A CONTRIBUTION TO THE BACTERIOLOGY OF ACUTE ANTERIOR POLIOMYELITIS.*

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PLATE 66.

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During the recent outbreak of acute anterior poliomyelitis in Philadelphia (1916) we began a series of bacteriological studies with the blood and cerebrospinal fluid collected from patients during life and with various tissues secured at eight autopsies.

Early in the work we obtained cultures of diplococci, streptococci, and other microorganisms, but on account of the consensus of opinion to the effect that these bacteria are of little or no importance in the etiology of the disease, we devoted our efforts toward the cultivation of the organism described by Flexner and Noguchi (1) in 1913. As the result of recent communications by Mathers (2), Rosenow and his associates (3), and Nuzum and Herzog (4), setting forth renewed claims for the etiologic relation of these cocci to acute anterior poliomyelitis, we have given the cultures further and more extensive study, and the purpose of this communication is to present the results of our work with many different cultures of these easily cultivated bacteria isolated from persons ill with acute anterior poliomyelitis.

* Part of the paper was read in the symposium upon acute anterior poliomyelitis before the Philadelphia County Medical Society, November 8, 1916. This work was instituted in the laboratory of the Philadelphia Hospital for Cutaneous Diseases and continued in the laboratories of Pathology of the University of Pennsylvania, with the cooperation of Dr. Allen J. Smith, Dr. Charles K. Mills, and others, as a part of a series of investigations bearing upon the microparasitology and clinical aspects of poliomyelitis.

It is probable that the micrococci recently described and regarded as the specific etiologic agent of acute anterior poliomyelitis were met with early in the course of investigations bearing upon the etiology of this disease. Bülow-Hansen and Harbitz (5) and Harbitz and Scheel (6, 7) found a diplococcus in the cerebrospinal fluid of several cases of acute anterior poliomyelitis and referred to the work of Geirsvold (8), who found a diplococcus in the cerebrospinal fluid of twelve cases and claimed to have produced paralysis and death in experimental animals with them. Pasteur, Foulerton, and Maccormac (9) reported the discovery of a micrococcus in the cerebrospinal fluid of a case during life, which produced in rabbits symptoms resembling the disease in human subjects. Leiner and von Wiesner (10) and Krause and Meinicke (11) reported that these micrococci were not the etiologic agents of acute poliomyelitis, as had Flexner and Lewis, who first showed that the etiologic agent was filterable through dense filters and probably belonged to the filterable viruses (12); simultaneous and similar results were observed and reported by Landsteiner and Levaditi (13). Dixon, Fox, and Rucker (14) also found a diplococcus in the cerebrospinal fluid, nose, and throat of patients with acute anterior poliomyelitis, and Rucker made one of the fullest studies of this diplococcus apparently identical with that recently described by Mathers (2) and Nuzum and Herzog (4). While the latter have reported the successful infection of various laboratory animals with these cocci and the reproduction of a disease with clinical symptoms and lesions similar to those of acute poliomyelitis, the experiments of the former were negative throughout; an inoculated monkey succumbed with a hemorrhagic meningitis, but without clinical or histological evidences of anterior poliomyelitis.

Rosenow (3) has recovered what he calls a "peculiar polymorphous streptococcus" from the tonsils, brain, cord, mesenteric lymph glands, and once from the blood, but never from the cerebrospinal fluid of cases of acute poliomyelitis, which produced lesions and symptoms among the lower animals regarded as those of acute poliomyelitis. He has described changes in size and staining reaction of these streptococci according to the culture medium employed, the age of the culture, and whether they have been grown aerobically or anaerobically. The cocci were said to become very small under anaerobic conditions and to approach in size the globoid bodies described by Flexner and Noguchi; the small forms were found to be filterable through Berkefeld filters while the larger forms were not.

We have found various microorganisms and especially a diplococcus and a streptococcus not only in the cerebrospinal fluid but also in the tissues of the central nervous system and in various other internal organs of fatal cases; in no instance, however, have we been able to produce anterior poliomyelitis in monkeys and rabbits with any culture by intracranial, intravenous, or intraperitoneal injection of these microorganisms.

Microorganisms Isolated.

From the cerebrospinal fluid of poliomyelitic patients during life and from various tissues after death, we have cultivated anaerobically four different varieties of microorganisms as follows: (*a*) streptococci, (*b*) diplococci, (*c*) diphtheroids, and (*d*) Gram-negative bacilli.

As stated above, we have divided the micrococci into two groups; namely, those which grow out into long chains and present the characteristics of streptococci and those which assume a diplococcus arrangement in small clumps and short chains. Apparently Rosenow believes that these are the same microorganism with a polymorphous nature. Before summarizing the results of our anaerobic cultures of the cerebrospinal fluid, blood, and tissues of poliomyelitic patients, the different microorganisms which we have recovered may be briefly described.

Streptococcus.

1. In cultures of tissues containing streptococci in an ascites-broth-kidney medium under sterile paraffin oil, macroscopic growth was first apparent after 5 to 9 days' incubation at 37°C.

2. Primary anaerobic and aerobic cultures and particularly subcultures of the streptococci grew in the form of a granular sediment at the bottom and sides of the tubes with a clear or but slightly cloudy supernatant medium. The diplococci, however, grew more diffusely.

3. Cultures under paraffin oil and in a Novy jar in an atmosphere of hydrogen grew somewhat more slowly than cultures under oil only.

4. Aerobic transplants from the anaerobic cultures to suitable solid or fluid media presented a visible growth within 48 hours.

5. Aerobic cultures of the streptococcus were readily secured by culturing emulsions of tissue in ascitic broth at 37°C. Anaerobic cultures required more time (at least 5 to 9 days, as stated above).

6. Films of 5 to 9 day anaerobic cultures showed short chains of Gram-positive cocci; longer chains were found later, although they may be found in the initial growth. As a rule, these cocci were round or slightly flattened; in a few instances the cocci in young anaerobic cultures have been elongated, while in older cultures the spherical shape was noted.

7. On defibrinated horse blood agar the colonies were small and of three varieties: (1) those which were non-adherent and produced a greenish pigment; (2) those which were non-adherent and hemolytic; and (3) those which were non-adherent and non-hemolytic.

8. Definite capsules were never found with any culture even after passage through mice, although films of cultures in ascitic broth stained after the method of Gram not infrequently showed slight unstained halos about the cocci suggestive of capsules.

9. In older anaerobic cultures the streptococci became smaller; after 14 days' incubation at 37°C. the majority of cocci were much smaller than those in fresh anaerobic and aerobic cultures. Large and small forms have been found together and the small cocci are easily decolorized during staining after the method of Gram.

10. Of sixteen cultures studied in relation to acid production with various carbohydrates, two were found to produce acid with inulin.

11. Aerobic and anaerobic cultures of streptococci were obtained from the cerebrum, cerebellum, pons, and cord after remaining in a 50 per cent mixture of pure neutral glycerol and sterile salt solution for periods of time varying from 10 days to 8 weeks.

The notable features of these streptococci were their slow multiplication in anaerobic cultures; the more rigid the anaerobiosis, the slower were the growths. The cocci became progressively smaller under anaerobic conditions, more easily decolorized with alcohol in the Gram method of staining, and more indefinite in outline as viewed microscopically.

Compared with films of mass cultures of the microparasite described by Flexner and Noguchi stained after the method of Gram but without counterstaining, our cocci were somewhat larger. The organism of Flexner and Noguchi occurs in short chains and irregular clumps, and while in our older anaerobic cultures we could find occasional cocci comparable with them in their minute size, yet the majority of our preparations showed uniformly distinctly larger forms.

Diplococci.

The most frequently cultivated microorganism in the cerebrospinal fluid and tissues of acute anterior poliomyelitis and apparently that first described occurs in the form of a Gram-positive diplococcus arranged in isolated pairs, tetrads, short chains, and irregular clumps. This diplococcus seems neither to have been named nor has its relation to known diplococci been established. The important morphological and biological characters of our cultures of these diplococci correspond closely with those described by various investigators.

1. In anaerobic cultures in ascites-broth-kidney medium of cerebrospinal fluid and emulsions of tissue containing this microorganism growth first appeared after 3 days' incubation at 37°C. Aerobic cultures presented macroscopic growth in 24 hours. In fluid medium the growths were diffuse with the gradual formation of a slimy sediment along the sides and bottom of the tubes.

2. Subcultures of the anaerobic cultures on ordinary culture media grew luxuriantly, producing whitish moist colonies resembling *Staphylococcus albus*.

3. Films of the aerobic and young anaerobic cultures showed a Gram-positive coccus usually arranged in diplococcus formation in chains of four or five pairs and in small clumps. Films of older anaerobic cultures (14 days or more) showed that the majority of these cocci had become smaller and many were easily decolorized by alcohol in the Gram stain.

4. Aerobic subcultures usually showed a staphylococcus grouping with a loss of the diplococcus formation. On solid media the tendency for the formation of short chains was lost.

As with the streptococci these diplococci tend to become smaller under strict anaerobic conditions as do cultures of *Staphylococcus albus* under similar conditions. These diplococci have many properties in common with varieties of diplococci found in the skin by one of us (15), and except for the reason that they have been found in tissues and cerebrospinal fluid collected under apparently aseptic conditions, we should be strongly inclined to regard them as microorganisms derived from the skin and mucous membranes.

Diphtheroid Bacilli.

These bacilli, which were short, solid, or occasionally granular in appearance and Gram-positive, did not present any difficulties in their recognition. They grew slowly under anaerobic conditions, but luxuriantly on appropriate media under aerobic conditions, producing large whitish colonies resembling the pseudodiphtheria bacillus rather than the true virulent bacillus. They were non-virulent for animals and their biological properties were those commonly found with cultures of diphtheroids recovered from enlarged lymphatic glands and mucous membranes.

Gram-Negative Bacilli.

In anaerobic and aerobic cultures of emulsions of poliomyelitic tissues and also of cerebrospinal fluid during life, two varieties of Gram-negative bacilli were found. (1) The usual variety was a short bacillus which morphologically and biologically appeared to be a member of the *B. coli* group. The majority produced gas with dextrose, produced indole, and coagulated milk. (2) In a few cultures

of the tissues a second Gram-negative bacillus was found which was much smaller than the preceding and resembled the influenza bacillus. Further statements regarding these bacilli cannot be made at this time except to state that we do not regard them as *B. influenzæ*.

Results of Anaerobic Cultures of Cerebrospinal Fluid in Acute Anterior Poliomyelitis.

Anaerobic cultures were made of the cerebrospinal fluid from 106 cases of acute anterior poliomyelitis by placing 1 or 2 cc. of the freshly drawn fluid collected under aseptic precautions in tubes of ascitic fluid or ascitic fluid and broth containing sterile rabbit kidney and covered with sterile paraffin oil. After incubation for 5 days or more the cultures were examined and studied; in Table I is given a summary of the results of these cultures, showing the different bacteria found. Not infrequently a culture of one fluid contained two or even three different microorganisms.

The majority of these cultures were made within a few hours after the admission of the patients to the Philadelphia Hospital for Contagious Diseases and at varying intervals after the onset of symptoms.

TABLE I.

Anaerobic Cultures of the Cerebrospinal Fluid of 106 Cases of Acute Anterior Poliomyelitis.

Microorganism.	Cultures showing presence of microorganism.
Sterile	16
Streptococci	None.
Diplococci	48
Gram-negative bacilli	22
Diphtheroids	22
B. subtilis	20

As shown in this table, definite streptococci were not found; the diplococcus was found in forty-eight fluids, or 45 per cent of those cultured. As previously stated, this microorganism may occur in pairs or short chains in the anaerobic cultures, but aerobically and on solid medium a staphylococcus arrangement is usual.

Gram-negative bacilli and diphtheroids were found in 20 per cent of cultures. *Bacillus subtilis* was also found in 19 per cent. This microorganism is of course a contamination, and not only indicates the difficulties of avoiding contamination in the collection of spinal fluids from a large number of small, sick children, but casts doubt on the series as a whole (16).[1]

Results of Anaerobic Cultures of Various Tissues in Acute Anterior Poliomyelitis.

Anaerobic cultures of various tissues from fatal cases of poliomyelitis were made during or immediately after autopsy, in ascites-kidney and ascites-broth-kidney media. All the cultures reported herein were cultivated under paraffin oil only and not in the Novy jar. The tissues removed at autopsy were collected with care to exclude bacterial contamination as much as possible, but the conditions were not such as always to accomplish this end. Hence the portion of tissue selected for culture was dipped momentarily in boiling salt solution or water before being cultured, obviously an imperfect method of securing even superficial sterilization of the tissues. Here again, as Table II shows, the variety of bacterial forms cultivated indicates that either postmortem or agonal invasion had taken place or unavoidable contamination had occurred during removal of the organs.

As shown in Table II, streptococci were found in the brain and cord of at least 50 per cent of our cases. The presence of streptococci in various other internal organs including the thymus gland, liver, spleen, mesenteric glands, kidneys, suprarenal glands, and pancreas was also noteworthy. While a streptococcus has been reported in the lymphatic glands, yet its widespread distribution in the tissues of poliomyelitic patients has not been determined heretofore, as indicated in our studies.

[1] The experience of the Department of Health of the City of New York with cultures of the cerebrospinal fluid is valuable in this connection (16). One difference in the results may be attributable to the fact that we employed the anaerobic method of cultivation which develops growth under conditions in which the aerobic cultures do not.

TABLE II.

Results of Anaerobic Cultures of Various Tissues in Fatal Cases of Anterior Poliomyelitis.

Organ.	Total No. examined.	Results of cultures.				
		Sterile.	Streptococci.	Diplococci (staphylococci).	Diphtheroids.	Gram-negative bacilli.
Cerebrum	8	2	4	3	0	3
Cerebellum	8	1	4	2	0	2
Pons and medulla	7	2	6	4	0	2
Cord	8	1	4	4	1	3
Exudate on cord	6	0	5	2	2	1
Thymus gland	2	0	2	0	0	0
Lungs	3	0	3	2	0	0
Liver	3	1	1	2	0	0
Spleen	3	1	3	1	0	0
Kidneys	3	0	2	1	1	0
Mesenteric glands	4	0	3	3	0	1
Pancreas	4	2	1	1	0	0
Suprarenals	2	1	1	0	0	0
Tonsils	2	0	2	2	1	0

The streptococci from the internal organs resembled those from the brain and cord, except that the former showed a tendency to grow in longer chains.

The diplococci were likewise found not only in the brain and cord but also in various internal organs.

The diphtheroids were found infrequently, while Gram-negative bacilli were found mostly in the cultures of the brain and cord.

Anaerobic Cultures of the Blood in Acute Anterior Poliomyelitis.

Blood cultures were made of twenty cases of anterior poliomyelitis during the acute stages of the disease by withdrawing 1 to 3 cc. of blood from a vein at the elbow under aseptic precautions and culturing in ascites-broth-kidney medium under paraffin oil. A streptococcus was recovered in one instance, while a staphylococcus was secured in ten. The latter in the fluid medium had a diplococcus arrangement while transplants on solid medium such as plain or glucose agar yielded profuse whitish colonies resembling *Staphylococcus*

albus. These cocci were practically indistinguishable from the micrococci cultivated from the spinal fluid. Since the blood has been cultured repeatedly without result, the question arises again whether contamination was not also the explanation of the presence of diplococci in the blood.

Filtration Experiments.[2]

Since Flexner and Lewis demonstrated that the etiologic agent of acute anterior poliomyelitis is filterable through filters which hold back such microorganisms as *Micrococcus prodigiosus*, filtration experiments are of considerable interest and importance in the study of the etiology of this disease. The later experiments of Flexner and Noguchi showed that the minute globoid bodies were filterable, and Rosenow has reported that the small forms of the polymorphous streptococcus are filterable through Berkefeld N filters.

We have conducted a number of experiments with salt solution emulsions of tissues containing diplococci and streptococci and with various pure cultures of these and other microorganisms. In the absence of reliable Berkefeld filters we have employed the small Kitasato (fine) and larger Pasteur-Chamberland (fine) candles. The tissues had been in 50 per cent glycerol and salt solution for varying intervals of time, but cultures of the emulsions made at the time of the filtrations showed the presence of viable microorganisms.

Pieces of tissue about the size of a bean were washed several times in sterile water and ground in sterile mortars with sterile sand, with the addition of about 15 cc. of sterile salt solution. The emulsion was then centrifuged or filtered through sterile paper and anaerobic cultures were prepared. The emulsions were then passed through the various sterilized filters with the aid of a suction pump and anaerobic cultures made of the first 3 cc. of filtrate and of larger amounts of filtrate.

Pure cultures of various microorganisms were first subcultured anaerobically and then passed through the candles and cultured in the same manner.

The results of these experiments are summarized in Tables III and IV.

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[2] We are indebted to Dr. Bertha M. Meine for assistance in conducting a number of these experiments.

TABLE III.

Results of Filtration Experiments with Emulsions of Poliomyelitic Tissues.

Material (glycerolated tissues).	Microorganisms present.	Filter used.	Amount of filtrate cultured.*	Results of anaerobic cultures of filtrates.†
Emulsion of cord (M).	Diplococci, streptococci.	Kitasato.	First 3 cc.	Sterile.
" " " (K).	Diplococci, streptococci, Gram-negative bacilli.	"	" 3 "	"
" " " (K).	Diplococci, streptococci, Gram-negative bacilli.	"	3 cc. of 15 cc. of filtrate.	Diplococci, streptococci, Gram-negative bacilli.
" " " (H).	Diplococci.	Pasteur-Chamberland.	First 3 cc.	Sterile.
" " " (C).	Streptococci, diplococci.	Pasteur-Chamberland.	" 3 "	"
" " " (C).	Streptococci, diplococci.	Pasteur-Chamberland.	3 cc. of 12 cc. of filtrate.	"
" " cerebrum(M).	Diplococci.	Kitasato.	First 3 cc.	"
" " " (H).	"	"	" 3 "	"
" " " (H).	"	Pasteur-Chamberland.	" 3 "	"
" " pons(M).	Streptococci (pure culture).	Kitasato.	" 3 "	"
" " " (M).	Streptococci (pure culture).	Pasteur-Chamberland.	" 3 "	"
" " " (K).	Streptococci, diplococci.	Kitasato.	3 cc. of 16 cc. of filtrate.	Growth of streptococci and diplococci.
" " " (K).	Streptococci, diplococci.	Pasteur-Chamberland.	3 cc. of 16 cc. of filtrate.	Sterile.

* Cultures in ascites-broth-kidney medium under paraffin oil.
† After 28 days' incubation at 37°C.

As shown in Table III, the filtrate of the emulsions of tissues with both the small Kitasato and larger Pasteur-Chamberland filters was invariably sterile if the first 3 cc. or less of filtrate were cultured; when larger amounts of emulsion were filtered and cultured, growths

TABLE IV.

Results of Filtration Experiments with Pure Cultures of Microorganisms from Poliomyelitic Tissues.

Culture* (acites-broth-kidney medium).	Microorganisms present.	Filter used.	Amount of filtrate cultured.†	Results of anaerobic cultures of filtrates.‡
5 day anaerobic.	Streptococci (large forms).	Kitasato.	First 3 cc.	Sterile.
5 " "	Streptococci (large forms).	"	3 cc. of 10 cc. of filtrate.	"
7 " "	Streptococci (mostly large forms).	Pasteur-Chamberland.	First 5 cc.	"
14 " "	Streptococci (many small forms present).	Kitasato.	" 3 "	"
14 " "	Streptococci (many small forms present).	"	3 cc. of 17 cc. of filtrate.	Streptococci.
17 " "	Streptococci (many small forms present).	Pasteur-Chamberland.	First 4 cc.	Sterile.
17 " "	Streptococci (many small forms present).	Pasteur-Chamberland.	4 cc. of 19 cc. of filtrate.	"
19 " "	Streptococci (many small forms present).	Pasteur-Chamberland.	5 cc. of 31 cc. of filtrate.	Streptococci.
8 " "	Diplococci.	Kitasato.	First 3 cc.	Sterile.
8 " "	"	"	3 cc. of 12 cc. of filtrate.	"
8 " "	Gram-negative bacilli.	"	First 3 cc.	"
8 " "	" "	"	3 cc. of 11 cc. of filtrate.	"

* Subcultures of all of these in ascites-broth-kidney medium preliminary to filtration showed the presence of viable microorganisms.

† In ascites-broth-kidney medium under paraffin oil.

‡ After incubation for 11 to 28 days at 37°C.

were sometimes secured with the Kitasato filtrates. The amount of filtrate used in these experiments is therefore of considerable importance.

Experiments with pure cultures of various microorganisms showed

in general that larger amounts of filtrate may contain microorganisms, while the first few cubic centimeters may be sterile; also that streptococci in older anaerobic cultures (containing many small forms) may be filterable, while younger cultures, containing mostly larger forms, are not. There is no doubt that the streptococci and also the diplococci secured by us from poliomyelitic tissues tend to become much smaller when grown in fluid medium under paraffin oil, and as shown in the above experiments these small forms may be filterable under conditions which hold back larger forms. Of even more importance, however, is the amount of culture passed through a candle, as the passage of large amounts may wash through a number of the microorganisms.

Results of Animal Inoculation Tests.

We have injected cultures of the various microorganisms into rabbits, and a few cultures of streptococci and diplococci into monkeys intracranially, intravenously, and intraperitoneally. All the animals were carefully observed for clinical evidences of poliomyelitis and after death sections were prepared of the brain and cord and examined for histological evidences.

Rabbit Inoculation Experiments.—The cultures injected into rabbits were derived from the following sources.

No. of cultures.	Cultures.	Source.
14	Streptococci.	Poliomyelitic cords.
5	"	" brains.
2	"	Spleen.
3	"	Mesenteric glands.
5	Diplococci.	Cerebrospinal fluids.
3	"	Poliomyelitic cords.
2	"	" brains.
4	Diphtheroids.	" tissues.
3	"	Cerebrospinal fluids.
6	Gram-negative bacilli.*	Poliomyelitic tissues.

* These bacilli were not of the colon group.

With the exception of two cultures all were transplants from the original anaerobic ascites-broth-kidney cultures, in order to eliminate

to a large extent the possible injection of original tissue present in the primary culture and to insure the injection of pure cultures. The aerobic cultures used for injection had been incubated at 37°C. for 24 hours, and in all instances stained films showed the presence of numerous microorganisms. The doses administered are given in Table V. The animals varied in weight from 1,400 to 1,600 gm.

TABLE V.

Results of Rabbit Inoculation Tests.

Microorganism.	No. of cultures injected.	Route of inoculation.	Dose.	Results.
			cc.	
Streptococci.	18	Intracranial.	0.5–0.8	No paralyses; six deaths 4, 18, 20, 21, 26, and 26 days later.
"	24	Intravenous.	1.0*	No paralyses; one arthritis; seven deaths 1, 2, 9, 15, 19, 35, and 56 days later.
"	24	Intraperitoneal.	2.0*	No paralyses; three deaths 3, 7, and 10 days later.
Diplococci.	7	Intracranial.	0.5–0.6	No paralyses; one death 28 days later.
"	10	Intravenous.	1.0	No paralyses; one death 3 days later.
"	10	Intraperitoneal.	2.0	No paralyses; no deaths.
Diphtheroids.	4	Intracranial.	0.5–0.6	No paralyses; one death 36 days later.
"	7	Intravenous.	1.0	No paralyses; one death 34 days later.
"	7	Intraperitoneal.	2.0	No paralyses; one death 36 days later.
Gram-negative bacilli.	3	Intracranial.	0.5–0.6	No paralyses; three deaths 11, 14, and 21 days later.
" "	6	Intravenous.	1.0	No paralyses; three deaths 1, 25, and 35 days later.
" "	6	Intraperitoneal.	2.0	No paralyses; one death 28 days later.

* Dose per kilo of body weight.

As shown in Table V, which summarizes the number of different cultures of various microorganisms injected in different routes, true clinical and histological evidence of poliomyelitis was not observed in a single instance.

The streptococci produced arthritis in one rabbit and the intracranial injections were followed in several animals by a slight meningitis with recovery of streptococci at autopsy.

The streptococci caused more deaths than the diplococci, diphtheroids, and Gram-negative bacilli.

The intravenous injections were followed in several instances by the development of fatal pleuritis and pericarditis, from which lesions and from the blood of the heart, streptococci were recovered. On the other hand, the deaths of many of these animals and particularly those succumbing 3 and 4 weeks or longer after injection were due to secondary and unrelated causes.

In several of the animals injected with the diphtheroids and Gram-negative bacilli and succumbing 2 weeks or more later, these microorganisms were not recovered, whereas cultures of the heart and various internal organs frequently showed the presence of streptococci, indicating that the animals are subject to independent and secondary fatal infections with streptococci.

Monkey Inoculation Experiments.—The inoculation experiments have been limited to four animals. The intracerebral injection of the recently cultivated diplococci failed to produce anterior poliomyelitis (No. 1); likewise the intracerebral injection of five different strains of streptococci failed, producing, however, in one case a meningitis (No. 2); likewise the intravenous (No. 3) and intraperitoneal (No. 4) injections of streptococci failed to produce symptoms or lesions of poliomyelitis. The brief protocols of these four experiments are as follows:

Monkey 1.—Macacus rhesus; inoculated intracerebrally under ether anesthesia with 0.8 cc. of a mixture of the first anaerobic ascitic broth subcultures of recently glycerolated cords of four cases of poliomyelitis. The cultures were incubated for 4 days and showed numerous diplococci arranged in pairs and short chains. The animal promptly recovered from the anesthetic and operation, showed a slight disinclination to move about for 3 days, but has never showed any weakness or paralysis (44 days' observation). Subcultures were employed to aid in the elim-

ination of the injection of original tissue; the diplococci were, therefore, 8 days in artificial medium before inoculation.

Monkey 2.—Macacus rhesus; inoculated intracerebrally under ether anesthesia with 0.8 cc. of a mixture of 3 day aerobic ascitic broth cultures of five different strains of streptococci recovered from two cords, one cerebrum, one spleen, and one thymus gland of five different cases of poliomyelitis. Two strains were in the first transfer, and the remaining three were in the third transfer from the original anaerobic cultures. The animal promptly recovered from the anesthetic and operation and developed a severe meningitis lasting over 2 weeks, with pus and streptococci in the cerebrospinal fluid. The animal did not move about or use its hind legs, but repeated tests failed to discover paralysis.

Monkey 3.—Cebus capucinus; inoculated intravenously with 5 cc. of a mixture of 4 day aerobic ascitic broth cultures of five different strains of streptococci in the second and third transfers from original anaerobic ascites-kidney-broth cultures of three cords, one pons, and one spleen of five different cases of poliomyelitis. This animal presented a mild rise in temperature over a period of 2 days following the injection, with no other disturbances and no weakness or paralysis (56 days' observations).

Monkey 4.—Cebus capucinus; inoculated intraperitoneally with 5 cc. of the same mixture of five different strains of streptococci given to Monkey 3. This animal has not shown weakness or paralysis (56 days' observations).

In considering this series of experiments on monkeys note should be taken of the fact that they reproduce chiefly the method of inoculation which succeeds with the filtered poliomyelitic virus. In one instance only was an intravenous injection given and in it the dose was below that employed by Rosenow. However, the large quantity of cultures inoculated should have sufficed to develop symptoms of poliomyelitis had the streptococci used possessed the power of inducing that disease.

DISCUSSION AND SUMMARY.

Four different varieties of easily cultivated microorganisms have been cultured from the cerebrospinal fluid and tissues of cases of acute anterior poliomyelitis; namely, a streptococcus, a diplococcus, diphtheroids, and Gram-negative bacilli. It is not contended that they were all inherent in the tissues; a part were doubtless extraneous.

The streptococci and diplococci may be considered as the most significant of the bacteria cultivated and are distinguishable from each other by biological tests.

The streptococci grew both aerobically and anaerobically; under anaerobic conditions growth was slow, the cocci became small and round, and were more easily decolorized with alcohol in the Gram stain. They were not found in the anaerobic cultures of 106 cerebrospinal fluids; they were found in one of twenty anaerobic blood cultures and frequently in the cerebrum, cerebellum, pons and medulla, cord, tonsils, lungs, liver, kidneys, spleen, pancreas, thymus gland, suprarenal glands, and mesenteric glands of fatal cases.

The diplococci are Gram-positive and, transplanted to solid media, yield luxuriant growths and a staphylococcus grouping. They grew aerobically and anaerobically, but more slowly under the latter condition, and the cocci became smaller and more rounded. Diplococci were found in the anaerobic cultures of 48 of 106 cerebrospinal fluids; also in the cerebrum, cerebellum, pons and medulla, cord, tonsils, lungs, liver, kidneys, spleen, pancreas, and mesenteric glands of fatal cases.

The filtrates of emulsions of tissues containing streptococci and diplococci passed through fine Kitasato and Pasteur-Chamberland filters were sterile unless large amounts of filtrates were collected. The amount of filtrate collected and cultured is therefore of considerable importance in filtration experiments.

The small forms of streptococci and diplococci in old anaerobic cultures are filterable with these filters, while young aerobic cultures containing large forms are not, unless large amounts of culture are filtered.

Intracranial, intravenous, and intraperitoneal injection of these easily cultivated streptococci, diplococci, diphtheroids, and Gram-negative bacilli failed to produce paralysis in rabbits or monkeys. With two exceptions all the cultures were transplants from the original anaerobic ascites-broth-kidney cultures of cerebrospinal fluid and various tissues. Arthritis and meningitis were produced by the streptococci, but there were neither clinical nor histological evidences of true poliomyelitis.

Occasional bacteriological studies since 1898 have shown that easily cultivated micrococci and bacilli may be present in the cerebrospinal fluid and tissues of the central nervous system of persons suffering with acute anterior poliomyelitis. The majority of bacteriol-

ogists have found the cerebrospinal fluid, blood, and nervous organs sterile. Opinions have varied in regard to the significance of the organisms and the micrococci in particular, but the consensus of opinion has been to the effect that they are secondary invaders and unable of themselves to produce poliomyelitis in the lower animals. After allowing for contaminations due to technical errors in securing specimens, the total number of observations indicates that easily cultivated micrococci occur sometimes in the brain and cord of persons suffering from epidemic poliomyelitis. Our studies have shown that they may be found not only in these locations, but also in the spleen, kidneys, suprarenal glands, and other organs. It is not known that they exert an influence in this disease, although they may possibly give rise to the production of antibodies, assuming their entrance not to be wholly agonal, as the cultures of streptococci are frequently of sufficient virulence to produce meningitis in rabbits and monkeys. Our experiments are in accord with those of other investigators who found that these microorganisms do not produce poliomyelitis in the lower animals, and are therefore in sharp contrast with the recent reports which would attribute an etiologic relationship of streptococci and allied organisms to that disease. At present this wide divergence of result cannot be accounted for, but it does not seem that it is possible for it to reside in any condition of the cultures employed by us as they were obtained from undoubted cases of epidemic poliomyelitis and inoculated in early generations.

As regards these easily cultivatable microorganisms, we agree at present with those who regard them as secondary and probably terminal invaders rather than the actual etiologic agent of the disease.

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EXPLANATION OF PLATE 66.

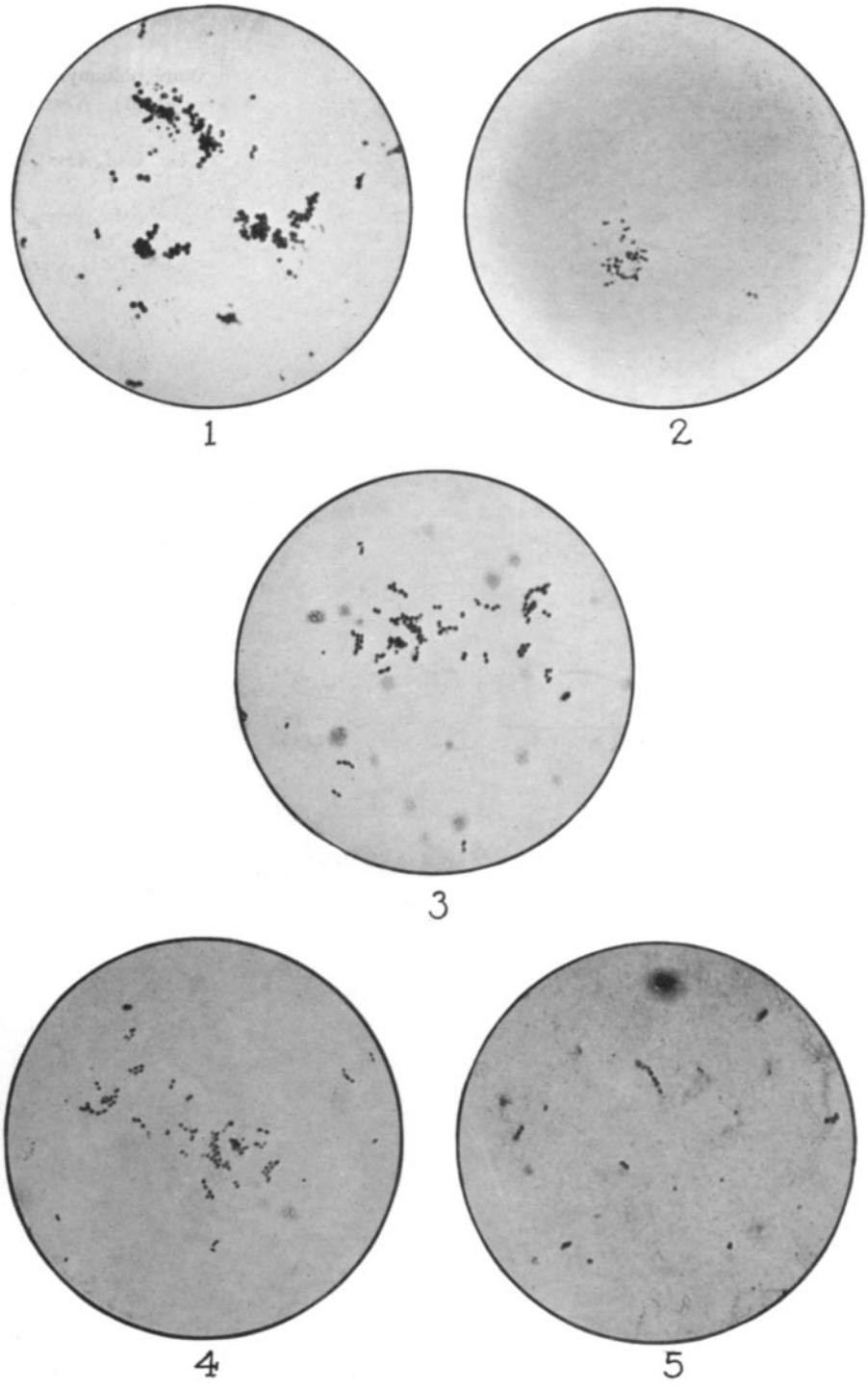
FIG. 1. Film of a 2 day aerobic subculture of diplococcus in ascites-broth-kidney medium; from an anaerobic culture of poliomyelitic cord in the same medium. Shows large forms. × 1,000.

FIG. 2. Film of an 18 day anaerobic culture of diplococcus in ascites-broth-kidney medium; same culture as shown in Fig. 1. The cocci have become smaller in size and more easily decolorized by alcohol in the Gram stain. × 1,000.

FIG. 3. Film of a 5 day anaerobic culture of streptococcus in ascites-broth-kidney medium; from the pons of a fatal case of acute anterior poliomyelitis. × 1,000.

FIG. 4. Film of an 11 day anaerobic culture of streptococcus in ascites-broth-kidney medium; same culture as shown in Fig. 3; some smaller forms of cocci tending to become Gram-negative are seen. × 1,000.

FIG. 5. Film of a 3 day aerobic culture of streptococcus in ascites-broth-kidney medium; from the thymus gland of a fatal case of acute anterior poliomyelitis. × 1,000.



(Kolmer: Bacteriology of Poliomyelitis.)