that according to most observers, feeding the virus (with the intestines uninjured) is seldom effective; its inoculation by the intravenous route is also nearly always ineffectual although recently Lennette and Hudson (1935) have described infection following repeated intravenous inoculation. On the other hand the experimental disease has often been produced when the virus has been given sub- or intracutaneously. With regard to the intracutaneous route, one observer (M. Brodie) has been quoted (International Committee, 1932, p. 83) as saying that:... "when a potent virus is used (such as the M.V. strain), infection may be obtained consistently by this route with an average incubation period of 8 days.... The total amount of virus to be used is injected in one bleb; 15,000

[1] Supported by a grant from the President's Birthday Ball Commission for Infantile Paralysis Research.

to 20,000 times as much infective cord material was required as for a successful intracerebral inoculation." It is unknown, whether or not Brodie's observation on the consistency with which massive doses of highly virulent "passage" strains will infect intracutaneously, has been repeated. However, the results of many active immunization experiments on monkeys with either live virus, or virus treated by chemical or physical agents, point to the fact that with the different doses employed in these experiments only a small percentage of the animals thus injected succumbed to the experimental disease as a result of this injection (International Committee, 1932; Brodie, 1935a and b; Kolmer, 1934). On the other hand Flexner (1935) has stated that: "When human virus is injected successively into the skin of *macacus* monkeys, it produces active immunity in the greater number, but paralysis in a proportion of the inoculated animals, just as the passage strains do."

Olitsky and Cox (1936) observed experimental poliomyelitis in 2 animals following the subcutaneous inoculation of virus, after it had been treated with tannin.

That some strains of poliomyelitis virus will differ in their ability to produce the experimental disease when injected intracutaneously in relatively small doses is the subject of this report. The observation was made during the course of a series of active immunization experiments on monkeys with six different strains of poliomyelitis virus. The technique consisted in the preparation of 10-per-cent cord suspensions representing each of the six different strains. One or more samples from each strain were exposed to 0.1 per cent formalin (one part 40 per cent formaldehyde in 1000 parts of saline solution) for 24 hours at ice-box temperature. Intracutaneous inoculations were made with two 2 cc. doses of the 10-per-cent virus suspensions, the second one being given two weeks after the first. The material was injected into the shaved skin of the abdomen in a series of 10 piqures. Daily temperature readings were taken over a period of six weeks on all animals thus inoculated. The effect of these inoculations in 20 monkeys is listed in table 1. In the majority of instances no symptoms followed the injections. With the F. strain (a virulent strain in its eleventh passage) 1 out of 4 inoculated animals

became febrile on the 9th day, and was completely paralyzed 2 days later. This animal had been inoculated with the virus untreated with formalin. Of the 5 animals inoculated intracutaneously with the Wfd. strain (a much less virulent strain, as far as its intracerebral infectiveness is concerned) 4 developed symptoms in from 9 to 17 days after the first inoculation. These symptoms were characterized by fever, tremor and ataxia. Two

TABLE 1

Incidence of experimental poliomyelitis in monkeys inoculated intracutaneously with six strains of the virus

STRAINS			ANIMALS INOCULATED WITH:		ANIMALS WHICH DEVELOPED SYMPTOMS FOLLOWING:		TOTAL INCIDENCE OF ANIMALS DEVELOPING EXPERIMENTAL POLIOMYELITIS
Name	Origin	Passages in monkeys	Formalinized virus	Live virus	Formalinized virus	Live virus	
Wfd.	Spinal cord. California, 1934	3 5	2	2 1	2	1 1	4/5
Mc.*	Nasopharynx. California, 1934	3	2	1	0	0	0/3
W†	Nasopharynx. New Haven, Ct., 1931	9	2	1	0	0	0/3
F.†	Spinal cord. New York, 1931	11	2	2	0	1	1/4
Park† (mixed strain)	Spinal cord	Many	2	1	0	0	0/3
Aycock†	Spinal cord	Many	1	1	0	0	0/2

* The isolation of this strain has been described recently by J. R. Paul, J. D. Trask and L. T. Webster: Isolation of poliomyelitis virus from the nasopharynx, Jour. Exp. Med., **62**, 245, August, 1935.

† The properties of these strains have been described by J. R. Paul and J. D. Trask: Neutralization test in poliomyelitis, Jour. Exp. Med., **61**, 447, April, 1935.

of the 4 monkeys also developed paralyses. Extensive paralyses did not occur and none of the animals died as a result of this illness. Only 1 of these 4 monkeys was sacrificed. Histological examination revealed extensive lesions characteristic of poliomyelitis, in the medulla, lumbar and cervical cord. The disease exhibited by these 4 monkeys did not differ materially from that produced by intracerebral inoculation of this same strain of virus in its earlier passages, except that after intracerebral inoculation

paralyses were more extensive and the incubation period was shorter.

The Wfd. strain is a so-called human strain. It was used on its third and fifth passage. It had been isolated in California in June, 1934, from the spinal cord and medulla of a fatal human case of poliomyelitis.[2] The first three passages to which it had been subjected were made in June and October, 1934; the fourth and fifth passages were done in July, 1935. The intracutaneous inoculations with the third passage were made in the period of February to June, inclusive, 1935; the one inoculation with the fifth passage was done in September, 1935. It will be noted that this Wfd. strain had been subjected to comparatively few monkey passages, but whether its enhanced infectivity by the cutaneous route is due to some specific property, or to its proximity to the human host is unknown. It is also of some interest to note that the formalinized virus (Wfd.) was infective, or in other words, the procedure of formalinization to which it had been subjected was insufficient to kill the virus completely. Its viability after exposure to formalin was not tested by intracerebral inoculation.

We do not believe that the cutaneous infectivity of this strain of low virulence is to be compared with that mentioned by Brodie and obtained by massive dosage. The cutaneous infectivity of the Wfd. strain seems to be a unique property which does not have to do with high virulence as tested by the intracerebral method. Whether this property will be retained in subsequent passages will be the subject of further study.

REFERENCES

BRODIE, M. 1935a Jour. Immunol., **28**, 1, 1935.

BRODIE, M. 1935b Amer. Jour. Pub. Health, **25**, 54.

FLEXNER, S. 1935 Science, **82**, 420.

International Committee 1932 Poliomyelitis by The International Committee for the Study of Infantile Paralysis. The Williams & Wilkins Company, Baltimore.

KOLMER, J. A. 1934 Amer. Jour. Med. Sci., **188**, 510.

LENNETTE, E. H., AND HUDSON, N. P. 1935 Proc. Soc. Exper. Biol. and Med., **32**, 1444.

OLITSKY, P. K., AND COX, H. R. 1936 Jour. Exper. Med., **63**, 109.

[2] We are indebted to the Superintendent and to the Medical and Pathological Staffs of the Los Angeles County General Hospital for the material from which this strain was obtained.