THE RÔLE OF STREPTOCOCCI IN EXPERIMENTAL POLIOMYELITIS OF THE MONKEY.

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Within the last 12 years, several investigators have reported the isolation of streptococci from poliomyelitic tissues of man and of animals. These workers, in view of the ease with which the streptococci could be recovered and the supposed fulfillment by the organisms of one or all of Koch's postulates, believe that the streptococci are either the incitants of, or are etiologically related to poliomyelitis.[1–4] It was stated, furthermore, that the microorganisms after repeated injection into horses, can produce a serum which has neutralizing, protective, and therapeutic properties in poliomyelitis.[5] Other experimenters, however, have failed to confirm this opinion.

Thus Bull[6] reported that streptococci found in cultures of brain and spinal cord from poliomyelitic monkeys induced in monkeys neither the clinical signs

[1] Rosenow, E. C., Towne, E. B., and Wheeler, G. W., *Science*, 1916, xliv, 614; *J. Am. Med. Assn.*, 1916, lxvii, 1202; 1917, lxviii, 280; *J. Med. Research*, 1917, xxxvi (N.S. xxxi), 175. Rosenow, E. C., Towne, E. B., and v. Hess, C. L., *J. Infect. Dis.*, 1918, xxii, 314. Rosenow, E. C., and Wheeler, G. W., *J. Infect. Dis.*, 1918, xxii, 281. Rosenow, E. C., and Gray, H., *J. Infect. Dis.*, 1918, xxii, 345. Rosenow, E. C., *J. Infect. Dis.*, 1918, xxii, 379.

[2] Nuzum, J. W., and Herzog, M., *J. Am. Med. Assn.*, 1916, lxvii, 1205.

[3] Hektoen, L., Mathers, G., and Jackson, L., *J. Infect. Dis.*, 1918, xxii, 89.

[4] Mathers, G., *J. Am. Med. Assn.*, 1916, lxvii, 1019; *J. Infect. Dis.*, 1917, xx, 113. Mathers, G., and Weaver, G. H., *J. Infect. Dis.*, 1918, xxii, 559.

[5] Rosenow, E. C., *J. Am. Med. Assn.*, 1917, lxix, 261, 1074; *J. Infect. Dis.*, 1918, xxii, 379. Nuzum, J. W., *J. Am. Med. Assn.*, 1917, lxviii, 24. Nuzum, J. W., and Willy, R. G., *J. Am. Med. Assn.*, 1917, lxix, 1247; *J. Infect. Dis.* 1918, xxii, 258.

[6] Bull, C. G., *J. Exp. Med.*, 1917, xxv, 557.

nor the histopathological lesions of the experimental disease which follows inoculation of the filtered poliomyelitic virus. Nor were monkeys protected against filtered virus after recovery from the effects of injection of streptococci. Bull also cultivated streptococci from the tonsils of man—a tissue frequently employed by others[1,2,4] as a source of the supposed cocci of poliomyelitis. Streptococci from the tonsils of 32 patients with poliomyelitis and similar bacteria from the tonsils of non-poliomyelitic patients showed no differences in their respective action in laboratory animals. Bull, therefore, regarded the streptococci as secondary bacterial invaders of nervous tissue.

Smillie[7] concluded that the streptococcus could not be implicated in the pathology of the poliomyelitic process. He found that it was a common contaminant or a secondary invader: streptococci occurred in animals which were etherized while moribund, or which had died some hours before autopsy, but were absent in virus-infected animals which were killed while still relatively strong. Amoss[8] maintained a similar attitude.

With reference to the specific neutralization of the poliomyelitic virus by streptococcus immune serum, reported by Rosenow,[5] by Nuzum and coworkers,[5] Amoss and Eberson[9] found that such serum had neither neutralizing powers *in vitro* nor *in vivo*, or any therapeutic property in monkeys.

Finally, Flexner and his coworkers,[10] Levaditi and Landsteiner,[11] and others[12] have found that the effects of the filtrable poliomyelitic virus could not be ascribed to the action of ordinary bacteria.

The discrepancy in the results of different investigators and the recent increased interest in the use of antistreptococcic serum for the prevention and treatment of infantile paralysis, led us to a restudy of cultivation of poliomyelitic tissues. The study concerned especially the source of streptococci and their relation to the etiology of the disease. In general, it included a comparison of strains of streptococci isolated from monkeys affected with experimental poliomyelitis;

[7] Smillie, W. G., *J. Exp. Med.*, 1918, xxvii, 319.

[8] Amoss, H. L., in Rivers, T. M., Filterable viruses, The Williams and Wilkins Company, Baltimore, 1928, 173.

[9] Amoss, H. L., and Eberson, F., *J. Exp. Med.*, 1918, xxvii, 309; 1918, xxviii, 323.

[10] Flexner, S., and Lewis, P. A., *J. Am. Med. Assn.*, 1909, liii, 2095; see also Bull.[6]

[11] Levaditi, C., and Landsteiner, K., *Compt. rend. Soc. biol.*, 1910, lxviii, 311.

[12] Zappert, J., v. Wiesner, R. R., and Leiner, K., Studien über die Heine-Medische Krankheit (Poliomyelitis Acuta), Leipsic and Vienna, 1911, 137. Kling, C., Petterson, A., and Wernstedt, W., *Communications Inst. méd. Etat Stockholm*, 1912, iii, 5.

with 3 strains of microorganisms generously supplied by Dr. Rosenow, and derived from poliomyelitic tissues; with 1 strain obtained from the American Type Culture Collection, also derived from poliomyelitis; and with 3 cultures of Rosenow poliomyelitic streptococci supplied by Eli Lilly and Company, whose kindness is gratefully acknowledged. In addition, cultures were made from the central nervous systems of poliomyelitic monkeys, with certain media recommended by Rosenow and others, media in which they obtained the so called poliomyelitic streptococcus. In view of the findings of two of the writers (Long and Olitsky[13]) that in so far as herpes virus encephalitis of rabbits and guinea pigs is concerned, the process of grinding permits contamination of tissues with streptococci and other ordinary bacteria, we cultured both fragments and emulsions of ground material from the same brain. Furthermore, cultivation tests were made with portions of the same brain in different rooms so as to check the results of one series against those of the other.

Mode of Procedure.

The materials cultured, the media and methods employed were as follows:

Source of Virus.—The poliomyelitic virus used consisted of the brains of 17 monkeys which showed the typical signs and pathological lesions of experimental poliomyelitis induced by the injection of either the M.A. strain[14] of poliomyelitic virus, or virus supplied us by Dr. Aycock.[15] The animals were killed at a time when the characteristic flaccid paralyses were clearly evident,[16] and tissues were removed for culture. In a few instances, the tissues were procured after the animal died from the typical experimental disease. In all cases, the diagnosis was checked by finding characteristic histopathological changes in the brain and spinal cord.[17] In addition the brain of a monkey which died from a non-poliomyelitic affection was also cultured.

The brains were removed from the monkeys under sterile precautions, and when fragments were used for cultures, 5 mm. cubes of tissue were cut for the purpose. When emulsions were employed, a portion of the brain was ground by

[13] Olitsky, P. K., and Long, P. H., *J. Exp. Med.*, 1928, xlviii, 199.

[14] Flexner, S., Clark, P. F., and Amoss, H. L., *J. Exp. Med.*, 1914, xix, 195.

[15] This strain was more active in monkeys than the M.A. strain.

[16] Full ether anesthesia was used in all experiments.

[17] Flexner, S., and Lewis, P. A., *J. Exp. Med.*, 1910, xii, 227.

means of a sterile mortar and pestle. To this, physiological saline solution was added to make a 10 per cent suspension. About 1 cc. of the suspension comprised the inoculum. It is to be emphasized that all procedures were carried out under the strict conditions underlying sterile bacteriological technique.

Media.—The media employed consisted of:

(1) 5 per cent rabbit's blood, beef infusion 1 per cent dextrose or plain agar in Petri dishes.

(2) Chopped meat medium, prepared according to the directions given by Evans.[18] The medium was used because it was employed by the latter to recover streptococci from epidemic encephalitis in man and experimental encephalitis in monkeys and rabbits.

(3) Ascitic fluid-dextrose broth medium in long tubes. The medium was prepared according to the directions of Rosenow and Towne.[19]

(4) Smith-Noguchi medium based on the principles outlined by Gates and Olitsky.[20]

(5) Hartley's modification of Douglas' tryptic digest broth.[21] The special broth served as a basis for the study of toxins of the streptococci isolated during the course of the experiments.

(6) Beef infusion broth either plain or containing 1 per cent dextrose, for the preparation of agglutinogens and for a study of cultural reactions.

(7) Sterile peptone solution containing the Andrade indicator and respectively 1 per cent of dextrose, maltose, lactose, saccharose, raffinose, salicin, inulin, and mannitol for fermentation reactions.

Plan of Experiments and Technique.—For the purpose of ascertaining the origin of microorganisms in the cultures, that is, whether they were derived from the tissues themselves or from sources outside the tissues, experiments were designed as follows: (*a*) the same material was cultured in different media all of which were favorable for the growth of streptococci; (*b*) one brain was subdivided under sterile precautions into two parts of which one portion was used for cultures of fragments and the other for cultures of emulsions; (*c*) to eliminate the personal factor in the results obtained, two of the writers separately cultured portions of the same material; (*d*) one series of tests was made under the usual conditions of sterility, that is, under a hood in the room used for routine work. Another series was made under extraordinary conditions of sterility: cultures were performed in a manner similar to that employed by Dr. Carrel at The Rockefeller Institute for inoculation and transplantation of living tissue cells. The worker clothed himself in sterilized gown, hood, face mask, and rubber gloves. The media,

[18] Evans, A. C., *Pub. Health Rep., U. S. P. H.*, 1927, xlii, 171.

[19] Rosenow, E. C., and Towne, E. B., *J. Med. Research*, 1917, xxxvi (N.S. xxxi), 175.

[20] Gates, F. L., and Olitsky, P. K., *J. Exp. Med.*, 1921, xxxiii, 51.

[21] Hartley, P., *J. Path. and Bact.*, 1922, xxv, 479.

material for cultivation, and instruments for cutting and grinding tissues were kept in sterile towels and treated as is done in modern surgical aseptic procedures. The room was especially adapted for elimination of air contaminants. It had no connection with the outside air and prior to the entry of the worker, motor-driven, powerful water sprays were put into action for the purpose of clearing the atmosphere of particles. The effectiveness of the procedures is exemplified by the fact that on one occasion blood agar medium in Petri dishes kept open for 35 minutes revealed no colonies of microorganisms.

All cultures were incubated at 37°C. and whenever any tube showed evidence of growth, it was examined; negative tubes were retained for 14 days before a final reading was made. It may be stated here that growth of the bacteria occurred as a rule from 18 to 72 hours after inoculation.

Cultivation Experiments.

Emulsions.—The first series of experiments comprised cultures of emulsions of brains from 18 monkeys in chopped meat medium and in ascitic dextrose broth. These were made by two of the writers, one working under the hood in the room usually employed for routine work, and the other in the special room already described, one worker alternating with the other in different tests. In two of the experiments a third person substituted for one of the original group.

The results of this experiment indicate that when the cerebral tissue was ground and emulsified and this material used for culture, a number of microorganisms, of different species, could be recovered. The number but not the species could be influenced by environmental conditions: when the usual, although strict sterile precautions were followed, the percentage of tubes of ascitic dextrose broth medium positive was 60, and of chopped meat medium, 57. When extraordinary care for sterility was employed, the positive tubes were respectively 21 and 13 per cent of all in each series.

Further analyses of Tables I and II show that the bacteria commonly encountered in the cultures of poliomyelitic brain emulsions in ascitic broth and meat media were diphtheroids, staphylococci, non-hemolytic streptococci, and spore-bearing rods. The same species were found on the medium in the Petri dishes exposed to the atmospheres of the two environments in which the cultures were made. The average time of exposure was about 30 minutes, that is, during the course of the setting up of a particular series of cultures. The average number of colonies on a plate exposed under the hood was

TABLE I.

Emulsions of Brain Cultured in Ascitic Dextrose Broth.

Monkey No.	Status	No. tubes		No. negative		No. positive		Non-hemolytic streptococci		Diphtheroids		Staphylococci		Spore-bearing rods		Miscellaneous		No. of colonies on opened plates	
		H	C	H	C	H	C	H	C	H	C	H	C	H	C	H	C	H	C
1	Experimental poliomyelitis	6		0		6		4		0		3 (*a*)		0		0			
2	" "	9		5		4				2		2		0		0			
3	" "	6	6	1	6	5	0	0	0	4	0	0	0	0	0	1 (*b*)	0		I = 0
4	" "	6	6	4	3	2	3	0	0	1	0	1	3	0	0	0	0	I(*c*) = 25	I = 6
																		II = 17	II = 10
5	" "	5	6	0	6	5	0	0	0	4	0	1	0	0	0	0	0	I = 59	I = 2
																		II = 47	II = 2
6	" "	5	6	3	5	2	1	0	0	2	1	0	0	0	0	0	0	I = 52	I = 2
																		II = 42	II = 4
7	" "	6	5	4	4	2	1	1	0	0	1	1	0	0	0	0	0	I = 16	I = 2
																		II = 16	II = 6
8	Normal	6		2		4		1		1		0		1		1 (*d*)		I = 18	
																		II = 16	
9	Experimental poliomyelitis	5	6	3	6	2	0	0	0	0	0	2	0	0	0	0	0	I = 14	I = 5
																		II = 8	II = 0
10	" "	6	6	4	0	2	6	0	6	1	0	1	0	0	0	0	0	I = 16	I = 4
																		II = 11	II = 2
11	" "	6		2		4		0		3		1		0		0		I = not counted	
																		II = 12	

12	"	"	6	6	1	6	5	0	0	0	5	0	1	0	0	0	0	0	I = 12 II = 16	I = 1 II = 12
13	"	"	6	6	0	5	6	1	0	0	5	0	0	1	0	0	1 (*e*)	0	I = 35 II = 37	I = 0 II = 0
14	"	"	6	6	2	4	4	2	0	0	2	2	1	0	1	0	0	0	I = 17 II = 15	I = 3 II = 0
15	"	"	6		5		1		0		0		0		0		1 (*f*)		I = 12	
16	"	"	6	6	5	5	1	1	0	0	0	1	1	0	0	0	0	0	I = 8 II = 10	I = 0 II = 1
17	"	"	6	6	0	6	6	0	0	0	3	0	3	0	0	0	0	0	I = 12 II = 12	
18	"	"	6		2		4		1		3 (*g*)		0		0		1 (*h*)		I = 14 II = 7	
Total 18			108	71	43	56	65	15	7	6	36	5	18	4	2	0	5	0		
	Per cent =				39+	78+	60+	21+					Average No. of colonies =						20+	3−

(*a*) In one case admixed with streptococci. (*b*) *M. tetragenus*. (*c*) No. of plate indicated by Roman numeral. The variations in numbers of colonies is due to length of time of exposure to the air which lasted throughout the period of the test. (*d*) Diplobacillus. (*e*) Streptobacillus. (*f*) Hemolytic streptococcus. (*g*) In one case admixed with streptococci. (*h*) Mould. H = cultures made in room used for routine work; C = cultures made in special (Dr. Carrel's) room.

about 20 and in Dr. Carrel's tissue culture room, about 3. There was evidently a correlation between the number of positive cultures with the number of bacteria in the air.

TABLE II.

Emulsions of Brain Cultured in Chopped Meat Medium.

Monkey No. (a)	No. tubes		No. negative tubes		No. positive tubes		Non-hemolytic streptococci		Diphtheroids		Staphylococci		Spore-bearing rods		Miscellaneous	
	H	C	H	C	H	C	H	C	H	C	H	C	H	C	H	C
1	6		0		6		4		1		1		0		0	
2	9		3		6		1		2 (*b*)		4 (*c*)		1 (*b*)		0	
3	6	6	1	6	5	0	1	0	3	0	0	0	0	0	1 (*d*)	0
4	6	6	5	4	1	2	0	0	0	0	1	2	0	0	0	0
5	5	7	1	7	4	0	0	0	4	0	0	0	0	0	0	0
6	5	6	0	6	5	0	0	0	1	0	3	0	1	0	0	0
7	6	5	2	4	4	1	1	0	1	1	1	0	1	0	0	0
9	5	6	4	5	1	1	0	0	0	0	1	0	0	1	0	0
10	6	6	4	2	2	4	0	4	0	0	2	0	0	0	0	0
11	6		5		1		0		1		0		0		0	
12	6	6	1	6	5	0	0	0	0	0	5	0	0	0	0	0
13	6	6	4	6	2	0	0	0	2	0	0	0	0	0	0	0
14	6	6	6	6	0	0	0	0	0	0	0	0	0	0	0	0
15	6		1		5		0		1		3		0		1 (*e*)	
16	6	6	4	5	2	1	0	0	2	1	0	0	0	0	0	0
17	6		0		6		0		2		4		0		0	
Total 16	96	66	41	57	55	9	7	4	20	2	25	2	3	1	2	0
Per cent =			42+	86+	57+	13+										

(*a*) For status of monkeys see Table I. Since these tests were made at the same time as those recorded in Table I, the control air cultures are not repeated here. (*b*) In one instance admixed with spore-bearing rod. (*c*) In one instance admixed with streptococci. (*d*) *M. tetragenus*. (*e*) Streptothrix. H and C as in Table I.

In addition it will be noted that although two portions of the same poliomyelitic or non-poliomyelitic monkey brain were employed, the results of cultures of each portion were in most instances wholly dissimilar. A striking example is that of Monkey 10. In one environment, 10 of 12 tubes of medium yielded non-hemolytic streptococci

(C, Tables I and II); in another room, of 12 tubes, 1 tube showed diphtheroids, 3 tubes staphylococci, and none revealed streptococci (H, Tables I and II).

Since the brains for culture were removed from monkeys while most of them were still relatively strong, although in different stages of flaccid paralyses, the notion that the bacteria were agonal invaders was not tenable. Moreover, the ground brain of the non-poliomyelitic monkey which was cultured at autopsy in ascitic broth also exhibited in different tubes diphtheroids, spore-bearing rods, diplobacilli, and non-hemolytic streptococci.

TABLE III.

Cultures of Fragments of Monkey Brain in Ascitic Broth and Chopped Meat Medium.

Monkey No.	Status	No. tubes chopped meat medium		No. tubes ascitic dextrose broth		No. of tubes positive in the meat medium		No. of tubes positive in the broth	
		H	C	H	C	H	C	H	C
2	Experimental poliomyelitis	9				0			
7	" "	6	5	5	6	0	0	0	0
8	Normal	6		6		0		0	
9	Experimental poliomyelitis	5	6	5	6	0	0	0	0
16	" "	6			6	0			0
17	" "	6			6	0			0
18	" "			6				1 (*a*)	
Total 7		38	11	22	24	0	0	1	0
		Total No. tubes 95				Total positive 1 tube			

(*a*) Diphtheroids. H and C as in Table I.

In general, the results of cultivation of emulsions of ground poliomyelitic and non-poliomyelitic cerebral tissues of monkeys parallel closely those of cultivation from herpes virus encephalitis of rabbits and guinea pigs, already reported.[13] In the last mentioned disease, it was shown that the various bacteria of common species, including the streptococci, were contaminants introduced during the process of grinding. Furthermore, in herpes virus encephalitis no growth of microorganisms was obtained when fragments, instead of ground

material, were used. How this applies in turn to poliomyelitic brain cultures is illustrated by Table III.

Fragments.—7 of the monkey brains which were cultured in the form of ground material were also cultured in the form of fragments.

Table III shows that of a total number of 95 tubes containing ascitic dextrose broth or chopped meat medium and fragments of monkey brains, 6 poliomyelitic and 1 non-poliomyelitic, only 1 exhibited growth. The positive tube contained diphtheroids. If a comparison be made with the cultures of emulsions of the same brains and the same media (Tables I and II), it will be plain that with poliomyelitic brains just as with herpes virus encephalitis brains, cultures of ordinary bacteria occurred as a consequence of emulsifying the cerebral tissues. Again it should be emphasized that the results shown in all three tables were derived from portions of the same brain.

In an additional experiment, a poliomyelitic brain from Monkey 15 was cut into fragments, the fragments placed in broth at 37°C. for 24 hours, and then transferred to 6 tubes of ascitic dextrose broth and 6 tubes of chopped meat medium. The latter medium was, after 14 days incubation, negative but the ascitic broth cultures showed nonhemolytic streptococci in 2 tubes, staphylococcus, diphtheroids, and streptothrix in single respective tubes. This test was made in the routine culture room. It should be compared with the findings in the same material as given in Tables I and II. It is obvious that the results in this case depended on the additional handling of the brain.

To summarize, a variety of ordinary bacteria including nonhemolytic streptococci can be recovered from cultures of poliomyelitic monkey brains. They appear irregularly in tests with different portions of the same brain, occurring sometimes in one favorable medium but not in another, frequently in one room but not in a second, often when only the usual bacteriological technique for sterility is followed but much less often when extraordinary precautions for sterility are maintained, and generally in emulsions of the brain but not in its fragments. Furthermore, the bacteria are similar to those often recovered from the air of the place in which the cultures are made. What has been said, therefore, of herpes virus encephalitis can be stated again as applying to experimental poliomyelitis in monkeys, namely, the bacteria are introduced into the material for culture during grinding.[13]

Smith-Noguchi Medium.—This opinion is further supported by results of cultures in the Smith-Noguchi medium, although the primary purpose of this series of cultivation tests was the recovery of the globoid bodies of Flexner and Noguchi.[22] The cultures were made with fragments of monkey brain in ordinary Smith-Noguchi medium[20]

TABLE IV.

Cultivation of Fragments in Smith-Noguchi Medium.

Monkey No. (*a*)	No. of tubes		No. of tubes positive		Diphtheroids		Non-hemolytic streptococci		Staphylococci		Miscellaneous		Globoid bodies	
	O	B	O	B	O	B	O	B	O	B	O	B	O	B
3	5	5	1	0	1	0	0	0	0	0	0	0	0	0
4	6	6	1	6	0	2	0	0	1	0	0	1 (*b*)	0	3
5	5	5	4	0	1	0	1	0	1	0	1 (*c*)	0	0	0
6	5	5	2	1	1	0	0	0	1	0	0	1 (*d*)	0	0
7	6	6	1	2	1	1	0	0	0	0	0	1 (*c*)	0	0
8	6	6	4	1	2	0	0	1	0	0	2 (*c*)	0	0	0
9	6	6	0	0	0	0	0	0	0	0	0	0	0	0
10	6	6	0	0	0	0	0	0	0	0	0	0	0	0
11	6	6	0	1	0	1	0	0	0	0	0	0	0	0
12	6	6	0	0	0	0	0	0	0	0	0	0	0	0
13	4	5	1	1	0	0	0	0	0	0	0	0	1	1
14	6	6	0	0	0	0	0	0	0	0	0	0	0	0
16	6	6	0	0	0	0	0	0	0	0	0	0	0	0
17	6	6	0	1	0	1	0	0	0	0	0	0	0	0
Total 14	79	80	14	13	6	5	1	1	3	0	3	3	1	4

(*a*) The status of these monkeys is given in Table I. Compare with Tables I to III for results on aerobic cultivation. (*b*) Streptothrix. (*c*) Spore-bearing rod. (*d*) Diplococcus. O = Ordinary Smith-Noguchi medium and B = Boëz' apparatus.

and similar medium having no petrolatum seal but placed in an anaerobic apparatus described by Boëz.[23] The results are given in Table IV.

There is general agreement on the difficulty of avoiding contamination in the Smith-Noguchi technique. In the series shown in Table IV 27 of 159 tubes exhibited different bacteria. The number of positives

[22] Flexner, S., and Noguchi, H., *J. Exp. Med.*, 1913, xviii, 461.

[23] Boëz, L., *J. Bact.*, 1927, xiii, 227.

might have differed if one instead of three workers had made all the tests. However, in the case of 2 monkeys (Nos. 5 and 6), emulsions of ground brain were also planted in Smith-Noguchi medium; 10 of 12 tubes showed different bacteria.

With respect to the isolation of globoid bodies, it is to be noted that they were obtained from the poliomyelitis brains of 2 of the 14 monkeys (Table IV). In 1 case, 3 of 12 tubes were positive, and in the other 2 of 9 tubes. 1 culture in its first generation was inoculated intracerebrally into a monkey; no signs of experimental poliomyelitis developed.[24]

The globoid bodies were found to be distinct microorganisms but differed from the streptococci recovered in the tests and from those obtained from Rosenow. They were definitely anaerobic; indeed, in one instance, a transplant from a culture in ascitic agar kept in the dark at room temperature for about 9 years was still viable and anaerobic. On the other hand, the inoculation of streptococci into Smith-Noguchi medium failed to bring about their conversion to globoid type. 9 strains of streptococci, including 3 of Rosenow's series, planted in the medium and examined after 14 days incubation showed death of 2, somewhat smaller forms in 3, and no change in the remaining strains. Transfer of the survivors to aerobic medium yielded profuse growths of typical large cocci or coccoid forms. These conditions prevailed through 3 successive subplants in Smith-Noguchi medium at 14 day intervals.

Properties of the Streptococci.

31 strains of non-hemolytic streptococci were collected for comparative study. In this collection were 7 strains of Rosenow, all of which were stated to be derived from poliomyelitis;[25] 1 was recovered from "ground" broth and 3 from ground chopped meat

[24] In this connection it is of interest to record a repeated experiment in which fragments of poliomyelitic brain were placed in broth and kept for 14 days under aerobic and anaerobic conditions. At the end of this time, the fragments were removed and ground in saline solution and injected intracerebrally in monkeys. Neither the aerobic nor the anaerobic material was found to be active.

[25] 3 of the strains were given us by Dr. Rosenow, 3 by Eli Lilly and Company, and 1 was obtained from the American Type Culture Collection.

medium, already described;[13] 2 from herpes virus encephalitis in rabbits;[13] 2 from normal guinea pig brain;[13] 5 from the air in the rooms in which the cultures were made; 1 from a non-poliomyelitic monkey brain; and 10 from poliomyelitic monkey brains. In addition there were included 1 strain of enterococcus and 1 of hemolytic streptococcus—the latter being recovered from the air.

Cultural Characteristics.—With few exceptions, the non-hemolytic streptococci produced on rabbit blood agar small, round, greenish tinged colonies with a very narrow zone of clearing; exceptional colonies did not produce this zone. On rabbit blood dextrose agar the colonies precipitated the medium to dark brown, just underneath and surrounding the colony. In fluid medium, consisting of chopped meat broth, ascitic dextrose broth, dextrose and plain broth, all the streptococci grew profusely within 18 to 48 hours. The dextrose media showed a granular appearance along the sides and a heavy, whitish precipitate; the other media revealed either granular or diffuse, but also luxuriant growths.

Morphologically long chained forms predominated but 2 of Rosenow's cultures and some selected streptococci obtained from the air and from other non-poliomyelitic sources revealed diplococcus forms often in short chains. All showed pleomorphism, and when a long chained form was inoculated into a rabbit, the tissues would usually reveal diplococcus forms; this happened irrespective of the source of the culture.

Tests for fermentation were made on 24 cultures of the non-hemolytic streptococci. Four distinct groups containing cultures having identical reactions could be determined; the reactions of each group as a whole, however, differed from those of the others. A fifth group contained 11 heterogeneous strains which were unrelated to any of the others. All cultures were classified as follows:

Group I.	1 strain of streptococcus obtained from air (Dr. Carrel's room).
	2 strains of Rosenow's series given us by Eli Lilly and Company.
Group II.	1 strain of streptococcus obtained from air (hood).
	1 strain from non-poliomyelitic brain.
	1 strain from poliomyelitic brain.
Group III.	1 strain (No. 349) of Rosenow's series from American Type Culture Collection.
	1 strain obtained from Rosenow.

Group IV. 3 strains from poliomyelitic brains.
2 strains obtained from Rosenow.

Group V. Heterogeneous. Includes 2 strains obtained from the air, 1 from Eli Lilly and Company, and 8 from poliomyelitic monkey brains. In this group only one culture fermented mannitol; 3, salicin; 6, inulin (3 faintly so); 3, raffinose; and all fermented dextrose, maltose, lactose, and saccharose.

It is noteworthy that there was no uniformity in fermentation reactions. Furthermore, when 2 or more cultures were recovered from the same brain, the individual growths showed different responses to the tests.

Serological Reactions.—With regard to direct and cross-agglutination reactions with rabbit immune serum, the different cultures of non-hemolytic streptococci, irrespective of source, also showed a marked heterogenicity.

Rabbits were immunized by the method of injecting intravenously first dead then living cultures. 3 to 4 inoculations were given at daily intervals and after a rest of 4 to 5 days, the daily series was continued, proceeding thus for over a month. 10 days after the last injection, the rabbits were exsanguinated. The blood serum then revealed agglutinins in dilutions of 1:320 to 1:5120 to homologous strains. In only one instance was the titre 1:40.

As antigens for the preparation of the rabbit serum, 10 strains were used, of which 7 were directly or indirectly obtained from Rosenow and were classified by him as "poliomyelitic" streptococci; 2 were recovered from cultures of "ground" broth or chopped meat medium;[13] and 1 was derived from cultures of poliomyelitic monkey brain. Agglutination tests were set up after the manner of Rosenow with the 10 sera and 25 different cultures. The latter included an enterococcus, a *Micrococcus tetragenus*, a hemolytic streptococcus, and 22 strains of non-hemolytic streptococci derived from the air, from media, from non-poliomyelitic guinea pig and monkey brains, and from poliomyelitic monkey brains.

A summary of the results reveals that 3 of the sera prepared with 3 cultures, 2 of which were derived respectively from "ground" media and 1 from a poliomyelitic monkey brain, agglutinated only the homologous cultures. The remaining 7 sera agglutinated, apart from the homologous strains, very few of the 25 cultures put to test. The latter 7 sera were all prepared with different strains ultimately derived from Rosenow. The positive results with these sera follow:

Serum 809, positive only with 2 strains of streptococci obtained from poliomyelitic monkey brains, in dilutions of 1:640 and 1:2560; and Serum 866, positive only with 1 similar strain (1:160). Serum 2254, positive only with 2 other Rosenow strains (1:160 and 1:320). Thus also Serum 349 (1:160 and

1:1280). Serum 3002, positive only with 1 other Rosenow strain (1:40); similar to this was Serum 3007 (1:1280). Serum 3005, positive only with 1 other Rosenow serum (1:320) and with a non-hemolytic streptococcus obtained from ground normal guinea pig brain.

One may therefore conclude that from the view-point of serological reactions the non-hemolytic streptococci, whether obtained from poliomyelitic or other material, form a class of bacteria of dissimilar antigenic function. Furthermore, pooled serum from monkeys recovered from experimental poliomyelitis (and similar serum from normal monkeys, employed as control) failed to agglutinate any of the non-hemolytic streptococci, including Rosenow's 7 strains. Finally, a similar heterogenicity among the poliomyelitic and among the non-poliomyelitic streptococci was revealed by precipitin tests.

Skin Reactions in Rabbits.—No skin reactions were noted in rabbits when the animals were injected intracutaneously with a filtrate from Hartley's modification of Douglas' tryptic digest broth[21] inoculated with Rosenow's strains of streptococci and incubated for 10 days, nor were skin reactions visible with lysates of these microorganisms prepared *in vivo* in immunized rabbits.

Pathogenicity for Rabbits.—It has been stated by Rosenow and others that rabbits dying of streptococcus infection showed signs and lesions identical with those of poliomyelitis in man and monkeys.[1,2,4]

36 rabbits were inoculated intracerebrally with 0.35 cc. to 0.4 cc. of 18 to 24 hour old broth cultures of non-hemolytic streptococci derived from the air, from non-poliomyelitic and poliomyelitic monkey brains. 18 different cultures were used and each was inoculated into 2 rabbits. The results are tabulated on page 446.

In spite of the fact that the filtrable virus of poliomyelitis cannot be successfully implanted on the nervous tissue of rabbits,[26] the streptococci, on the other hand, readily induced a reaction which is not characteristic of the virus of poliomyelitis but is typical of streptococcus infection. It is to be noted that the microorganisms derived from poliomyelitic and non-poliomyelitic tissues; or from the air; or, as has been previously reported,[13] from "ground" medium, and from ground normal or herpes virus-infected brain, show no distinctive effects in rabbits. Many of the cultures of streptococci,

[26] Flexner, S., and Lewis, P. A., *J. Am. Med. Assn.*, 1910, liv, 45.

obtained from different sources, are pathogenic for rabbits, producing purulent meningoencephalitis complicated by streptococcic septicemia, and few of these bacteria are apparently non-pathogenic. It is noteworthy that some rabbits are resistant to pathogenic strains —an observation also made in the studies on herpes virus encephalitis.[13]

Strain	Source	Results in each of 2 rabbits
3002 (*a*)	Rosenow (from poliomyelitic tissue)	1 died within 24 hrs.; the other survived
3005	" "	Both survived
3007	" "	" died within 24 hrs.
349	" "	1 died within 24 hrs.; the other survived
809	" "	Both died within 24 hrs.
866	" "	" " " " "
2254	" "	" survived
1	Experimental poliomyelitis of monkey	" died within 48 hrs.
3	" "	1 died within 48 hrs.; the other survived
6	" "	" "
7	" "	Both died within 24 hrs.
15	" "	" survived
10	" "	" died within 24 hrs.
10E5	" "	1 died on the 5th day; the other survived
8	Non-poliomyelitic monkey	Both died within 24 hrs.
Air C	Air Dr. Carrel's room	" " " " "
Air H	Air routine laboratory	" survived
Air H 2	" " "	1 died within 24 hrs.; the other survived

(*a*) Strains 3002, 3005, 3007 were obtained from Eli Lilly and Company; 349, from the American Type Culture Collection; and 809, 866, and 2254 directly from Dr. Rosenow.

The rabbits that died exhibited purulent meningoencephalitis. Their brains, and in most instances the heart's blood, yielded pure growths of streptococci. In respect to the action of streptococci in rabbits, we have confirmed in detail the previous findings of Bull,[6] to whose work the reader is referred for a comprehensive description of the signs and histopathology in injected animals. These experiments are also in complete agreement with those reported by Olitsky and Long on the rôle of streptococci in herpes virus encephalitis,[13] and the reader is also referred to this paper for a fuller account of the action of streptococci in rabbits.

DISCUSSION AND SUMMARY.

It is the opinion of Bull,[6] that the streptococci recovered from poliomyelitic tissues, while having no etiological or pathological relationship to the virus of poliomyelitis, occur as secondary invaders in the disease. Smillie[7] and Amoss[8] indicated that the bacteria may be agonal invaders.

The results of the experiments reported in this paper point to another source of the streptococci. They occur as contaminants which are introduced into the cultures during the process of grinding tissues. The source of the streptococcus may therefore be the air of the place in which the cultures are made. We have come to this conclusion because first, the tissues of which cultures yielded streptococci were derived from a number of monkeys with experimental poliomyelitis still in a vigorous state. Secondly, when the tissues were ground bacteria were noted much more frequently in their cultures than in those in which fragments of the same brains were used. Thirdly, microorganisms occurred more often in cultures made in the routine laboratory than in a special room where asepsis was carried to the extreme of a major surgical operation on man. Fourthly, streptococci were obtained from the air of the places where cultures were made. Finally, there is no correlation between the cultures of two portions of the same brain.

The streptococci occurred in some cultures in pure growth and in others admixed with other ordinary species of bacteria. The latter were often found, in turn, in pure culture and what applies to streptococci, as mentioned in the preceding paragraph, applies equally to the staphylococci, diphtheroids, spore-bearing rods, and other miscellaneous, familiar microorganisms.

We could not determine that there exists any etiological relation of the streptococci to poliomyelitis. The fermentation reactions of the microorganisms obtained from the air, from non-poliomyelitic and poliomyelitic monkey brains indicate that bacteria from any of these sources are markedly different. So also with the serological reactions of agglutination and precipitation. Furthermore no agglutination was observed when the serum of monkeys convalescent from experimental poliomyelitis was mixed with any of the streptococci

recovered or those received directly or indirectly from Rosenow. Moreover, the intracerebral injection with cultures, irrespective of their source, induced in rabbits a purulent type of meningoencephalitis, often associated with streptococcic septicemia. This result is at marked variance with any known effects of the true filtrable virus of poliomyelitis in man and in the monkey.