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Identification of a Strain of Poliomyelitic Virus from Feces in Non-Paralytic Poliomyelitis. I. Immunologic Tests.*

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In 1937 we recovered a virus from the stools of a child with non-paralytic poliomyelitis.[1] Stools passed on the 1st, 14th, and 25th days after the onset of his mild illness were positive, but virus was not recovered from 2 specimens passed on later dates. For the original identification of this virus, 3 criteria were used: (i) The production of the usual signs of experimental poliomyelitis in monkeys; (ii) the demonstration of typical histological lesions in the monkey's spinal cord; (iii) passage to another monkey. Subsequently this strain, known as the SK strain, has been passed to its 8th generation and has produced typical experimental poliomyelitis in more than 40 monkeys. Intracerebral, intraperitoneal, intracutaneous, intranasal and oral inoculations have been successful.[2]

Further identification of this virus is presented in this report which includes the results of neutralization tests, reinoculation tests in monkeys, and also susceptibility tests in animals usually resistant to poliomyelitis.

The neutralization tests appear in Table I. "Anti-sera" prepared by immunization of monkeys with 6 different strains of poliomyelitic virus, whose properties have been described previously,[3] were used. Two series of tests were run: one on April 21, 1938, with the strain in its first generation; the other on November 8, 1938, with the strain in its fourth generation and with its infectivity considerably enhanced. In all neutralization tests equal parts of a suspension of 10% virus, and undiluted serum were mixed and incubated for 2 hours at 37°; 0.5 cc of the resulting mixtures was then inoculated intracerebrally into one monkey each. With each series there was

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1 Trask, J. D., Vignec, A. J., and Paul, J. R., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 147; *Ibid., J. Am. Med. Assn.*, 1938, **111**, 6.

2 Vignec, A. J., Paul, J. R., and Trask, J. D., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 246.

3 Trask, J. D., Paul, J. R., Beebe, A. R., and German, W. J., *J. Exp. Med.*, 1937, **65**, 687.

TABLE I.

Neutralization Tests Between "Antisera" for 6 Strains and SK. Virus in Its 1st and 4th Generations.

*Immune sera prepared from following strains	1st Test—(April 21, 1938) SK. virus: Generation 1 (pool of cords of No. 849, No. 860, and No. 901)					2nd Test—(Nov. 8, 1938) SK. virus: Generation 4 (Cord of No. 1020—*M. mordax*)				
	Dose 0.5 cc, %	No. *Mac. rhesus*	Result	First day of Fever	First day of Paral.	Dose 0.5 cc, %	No. *Mac. rhesus*	Result	First day of Fever	First day of Paral.
Aycock, 1920	5	960	—			5	1076	—		
Park, Mixed	5	961	P	12	14	5	1078	P	7	13
Flexner, 1931	5	962	—			5	1082	P	7	10
We., 1931	5	964	P	15	18	5	1083	P	5	9
Wfd., 1934	5	966	—			5	1084	—		
McC., A., 1934	5	967	—							
McC., B., 1934						5	1085	—		
Control Sera:										
Convalescent Human	5	939	—			5	1074	—		
Normal Monkey	5	948	P	7	12	5	1086	P	5	9
" "	5	968	—			5	1087	P	5	9
" "	5	971	P	12	14					
" "	0.5	972	—			0.5	1088	P	8	12
" "	0.5	973	—			0.5	1089	P	7	14

Legends.—Result: P = The monkey contracted experimental poliomyelitis and therefore the virus was not neutralized.

— = The monkey did not contract experimental poliomyelitis and therefore the virus was neutralized, or (as in the case of titrations) it was of insufficient virulence (or dosage) to infect.

First Day of Fever: 12 = Fever began on the 12th day of the experiment.

*For their adequate interpretation these results should be compared with our previous cross immunization tests.[3] It may suffice to say that in such tests, 5 sera neutralized their homologous strains. The We. and McC. (B) samples have not been described before. However, the former neutralized the Park and Flexner strains in June and July, 1936, and was not tested with its own strain.

included: (i) a control neutralization test with human serum (made up of pooled samples from 5 convalescent paralytics from the epidemic of 1931), and (ii) infectivity controls in which virus suspension of 2 different concentrations were mixed with normal monkey serum. In the second series of tests (November, 1938) the infectivity of the virus proved to be at least 10 times greater than that used in the first. What differences exist between the results of the 2 series seem largely explainable on this basis.

The results in Table I indicate that the SK. strain is related immunologically to the Aycock strain of 1920 and to the Wfd. and McC. strains which came from the California epidemic of 1934. On the other hand, there was at least some immunologic difference between the SK. strain and the Park strain and two eastern strains of 1931.

The series of reinoculation tests, in which the SK. strain was tested on monkeys which had been paralyzed previously by various poliomyelitis viruses, appear in Table II. Here, again, 2 sets of tests were run. In the first, with the weaker (1st generation) material of April, both the paralyzed, heterologously "immunized" monkeys, as well as the homologously immunized monkeys, survived reinoculation with the SK. strain without infection. However, not all of the normal controls were infected in this series. In the second series with the stronger (4th generation) material of November, 4 of the 6 para-

TABLE II.
Reinoculation of Paralyzed Monkeys Convalescent from Homologous and Heterologous Strain Infections.

Convalescent Monkeys				Reinoculation with SK. strain 0.5 cc. 5% virus intracerebrally	
Date of first paralysis, 1938	*M. rhesus* No.	Strains (All 1937)	Source of Strain	April 21, 1938* Paralysis	Nov. 8, 1938* Paralysis
2/20	921	SK. New Haven Co.	Feces	—	—
3/31	940	G.W. " " "	Spinal cord	—	—
4/13	934	Wn. " " "	" "	—	—
4/22	954	G.W. " " "	" "	Not done	—
7/27	996	Toomey, Cleveland†	" "	" "	+ (mild, delayed 3 days)
9/1	995	Ah., Toronto†	" "	" "	+ (mild, delayed 5 days)
9/11	973	Fx., Toronto†	" "	" "	—

*Same lots of virus as those used in experiments in Table 1. (See virulence controls.)

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TABLE III.
Inoculation of Rabbits, Guinea Pigs, and Swiss Mice.

Date, 1938	Animals	Total dose of SK. virus* cc	%	Routes	Results Symptoms during 4 wks	Histology Brain or Cord	Meninges
Apr. 21	2 rabbits	1	5	Intracerebral Intraäbdominal Corneal	1 remained well 1 wry neck	— +	— +
Apr. 21	4 guinea pigs	0.7	5	Intracerebral Intraäbdominal Corneal	4 dead with peritonitis on 3rd, 6th, 10th and 25th days	All negative	
Apr. 21	6 mice	0.03	5	Intracerebral	Remained well	,,	,,
June 1	2 rabbits	0.5	10	,,	1 remained well 1 dead—brain abscess, 7 days	— —	— Purulent
June 1	3 guinea pigs	0.2	10	,,	1 remained well 1 brain abscess 1 equivocal fever	— — —	— Purulent —
Nov. 8	2 rabbits	0.35	10	,, Corneal	1 remained well 1 snuffles	— —	— —
Nov. 8	3 guinea pigs	0.1	10	Intracerebral	3 equivocal fever: onset 11th and 12th days; 1 sick with pelvic abscess, killed 28th day	— — —	— — —
Nov. 8	6 mice	0.03	10	,,	5 remained well 1 dead 3d day	5 negative +	 +
Dec. 15	12 mice	0.03	10	,,	Remained well	All negative	

*For control of activity of virus used April 21st and November 8th, see Table I; virus used June 1st was active in a monkey inoculated that day, but a larger dose was used; 4 cc—10% virus; virus used December 15th was freshly prepared from the cord used November 8th, see Table I.

+ = Accumulation of round cells. — = No lesions.

lyzed, heterologously "immunized" monkeys survived reinoculation without infection. Two, however, out of these 6 "immune" monkeys (Nos. 996 and 995) developed mild, delayed attacks of poliomyelitis. The heterologous reinfection of monkeys with poliomyelitic virus has been described.[4, 3] This indicates an immunologic similarity among strains from New Haven County in the autumn of 1937. It appears to suggest a difference between the SK. strain and strains collected during the outbreaks in Toronto and Cleveland in 1937, though obviously factors other than immunologic differences might have determined the results. Immunologic similarity among strains from the same epidemic have been described before by means of neutralization tests.[3]

As a third means of identification (or of eliminating other viruses) rabbits, guinea pigs and Swiss mice were inoculated. These tests appear in Table III. After recovery from the trauma of inoculation, these animals presented no consistent or characteristic signs or symptoms, and no consistent microscopic evidences of diffuse or localized nonsuppurative lesions. A variety of lesions were detected in some of the 40 animals which were used in these experiments. Some of these were thought to result from the trauma of inoculation. Some were unexplained. None of them have been attributed to the SK. virus and we have presumed that this virus was non-infective for these 3 species by the routes and in the doses used. No reactions were produced in the eyes of 4 rabbits inoculated on the scarified cornea.

Summary Strains of virus, recovered from the stools of a child (SK.) with nonparalytic poliomyelitis, produced typical experimental poliomyelitis in *Mac. rhesus* monkeys. This SK. strain appears to be related immunologically to other strains of poliomyelitis virus recovered from the same epidemic, and to be related to the Aycock strain of 1920, and to some Californian strains of 1934. On the other hand, there appears to be at least some immunologic difference between the SK. strain and the Park strain, two eastern strains of 1931, and strains from Toronto and Cleveland of 1937.

The SK. strain produced no corneal reaction in rabbits and no consistent type of infection on intracerebral inoculation into rabbits, guinea pigs, and Swiss mice.

These tests yield further evidence, in addition to that already reported,[1] that the SK. strain is an example of poliomyelitic virus.

[4] Paul, J. R., and Trask, J. D., *J. Exp. Med.*, 1933, **58**, 513.