

STAFF MEETINGS OF THE MAYO CLINIC Jan. 13, 1943

ISOLATION OF SPECIFIC TYPES OF STREPTOCOCCI AND  
VIRUS FROM THE STOOL IN STUDIES OF EPIDEMIC  
POLIOMYELITIS AND ENCEPHALITIS, AND THE PRO-  
DUCTION OF VIRUS FROM THE "POLIOMYELITIC"  
STREPTOCOCCI

E. C. Rosenow, M. D., Division of Experimental Bacteriology: In earlier studies streptococci have been consistently isolated from tissues shown to contain virus such as brain and spinal cord in epidemic<sup>1,2,3</sup> and experimental poliomyelitis<sup>4,5</sup>, and in epidemic encephalitis<sup>6,7</sup> and epidemic equine encephalomyelitis<sup>8</sup>. In more recent studies<sup>9</sup> by the use of a serial dilution method, it has been possible to isolate parent organisms lysed by phage and pure cultures of streptococci in high dilutions without loss of specificity from emulsions and filtrates of virus and from cerebrospinal fluid in epidemic and experimental poliomyelitis and encephalitis. The method has been modified somewhat and has been found useful for the isolation of specific types of streptococci from badly contaminated material such as stool. It is the object of this paper to report details of the method used and the results obtained in the isolation and study of specific types of streptococci from the stools of patients during the acute stage of epidemic poliomyelitis and encephalitis.

METHODS

The stools were collected directly in paraffin cartons or other containers or from toilets by using a sterile wooden blade and placing approximately 1 c.c. of stool substance in wide mouthed glass screw-cap jars. The specimens from cases that occurred in Rochester, Minnesota, were sent directly to the laboratory and those from cases

1. Rosenow, E. C. and Wheeler, G. W.: The etiology of epidemic poliomyelitis. *J. Infect. Dis.* 22: 281-312, 1918.
2. Rosenow, E. C.: Streptococci in the spinal fluid in acute epidemic poliomyelitis, preliminary report. *J. A. M. A.* 91: 1594-1595 (Nov. 24) 1928; *Proc. Staff Meet., Mayo Clin.* 3: 330-331 (Nov. 14) 1928.
3. Rosenow, E. C., Heilman, F. R. and Pettet, C. H.: Observations on the epidemic of polio-encephalitis in Los Angeles, 1934. *Proc. Staff Meet., Mayo Clin.* 9: 448-451 (July 25) 1934.
4. Rosenow, E. C.: Streptococci in the lesions of experimental poliomyelitis in monkeys. *Proc. Soc. Exper. Biol. & Med.* 27: 444-445 (Feb.) 1930.
5. Rosenow, E. C.: Streptococci in the spinal fluid of experimental poliomyelitis in monkeys. (Abstr.) *J. Bact.* 19: 27-28 (Jan.) 1930.
6. Rosenow, E. C.: Isolation of streptococci in a study of the epidemic of encephalitis in St. Louis. *Proc. Soc. Exper. Biol. & Med.* 13: 285-286 (Nov.) 1933.
7. Rosenow, E. C. and Caldwell, H. W.: Studies on the etiology and serum treatment of encephalitis during the epidemic in North Dakota and Minnesota, 1941. *Proc. Staff Meet., Mayo Clin.* 16: 557-558 (Sept. 10) 1941.
8. Rosenow, E. C. and Schlotthauer, C. F.: Studies on the relation of streptococci to the etiology of equine encephalomyelitis; preliminary report. *Proc. Staff Meet., Mayo Clin.* 12: 631-636 (Oct. 6) 1937.
9. Rosenow, E. C.: Isolation of bacteria from virus and phage by a serial dilution method. *Arch. Path.* 26: 70-76 (July) 1938.

which occurred elsewhere were shipped by regular or air mail in the glass jars well sealed and packed in screw-cap cartons.

A 10 per cent emulsion of the stools in physiologic salt solution was made by grinding the stools with alundum powder in a mortar in a ventilated hood. The emulsion and cultures were made within two to twenty-four hours after collection in most instances, and after twenty-four hours in a few cases. Control cultures were made in identical manner from emulsions of the stools of patients of about the same age but ill with miscellaneous diseases, and from well persons, both groups remote from poliomyelitis. Cultures were made routinely by spreading on the surface of blood-agar a few drops of the first dilution (1:10,000) of the emulsion, made in dextrose-brain broth and by making serial dilutions alternately in tubes of dextrose-brain broth and soft dextrose-brain agar, each containing 20 c.c., for seven or fourteen consecutive dilutions, each at 1:10,000 or  $10^{-4}$ .

The method of making the serial dilutions was as follows: A sterilized twenty-six gauge nichrome wire was dipped into the emulsion. The adherent material (about 2 cubic millimeters) was inoculated into the first tube of dextrose-brain broth, the inoculating wire being shaken vigorously. The wire then was sterilized in the Bunsen flame. Meanwhile the inoculated tube was tilted once. The wire was dipped into this tube and approximately 2 cubic millimeters was transferred into the second tube. Transfers then were made consecutively into the remaining tubes, without heating the wire, by shaking and twirling the wire vigorously in each tube. The elapsed time between consecutive transfers or dilutions was about ten seconds. The tubes containing the dextrose-brain broth and dextrose-brain agar had been steamed to dispel dissolved oxygen and to melt the 0.2 per cent dextrose-brain agar and then cooled to 40° C. in a water bath immediately before they were inoculated. The test tube racks were sterilized with a mercuric chloride solution, 1:2,000. The operator wore sterile rubber gloves and the transfers were made in sterilized air in a nonstacked copper hood. Control transfers made in dextrose-brain broth and dextrose-brain agar in identical manner beginning with sterile material never yielded streptococci.

The cases in which the stools were obtained occurred in four widely separated epidemics of poliomyelitis and one widespread epidemic of encephalitis during the summer and autumn of 1940 and 1941, respectively. I am indebted to the physicians for permitting me to study their cases and for making the stools available for cultures. The diagnosis in all cases was established by clinical history, physical examination and spinal fluid findings. Cultures were made from the stool in altogether sixty-three cases of poliomyelitis, in twenty-three cases of encephalitis, from forty-five persons remote from poliomyelitis

having miscellaneous diseases and from fifty-six well persons remote from poliomyelitis (table 1 and fig. 1). Forty-five of the patients suffering from poliomyelitis were in the acute or active stage of the disease (one to fifteen days) and eighteen were convalescent or had recovered a short time before (sixteen to thirty-five days). The average duration of the disease in the former group was seven days and in the latter group thirty-two days. Fifty-two of the sixty-three patients suffering from poliomyelitis did not receive the poliomyelitis anti-streptococcic serum at any time. In eleven cases cultures not included in table 1 were made before and after serum treatment. The duration of the disease in the group of cases of encephalitis ranged from one to fifteen days and averaged six days. Serum treatment was not given in any of these cases before stools were obtained for culture.

Table 1  
Streptococci in serial dilution cultures of the stool in acute epidemic poliomyelitis and encephalitis, chronic diseases and normal controls

Source of stool	Cases	Serial dilutions, alternately in dextrose brain broth and dextrose brain agar. Percentage incidence of growth of streptococci.															
		1:10	1:100	1:1,000	1:10,000	1:100,000	1:1,000,000	1:10,000,000	1:100,000,000	1:1,000,000,000	1:10,000,000,000	1:100,000,000,000	1:1,000,000,000,000	1:10,000,000,000,000	1:100,000,000,000,000	1:1,000,000,000,000,000	
Persons having epidemic poliomyelitis, 1940	1 to 15 days	45	100	91	84	76	73	67	47	34	56	44	47	41	26	26	24
	16 to 135 days	18	94	33	22	33	22	22	5	11	0	0	0	0	0	0	0
Persons having epidemic encephalitis, 1941, 1 to 15 days	23		83	61	30	39	14	36	23	26							
Persons having chronic diseases remote from poliomyelitis and encephalitis	45		76	29	9	9	7	2	2	9	33	22	23	11	11	11	11
Well persons remote from poliomyelitis and encephalitis	56		86	18	7	0	2	0	0	8	0	0	0	0	0	0	0

\*Also cultured in dilutions of 10-4 to 10-28

RESULTS

The results summarized in table 1 and figure 1 represent specimens of stool obtained from patients who did not receive the poliomyelitis antistreptococcic serum at any time or obtained before serum treatment was given in the case of those that did receive the serum. The incidence of isolations of streptococci in the first dilution of 1:10,000 was nearly equally high in the different groups but with increasing dilutions a marked difference in isolations of streptococci occurred. In the high dilutions streptococci were isolated most frequently from the stools of persons in the acute or active stage of poliomyelitis, next in persons in the acute stage of encephalitis, third in persons during convalescence from poliomyelitis, fourth in persons remote from poliomyelitis and ill with nonepidemic diseases, and least frequently in well persons remote from poliomyelitis.

The underlying mechanism responsible for growth, in dextrose-brain broth and dextrose-brain agar cultures, of streptococci from the stool in cases of poliomyelitis and encephalitis far beyond what would be possible from mathematical calculations on the basis of the numbers of streptococci considered as inert particles, about 2,000,000,000 per cubic centimeter, has thus far defied analysis. However, the evidence obtained indicates that it is due in part to growth or biogenesis of the streptococci or of submicroscopic components as the dilutions

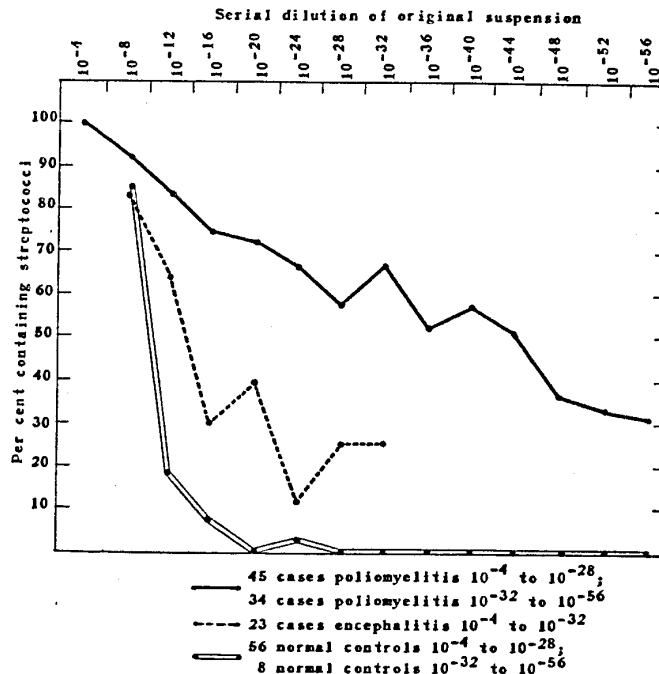


Fig. 1. Percentage incidence of growth of streptococci in serial dilution cultures of stools.

were made, to differential adhesion of the streptococci as such to the transferring wire or walls of the pipet, or to the adhesion of submicroscopic components. The magnitude of the dilution of 1:10,000 or  $10^{-4}$  at every step applies to the liquid and not to the suspended organism. This phenomenon occurs especially when materials containing epidemic or virulent strains of streptococci are subjected to serial dilutions in dextrose-brain broth and dextrose-brain agar. Growth of nonvirulent streptococci beyond that expected from the number in the original inoculum in the dilutions usually does not occur. It does not occur or occurs to a far lesser degree for virulent or non virulent streptococci when dextrose-broth or blood-agar mediums are used.

Growth of streptococci from the stool occurred in dilutions of  $10^{-32}$  to  $10^{-56}$  in from 56 to 24 per cent of cases of poliomyelitis in the acute or active stage of the disease (fig. 2a) or, as perhaps more accurately expressed after eight and twelve successive washings of the inoculating wire, whereas no growth occurred at these dilutions in the group of patients that were convalescing from poliomyelitis or that had recovered a short time before and in the group of well persons remote from poliomyelitis. Dilutions were not made beyond  $10^{-32}$  in the group having encephalitis but the incidence of isolations was high up to and at this dilution (figs. 1 and 2b). Isolations of streptococci at dilutions of more than  $10^{-26}$  in the group having miscellaneous diseases were

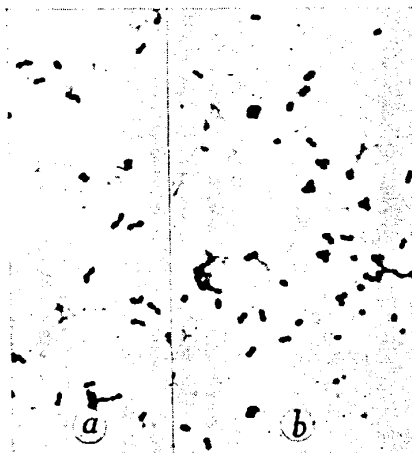


Fig. 2. Streptococci isolated from the stools of patients in the acute stage; a, of poliomyelitis and b, of encephalitis (Gram stain,  $\times 1,000$ ).

from one case of ulcerative colitis and two cases of postoperative persistent hiccup. Cultures from only one of the fifty-six well persons yielded streptococci in dilutions of more than  $10^{-12}$  and that was at  $10^{-20}$ . Only nonvirulent streptococci resembling *Streptococcus faecalis* were isolated from the blood-agar plates inoculated with 1:10,000 dilutions of the emulsions. Interestingly, by the use of shake cultures in ascites-dextrose agar, the poliomyelitic type of streptococcus was isolated from the stool in three cases of poliomyelitis associated with diarrhea in the epidemic of poliomyelitis in New York in 1916.

Microscopic examination of Gram-safranin-stained films from the emulsions of most of the stools of the different disease groups revealed a similar admixture of large numbers of bacteria including diplococci and streptococci. In some of the films from acute cases of poliomyelitis and encephalitis there were found extremely large numbers of

diplococci or streptococci resembling those isolated in dilution cultures. These often occurred in clumps, especially in constipated stools. It was evident that the much higher incidence of isolation of streptococci from the stool in high dilution during the acute stage of poliomyelitis or encephalitis than from the other groups was not due wholly to the greater numbers of streptococci as such but to their ability to "grow" or synthesize or to adhere to the transferring wire as the dilution cultures were made. Gram-negative, motile, gas forming bacilli, *Escherichia coli*, grew in the low dilutions in all instances and there was no difference in the various groups in the incidence of growth of this organism in the higher dilutions. The end point of growth of streptococci was usually less than  $10^{-16}$  although in a few instances growth occurred in the higher dilutions, often after skips of no growth in one to even four dilutions. Similar skips of growth of streptococci were noted in the high dilutions in the group of patients in the acute stage of poliomyelitis and encephalitis. Gram-positive bacilli resembling *Clostridium welchii* grew in both dextrose-brain broth and dextrose-brain agar in the low dilutions but usually were not found in dilutions of more than  $10^{-12}$ . However, from a few specimens these grew as large colonies in dextrose-brain agar up to dilutions of  $10^{-22}$ . Staphylococci and *Bacillus subtilis* grew only infrequently in the low dilutions and were never encountered at dilutions of more than  $10^{-12}$ .

The streptococcus grew in cultures from the stools in dilutions of  $10^{-12}$  or more in all of eleven cases of acute poliomyelitis before, and in only four of these two or more days after, the patient had a therapeutic injection of the poliomyelitis antistreptococcic serum. This diminution in incidence of growth accompanied especially marked clinical improvement.

In previous studies it was found that alpha or green-producing streptococci associated with epidemic disease often resisted pasteurization in milk or heating to  $63^{\circ}$  C. for thirty minutes. Hence the emulsions in saline solution of stools of patients having poliomyelitis and encephalitis and of stools of well persons which had been cultured by the serial dilution method were diluted threefold in saline solution and heated in sealed vials at  $63^{\circ}$  C. for thirty minutes and then cultured in dextrose-brain broth. Streptococci, nearly always in mixture with gram-positive bacilli resembling *Clostridium welchii* and almost never with gram-negative bacilli, were isolated from twenty-two of sixty-seven specimens from sixty-two persons having poliomyelitis or encephalitis and, in sharp contrast, from only two of sixty-one specimens from sixty-one well persons remote from poliomyelitis. This high resistance to heat of the streptococci in the stools of persons having poliomyelitis or encephalitis is in keeping with the fact that virulent or epidemic strains of streptococci often resist pasteurization in milk as now practiced, while nonvirulent strains and epidemic

strains after loss of virulence from prolonged cultivation on artificial mediums do not resist pasteurization. The streptococci isolated from the stool, as from nasopharynx, cerebrospinal fluid, brain and spinal cord in epidemic poliomyelitis and encephalitis were of alpha green-producing type. They were similar in size, growth requirements and cultural characteristics and were not isolated by usual aerobic methods.

#### RESULTS IN RABBITS FOLLOWING INOCULATION OF THE STREPTOCOCCI

Pure cultures of the streptococci, usually from single colonies of extremely high dilutions, were inoculated into rabbits to measure specific neurotropic virulence. A tenth of a cubic centimeter, of a 1:10,000 dilution of young cultures in dextrose-brain broth was routinely inoculated intracerebrally and the results obtained are summarized in table 2. Strains from nasopharynx and stool had similar

Table 2  
Mortality rate and symptoms in rabbits following intracerebral inoculation of streptococci isolated from nasopharynx and stools of patients having epidemic poliomyelitis or encephalitis

Source of streptococci		Strains	Rabbits		Symptoms	
			Inoculated	% that died	Tremors, spasms, ataxia, etc., %	Flaccid paralysis, %
Epidemic poliomyelitis, 1940	Nasopharynx	26	42	83	38	83
	Stools	18	16	54	36	76
Epidemic encephalitis, 1941	Nasopharynx	39	46	54	87	13
	Stools	11	14	79	86	14
Nasopharynx of well persons remote from poliomyelitis and encephalitis		37	59	36	12	2

high degree of neurotropic virulence and of specificity. This was not the case with streptococci from persons remote from the disease.

#### SEROLOGIC SPECIFICITY OF THE STREPTOCOCCI

As shown in table 3, the strains of streptococci from nasopharynx and stool of persons having poliomyelitis and encephalitis were specifically agglutinated by the poliomyelitis and encephalitis antistreptococcic serums respectively, and as shown in table 4, precipitation reactions with extracts of nasopharyngeal swabbings, filtrates from stools and extracts of streptococci and the respective antistreptococcic serums were likewise highly specific.

The successful isolation of a specific type of streptococcus from the stools of patients having poliomyelitis is of special interest because of the demonstration of a toxic factor<sup>10,11</sup> and of poliomyelitic virus

10. Toomey, J. A.: Demonstration of a toxic factor in the stools and urines of poliomyelitis patients. *J. Prev. Med.* 6: 379-386 (Sept.) 1932.
11. Toomey, J. A. and Takacs, W. S.: A toxic factor in the stools of patients ill with poliomyelitis. *Am. J. Dis. Child.* 61: 35-40 (Jan.) 1941.

in the stool<sup>12-17</sup> and sewage<sup>18</sup> in relation to poliomyelitis, thus adding one more material in which the streptococcus and virus are found to be associated. Experiments were conducted to determine whether virus in the stool might be virulent for mice and if so, whether the poliomyelitis type of streptococcus could be isolated from the brain and spinal cord of mice that succumbed to virus.

#### DETECTION IN STOOL OF VIRUS VIRULENT FOR MICE

Five per cent emulsions from stool or filtrate of emulsions or both of fifty-four cases of poliomyelitis were inoculated intracerebrally (0.03 c.c.) or intranasally (0.03 c.c.) or both into 408 mice of which seventy-six (19 per cent) died in from three to twenty-one days without development of gross lesions of brain, lungs or other viscera. Many of the remaining mice succumbed in from one to four or five days from

Table 3  
Agglutination of streptococci isolated from nasopharynx and stools of patients having epidemic poliomyelitis or encephalitis

Source of streptococci	Strains	Percentile incidence of specific agglutination by anti-streptococci serums for:				Control, normal horse serum	
		Polio-myelitis	Encephalitis	Arth-ritis	Infl-u-enza		
Epidemic poliomyelitis, 1940	Nasopharynx	36	86	4	7	3	0
	Stools	58	80	7	3	3	0
Epidemic encephalitis, 1941	Nasopharynx	98	7	89	0	3	0
	Stools	14	0	79	0	7	0
Nasopharynx of well persons remote from poliomyelitis and encephalitis	53	0	15	0	21	0	

bronchopneumonia associated with a mixture of organisms while others remained well. Sections of the brains of sixty-seven of the seventy-six that had died without gross lesions of the lungs revealed degeneration, perivascular and parenchymatous infiltration and other lesions of polioencephalitis. These sixty-seven mice had received emulsions from the stools of thirty-four patients.

Successive passage experiments were done with emulsions (0.03 c.c. intracerebrally) of the brains of mice that succumbed to polioencephalitis after inoculation of the stools of nine patients ill from

12. Trask, J. D., Vignec, A. J. and Paul, J. R.: Poliomyelitis virus in human stools. *J. A. M. A.* 111: 6-11 (July 2) 1938.
13. Toomey, J. A.: Attempts to isolate poliomyelitis virus from the stools of human beings and the experimental animals. *J. Arch. Pediat.* 56: 693-698 (Nov.) 1939.
14. Sabin, A. B.: Etiology of poliomyelitis. *J. A. M. A.* 117: 267-269 (July 26) 1941.
15. Howe, H. A. and Bodian, David: Poliomyelitis in the chimpanzee: A clinical-pathological study. *Bull. Johns Hopkins Hosp.* 69: 149-182, 1941.
16. McClure, G. Y.: A syndrome in *Macacus rhesus* after inoculation of stool from carriers of poliomyelitis virus. *Science* 94: 307-308 (Sept. 26) 1941.
17. Kramer, S. D., Gilliam, A. G. and Molner, J. G.: Recovery of the virus of poliomyelitis from the stools of healthy contacts in an institutional outbreak. *Pub. Health Rep.* 54: 1914-1922 (Oct. 27) 1939.
18. Paul, J. R., Trask, J. D. and Culotta, C. S.: Poliomyelitic virus in sewage. *Science* 90: 258-259 (Sept. 15) 1939.



two to twenty-one days. Twenty-seven of forty-eight mice inoculated with the stool in the first passage, eleven of twenty-nine and five of sixteen in the second passage, inoculated respectively with emulsion or filtrate of emulsion of the brain of mice that had succumbed to inoculation of stool, three of four and nine of twenty-eight inoculated respectively with emulsion or filtrate in the third passage, four of twelve inoculated with filtrate in the fourth passage, eleven of twelve and two of eight inoculated respectively with emulsion or filtrate in the fifth passage, and six of seven inoculated with filtrate in the sixth passage succumbed in from three to twenty-one days without gross lesions. Thus fifty-two (56 per cent) of ninety-three mice that received emulsion, and twenty-six (37 per cent) of seventy-one mice that received filtrate or a total of seventy-eight (48 per cent) of 164

Table 4  
Precipitation reaction with cleared extracts of nasopharyngeal swabbings and filtrates of stools of patients having epidemic poliomyelitis and encephalitis and with the respective antistreptococcal serums

Type and source of antigen			Cases	Percentile incidence of precipitation with anti-streptococcal serum for:				Control, normal horse serum
				Poliomyelitis	Encephalitis	Arthritis	Influenza	
Cleared extracts of nasopharyngeal swabbings or filtrates of emulsions of stools of:	Epidemic poliomyelitis 1 to 20 days, 1941	Nasopharynx	60	88	42	23	12	0
		Stools	20	50	15	5	10	0
	"Convalescent" poliomyelitis		9	0	0	11	0	0
	Persons ill and well remote from poliomyelitis and encephalitis		11	10	0	20	0	0
	Epidemic encephalitis, 1941	Nasopharynx	114	21	78	5	0	0
		Stools	15	13	60	7	0	0
Filtrates of extracts of streptococci isolated from stools of:	Epidemic poliomyelitis		25	48	0	4	0	0
	Persons ill and well remote from poliomyelitis		38	5	0	8	0	0

mice inoculated with virus in these passage experiments succumbed presumably to poliomyelitis. Cultures of the brain yielded the streptococcus in eleven of twenty-one mice that received emulsions and in four of thirteen that received filtrates, or a total of fifteen (44 per cent) of thirty-four mice. The brain and spinal cord revealed typical lesions of poliomyelitis (fig. 3).

Filtrates or emulsions or both of stools from eight persons who were convalescing from poliomyelitis and from forty-one persons having chronic diseases were inoculated into 191 mice; only three died in from three to twenty-one days. Sections of their brains were free from lesions.

#### PRODUCTION OF VIRUS FROM THE STREPTOCOCCUS FROM STOOL

From these and other studies it was thought possible that virus might be produced by the streptococcus in the intestinal tract. Experiments were performed to test this possibility. Pure cultures of the streptococcus from the stools of eighteen patients suffering from acute poliomyelitis were inoculated intracerebrally into eighty-two mice.

These cultures were far removed from original source, representing dilution of original material never less than  $10^{-20}$ . Twenty-two (27 per cent) of the mice, representing eleven cases, succumbed in from three to twenty-one days, presumably to encephalitis or poliomyelitis. The 5 per cent emulsion or filtrate of emulsions of the brain and spinal cord of one or more mice that succumbed after inoculation of these eleven strains was used for from one to eight additional passages.

The results according to animal passage of these strains were as follows: thirty of fifty-two mice in the first passage, forty of 104 mice in the second passage, thirty-four of sixty-four mice in the third passage, fifty-six of seventy-two mice in the fourth passage, fifty-six of

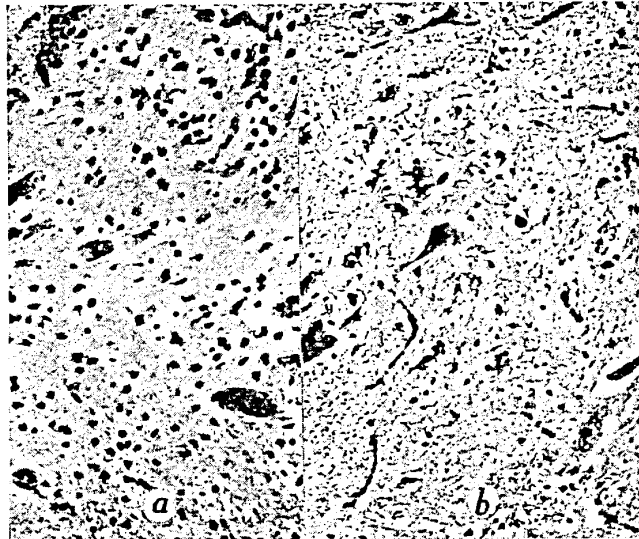


Fig. 3a. Lesions in the brain, and b, anterior horn of the spinal cord, of a mouse that succumbed to polioencephalitis after inoculation of virus in the third mouse passage, derived from the stool of a patient in the acute stage of poliomyelitis (hematoxylin and eosin,  $\times 185$ ).

eighty-four mice in the fifth passage, thirty-two of fifty-eight mice in the sixth passage, one of four mice in the seventh passage, two of four mice in the eighth passage and none of four mice in the ninth passage succumbed in from three to twenty-one days without gross lesions of viscera. Thus 251 (56 per cent) of the 446 mice employed succumbed.

The results with one of these strains obtained from the stool will serve for illustration. The streptococcus in the second culture generation from the stool of a patient during the acute stage (fourth day) of paralytic epidemic poliomyelitis consisting of a 1:200 dilution in dextrose-brain agar of a single colony at a dilution of  $10^{-52}$ , was inoculated intracerebrally (0.03 c.c.) and intralingually (0.1 c.c.) into four mice.

Two of these succumbed, one on the third day and one on the ninth day after inoculation. Eight mice were inoculated with emulsions of the brain of the mouse that had succumbed on the ninth day in the first passage. In five of these symptoms of encephalitis developed. Ten mice were inoculated with filtrates of the brain emulsion of the mouse that had succumbed on the third day in the first passage. In two of these tremors, spasms and other symptoms of encephalitis developed, from which death occurred. Emulsion of the brain of one of these and filtrate of the brain emulsion of the other were then passed successively through two sets of mice for four passages, one set receiving emulsion consecutively and the other set filtrate of emulsion of

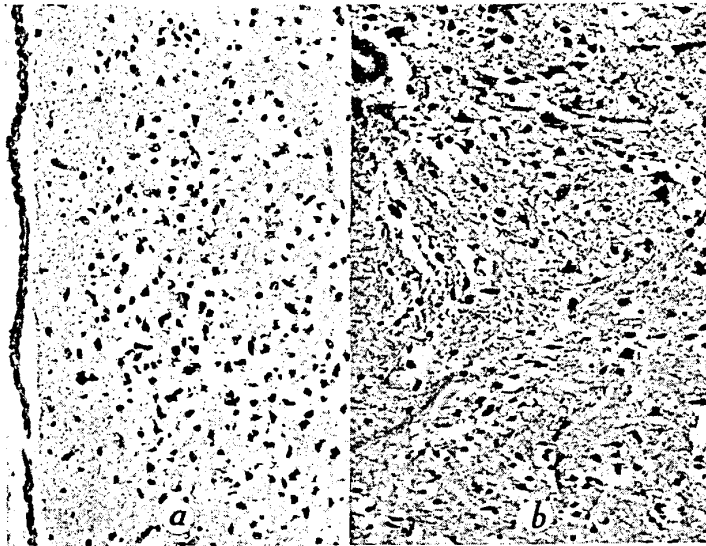


Fig. 4a. Lesions in the pons, and b, anterior horn of the spinal cord, of a mouse that succumbed after inoculation with virus in the fourth mouse passage, derived from the streptococcus isolated from the stool of a patient in the acute stage of poliomyelitis (hematoxylin and eosin,  $\times 185$ ).

the brain from preceding mouse passages. In the third passage two of four that received emulsion and two of four that received filtrate, in the fourth passage all of four that received emulsion and three of four that received filtrate, in the fifth passage one of four that received emulsion and three of four that received filtrate, in the sixth passage all of four that received emulsion and all of four that received filtrate, in the seventh passage one of four that received emulsion, and in the eighth passage two of four that received filtrate succumbed in from three to twenty-one days. In the ninth passage none of four that received filtrate succumbed and the virus was lost.

Cultures were made at necropsy from the pipettings of brain and spinal cord of altogether ninety-two mice that had succumbed, presumably to virus, in these passage experiments with virus derived from the streptococcus. Forty-seven mice (51 per cent) yielded the streptococcus. In twenty-five (58 per cent) of forty-three that received the streptococcus in the first passage and emulsions of brain and spinal cord in subsequent passages, and in twenty-two (45 per cent) of forty-nine that received filtrates of stool intracerebrally and emulsions of stool intranasally or emulsions only intranasally and emulsion or filtrates of emulsion of brain and spinal cord in passage experiments, symptoms of encephalitis or poliomyelitis were observed before death. Sections of the spinal cord or brain or both of 117 mice that presumably had succumbed to encephalitis or polioencephalitis were examined. Lesions characteristic of polioencephalitis (fig. 4) or encephalitis in varying degrees were found in 106. Brains of all mice used in propagating the virus revealed characteristic microscopic changes. In some mice with flaccid paralysis, lesions of the spinal cord were typical of poliomyelitis<sup>19</sup>, but usually lesions of the brain were maximal and lesions of the spinal cord minimal or absent.

#### SUMMARY AND CONCLUSIONS

Specific types of streptococci were isolated consistently by a serial dilution method from the stools of patients having epidemic poliomyelitis or encephalitis. Virus virulent for mice was obtained from the stools and virus was produced with streptococci isolated from the stools of patients having acute poliomyelitis. The possibility that virus obtained in the experiments beginning with streptococci represented virus from the stool and not from streptococci is extremely unlikely because the dilution of suspensions of stool material from which the streptococci were isolated was extremely high, never less than  $10^{-20}$  or 1:100,000,000,000,000,000,000. Moreover, in previous studies virus has been produced from neurotropic streptococci isolated from material not associated with virus<sup>20</sup>. The period of incubation, the incidence of deaths from encephalomyelitis, and of isolations of the streptococcus from the brains of animals that had succumbed and the symptoms and lesions on successive passages of the "natural" and experimentally produced virus were similar and chiefly those of encephalitis. This study adds one more material—the stool—in poliomyelitis to those previously reported in which the streptococcus and virus are associated. It is suggested that growth of the streptococcus in the intestinal tract may be an important source of specific toxic products and of virus in epidemic poliomyelitis.

19. Lillie, R. D. and Armstrong, Charles: Cerebrospinal pathology of experimental poliomyelitis in the eastern cotton rat, *Sigmodon hispidus hispidus*, and in the white mouse, *Mus musculus*. Pub. Health Rep. 55: 718-725 (Apr. 26) 1940.
20. Rosenow, E. C.: The relation of streptococci to the viruses of poliomyelitis and encephalitis: preliminary report. Proc. Staff Meet., Mayo Clin. 10: 410-414 (June 26) 1935.