

THE ETIOLOGY OF EPIDEMIC POLIOMYELITIS.

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Since 1840, when Heine (1) first definitely separated epidemic poliomyelitis, or, as he first named it, spinal infantile paralysis, from other more or less similar conditions of cerebrospinal origin, many theories have been advanced to explain the nature of the cause of the disease. Colmer (2) attributed a small epidemic in West Feliciana, Louisiana, in 1841 to teething. A few years later Kennedy (3) drew a similar conclusion. Even Heine held the view that difficult dentition was at the root of the trouble. More recently other theories have been advanced, such as vertebral subluxation (4), glandular inefficiency (5), and still others (6).

In 1871, Roger and Damaschino (7) concluded, from the three autopsies which they studied, that the primary lesion in the cord was vascular and the process an inflammatory softening, of the nature of a myelitis. Schultze (8) in 1876 studied the spinal cord from a case which died at the age of 23, or 20 years after the actual attack, and found changes in the lateral tracts and Clarke's columns in addition to the usual lesions, and regarded the process as inflammatory in type. Wickman (9) has recently drawn the same conclusion after an histological study of many cases. In 1884, Strümpell (10) suggested that the symptoms of epidemic poliomyelitis were those of an infection of an independent nature, and this theory was later experimentally shown to be correct by Landsteiner and Popper (11), Flexner and Lewis (12), and others. However, the infective agent is still undetermined; on the one hand, many investigators, such as Leiner and von Wiesner, Landsteiner and Levaditi, Römer, and others, have failed to find microorganisms which they could regard as the cause of the disease in either the spinal fluids or autopsy materials from cases of epidemic poliomyelitis; while, on the other hand, an etiological relation to the disease has been claimed by a number of experimenters of several different types of microorganisms, such as bacilli (13, 14), cocci (15-21), spirochetes (22), rhizopods (23), hemoprotozoa (24), intranuclear bodies (25), globoid bodies (26), and many others (27). Among all these, cocci and globoid bodies have come into more serious consideration. The present paper is a report of the results obtained in an investigation of the etiology of poliomyelitis, together with a discussion of the findings obtained by others.

Technique of Cultivation.

The inoculated animals were, when possible, etherized just before death. With animals that died during the night this part of the procedure could not, of course, be carried out. Our purpose was to make sure, as far as possible, that any organisms cultivated from the brains were not postmortem invaders, but were present during the life of the animal. A median incision was made through the skin over the skull, running from the tip of the nose to the back of the neck, and the skin dissected back on both sides of the head. The skull was disinfected with tincture of iodine, and the head and body were covered with three layers of gauze soaked in lysol. A small hole was made in the gauze so as to expose the upper part of the skull. The skull was next opened, and, further to insure sterility, the surface of the brain was seared with a scalpel. A large piece, sometimes one-half of the brain, was removed with a pair of long forceps and then immediately put into a Rosenow sterile air chamber (28) (sterilized in the hot air sterilizer for 1 hour at 160°C.) where it was emulsified. The emulsion was poured into a large tube from which six test-tubes each of the different media were inoculated with pipettes. The tubes, all of which were incubated aerobically at 37.5°C., were examined after 2 days and, if they were found to be sterile, were again examined 2 or 3 days later.

The media used for this set of cultures were plain broth, ascitic broth, glucose broth, ascitic glucose broth, plain tissue broth, ascitic tissue broth, glucose tissue broth, ascitic glucose tissue broth, glucose agar, and ascitic glucose agar. The amount of glucose in the broth was 0.2 per cent and that in the agar 1 per cent. The reaction of all the media was 0.6 to 0.8 per cent acid.

A part of the tubes inoculated in each case was prepared by methods simulating as exactly as possible those employed by Rosenow (16).

For anaerobic cultivation we have used chiefly the methods of Noguchi (26). In their original paper Flexner and Noguchi (26) advise the use of fresh kidney of the normal rabbit, while Heist, Solis-Cohen, and Kolmer (29) state that the rabbit kidney which had been kept for several weeks before the ascitic fluid was added gave better results. We have used both the fresh and kept kidneys and inoculated at each time from forty to seventy tubes with both fresh and glycerolated brain tissue from poliomyelitic cases. Because of the ready contamination on removal, only glycerolated spinal cords have been cultured. All the tubes were incubated for from 12 to 15 days at 37.5°C. in jars made anaerobic by a combination of air exhaustion, oxygen absorption, and hydrogen displacement. We have also employed the anaerobic methods devised by Sellards (30) and by Smillie (31); the former depending upon the efficient absorption of oxygen by phosphorus, and the latter upon the catalytic action of platinized asbestos upon hydrogen and oxygen. It may be stated here that whichever of these methods was used the results obtained were the same.

Experiments on Monkeys.

Since 1909, when Landsteiner and Popper (11), Flexner and Lewis (12), and others first succeeded in transmitting poliomyelitis to monkeys, these animals have been generally used by all investigators in their search for the causative agent of the disease. In the culture work we employed eight *rhesus* monkeys and, of this number, seven were injected with glycerolated poliomyelitic virus alone—five intracerebrally, one subcutaneously, and one both intracerebrally and intraperitoneally. The eighth monkey was given two intracerebral injections; the first streptococci, and the second glycerolated poliomyelitic virus. Both aerobic and anaerobic cultures of the brain and cord emulsions used for the inoculations were made at the time of injection on ascitic glucose agar slants. No growth occurred in any of the tubes. When they showed signs of illness or of poliomyelitis, the monkeys were etherized from 4 to 16 days after injections; some succumbed to the disease. Five of the eight monkeys showed histological changes of poliomyelitis and three did not; nevertheless streptococci were isolated from the brains of all. Because of the easy contamination of the spinal cord, all the cultures were made from the brains. The following protocols show the isolation of streptococci from monkeys.

Monkey 1.—Aug. 29, 1916. Injected intracerebrally with glycerolated poliomyelitic virus obtained from The Rockefeller Institute. Sept. 4. Flaccid paralysis of all limbs. Sept. 5. Paralysis remained; moribund; etherized. Cultures of the brain yielded streptococci.

*Histological Examination.*¹—Changes of typical acute anterior poliomyelitis.

Monkey 2.—Nov. 3, 1916. Injected intracerebrally with Rockefeller Institute virus. Nov. 6. Right hind leg was paralyzed. Nov. 7. Complete paralysis; moribund; etherized. Cultures of the brain yielded streptococci.

Histological Examination.—Changes of typical acute anterior poliomyelitis.

Rosenow (15, 16) and others have reported the transmission of poliomyelitis to monkeys, rabbits, and guinea pigs by the injection of streptococci isolated from human cases of the disease, but we have not been able to corroborate their results. The following two protocols illustrate our findings.

¹The sections were kindly examined for us by Dr. H. C. Howe of the Departments of Medicine and Neurology of the College of Physicians and Surgeons.

The first protocol illustrates the action of streptococci on rabbits and guinea pigs.

Monkey 3.—Oct. 20, 1916. Injected intracerebrally with Rockefeller Institute virus. Nov. 5. Found dead in the morning. Cultures of the brain yielded streptococci.

Histological Examination.—Changes of typical acute anterior poliomyelitis.

Animal Injection.—Nov. 12, 1916. Two rabbits and two guinea pigs were injected intravenously with a 20 hour culture of the streptococci which were in all respects similar to those described by Rosenow.² Rabbit 1 and Guinea Pig 1 were injected each with the growth from 22.5 cc. of ascitic glucose broth suspended in 1.5 cc. of sterile salt solution, Rabbit 2 with that from 30 cc., and Guinea Pig 2 with that from 15 cc. of the same medium suspended in 2 and 1 cc. of sterile salt solution respectively. Both aerobic and anaerobic cultures of the bacterial suspension were made on ascitic glucose agar and blood agar slants at the time of injection. The streptococci were found to be alive and free from contamination. On Dec. 2, Rabbit 1 was again injected intravenously with a 20 hour culture of streptococci from Monkey 4. This time it received the growth from 45 cc. of ascitic glucose broth suspended in 3 cc. of sterile salt solution. None of the four animals showed any signs of illness in the 4 months during which they were kept under observation.

The second protocol shows the result of injection of the streptococci into the monkey.

Monkey 5.—Jan. 4, 1917. Injected intracerebrally with a cord emulsion from Monkey 2. Jan. 9. There was general muscular tremor, stiffening of neck, and weakness of limbs. The animal was etherized and cultures of the brain were made. Streptococci were obtained.

Histological Examination.—Changes of typical acute anterior poliomyelitis.

Animal Injection.—Feb. 17, 1917. After subculturing a number of times, 0.4 cc. of an ascitic fluid tissue culture containing the streptococci isolated from this monkey was injected intracerebrally into Monkey 6. The animal remained well from Feb. 18 to Apr. 9. Apr. 10. Injected intracerebrally with 0.5 cc. of poliomyelitic virus (Willard Parker virus). There was no sign of illness from Apr. 11 to 17. Apr. 18. Legs completely paralyzed; arms weak; etherized; and cultures of the brain made. Streptococci were recovered.

Histological Examination.—Changes of typical acute anterior poliomyelitis.

The above protocols show that streptococci, although they were the only organisms repeatedly found present in the brains of the experi-

² This culture was stated by Dr. Rosenow, who saw it in our laboratory, to be similar to his.

mental monkeys, do not seem to have any etiological relation to poliomyelitis; they not only did not induce poliomyelitis in rabbits, guinea pigs, and monkeys, but were also unable to produce, in the monkey, antibodies that neutralized the virus which was subsequently injected. Furthermore, we have isolated streptococci from monkeys which, although they had been injected with poliomyelitic virus, did not show changes of this disease, and these animals served, therefore, as our controls.

Monkey 4.—Nov. 11, 1916. Injected intracerebrally with Willard Parker virus. Nov. 13. Partial paralysis of right limbs. Nov. 14 and 15. Condition remained the same. Nov. 16. Excitable in the morning; looked sick; refused food. Condition was worse in the afternoon. There was general muscular tremor. The animal was etherized and cultures of the brain were made; streptococci were found.

Histological Examination.—Multiple abscesses of the brain and cord.

Animal Injection.—A rabbit injected intravenously with the streptococci isolated from this monkey did not show any sign of illness in the 5 months during which it was kept under observation.

Monkey 7.—This monkey, which withstood an intracerebral injection of the brain emulsion from Monkey 2, did not show typical changes of poliomyelitis after death following subsequent intraperitoneal injections. Dec. 18, 1916. Injected intracerebrally with the virus from Monkey 2. Jan. 10. No signs of illness. From this date to Feb. 5 given five intraperitoneal injections of the same material (for immunization) at intervals of 6 to 7 days. Feb. 11. Found dead.

Histological Examination.—While certain parts as the medulla, showed the changes of acute poliomyelitis, other portions, as the cerebellum, showed inflammatory changes which were more probably due to other agents than the virus of poliomyelitis.

Monkey 8.—Feb. 16, 1917. Injected subcutaneously with poliomyelitic virus. Feb. 20. Found dead.

Histological Examination.—Acute cloudy swelling of the ganglion cells of the cord of unknown origin.

Experiments on Rabbits.

The experiments on rabbits were done with the intention of clearing up two much disputed questions: first, the transmission of poliomyelitis to rabbits; and, second, the isolation of any microorganism whatever, from the brains of rabbits that died after injections of poliomyelitic virus and of streptococci.

With regard to the first question, Krause and Meinecke (32), Lentz and Huntmüller (33), Marks (34), and Rosenau and Havens (35) have made various claims regarding the passage of the virus through rabbits. The first two sets of investigators claim to have carried the virus back to the monkey, while Rosenau and Havens induced indefinite lesions only in the monkey with the rabbit material. Marks alone reinoculated the rabbit material in its 2nd, 4th, and 6th generations successfully in monkeys in which animals typical lesions occurred. But no typical lesions were ever found in the rabbits.

More recently Rosenow (15) and Nuzum and Herzog (18) have reported the production of poliomyelitis in rabbits by the injection of streptococci isolated from cases of that disease. Mathers (20), who also isolated the streptococcus, is non-committal on its etiological significance. On the other hand, many investigators, as Landsteiner and Levaditi (36), Leiner and von Wiesner (37), Römer and Joseph (38), Flexner and Lewis (39, 40), and others have obtained only negative results in their attempts to produce poliomyelitis in rabbits.

We used fifty-six rabbits. Forty-three were inoculated with either glycerolated poliomyelitic virus or streptococci, while thirteen were kept as controls. The animals were mostly young. None of the animals that succumbed to the injections showed any changes characteristic of poliomyelitis of man or monkey. Micrococci arranged in pairs, chains, and groups were the only organisms found, and they were isolated from the brains of nearly all the animals from which we have made cultures. The experiments were carried out in the following manner.

Series I.

Rabbits Injected with Glycerolated Poliomyelitic Virus from Monkeys.

—Eleven rabbits were injected with brain and cord emulsions from Monkey 2: two with cord intracerebrally; three with brain intravenously; three with brain intracerebrally; two with brain intracerebrally twice; and one with brain both intracerebrally and then, after 30 days, intravenously. Of this number, six have died from 6 to 20 days after injection and five remained well.

Rabbit 3.—Weight 865 gm. Dec. 18, 1916. Injected intracerebrally with 0.3 cc. of brain emulsion. It did not show any signs of illness after injection and on Jan. 17 was given another dose of the same size and through the same route. Jan. 17 to Feb. 5. Remained well. Feb. 6. Found dead in the morning, outside the cage. Because of the uncertainty of the cause of death no cultures were made at the time of autopsy.

Histological Examination.—Cerebrum and cord appeared normal. One small area of cellular accumulation in the cerebellum was found.

Rabbit 4.—Weight 550 gm. Jan. 4, 1917. Injected intracerebrally with 0.3 cc. of cord emulsion. Feb. 27. Found lying flat on the chest. Front legs paralyzed and hind legs very weak. Autopsied 10 minutes after death.

Histological Examination.—Cerebrum, cerebellum, and cord were found to be normal both macroscopically and microscopically.

Rabbit 5.—Weight 610 gm. Jan. 17, 1917. Injected intravenously with 0.5 cc. of brain emulsion. Remained well up to Jan. 27. Jan. 28. Found dead.

Histological Examination.—With the exception of the slight vascular congestion in the cerebrum there was no change in the nervous system.

Rabbit 6.—Weight 430 gm. Jan. 17, 1917. Injected intracerebrally with 0.3 cc. of brain emulsion. Remained well up to Jan. 29. Jan. 30. Found dead.

Histological Examination.—In the cerebrum there were slight perivascular infiltration and a few areas of cellular accumulation. In the cord there was one area of cellular accumulation in one posterior horn, but the meninges and vessels were normal. In another section of the cord there was a large hemorrhage in the posterior horn.

Rabbit 7.—Weight 605 gm. Jan. 17, 1917. Injected intracerebrally with 0.3 cc. of brain emulsion. Jan. 24. Found dead. No previous sign of illness observed.

Histological Examination.—With the exception of slight congestion of the vessels of the pia and those in the substance of the cerebrum, there was nothing abnormal in the central nervous system.

Rabbit 8.—Weight 335 gm. Jan. 17, 1917. Injected intracerebrally with 0.3 cc. of brain emulsion. Jan. 29. Found dead. No previous signs of illness observed.

Histological Examination.—Cerebrum: Cellular infiltration of pia and in adventitial lymph spaces of vessels throughout section; many localized areas of cellular infiltration. Cord: Area of necrosis in left anterior horn and accumulation of polyblasts; a similar accumulation of polyblasts in the white matter just adjacent to the anterior horn on the right side. No vascular changes. Meninges and ganglion cells normal.

Series II.

Rabbits Injected with Brain Emulsions of Other Rabbits That Died after Injections with Poliomyelitic Virus.—Nine rabbits were injected either intracerebrally or intravenously; two died and seven remained well.

Rabbit 9.—Weight 600 gm. Jan. 24, 1917. Injected intracerebrally with 0.3 cc. of brain emulsion from Rabbit 7. An abscess developed at the site of injection and the animal died on Feb. 18. No autopsy.

Rabbit 10.—Weight 700 gm. Jan. 24, 1917. Injected intravenously with 0.5 cc. of brain emulsion from Rabbit 7. Jan. 25 to Feb. 1. No signs of illness. Feb. 2, 1.30 p.m. Found lying flat on one side. The limbs were not actually paralyzed but they were very weak, as shown by the unsteadiness and drooping when the animal was put on its legs. The ear veins were dilated. After about 10 minutes the animal fell down on one side with paroxysmal stretching and convulsive movements of the limbs. Then it had labored gasps and, at the same time, the head was strongly drawn back. It died at 2 p.m.

On autopsy the brain was found to be congested, edematous, and soft. Three hemorrhagic spots were found on the large intestinal wall.

Histological Examination.—There were a few small hemorrhages in the cerebral cortex, otherwise the cerebrum, cerebellum, and cord were normal.

Series III.

Rabbits Injected Intravenously with Streptococci Isolated from Monkeys.—Six rabbits were injected; four died and two remained well. To avoid unnecessary repetition, it may be stated that all the bacterial suspensions were made in sterile normal salt solution. Both anaerobic and aerobic cultures of all the suspensions were made on ascitic glucose agar and blood agar slants at the time of injection and the bacteria were always found to be alive and free from contamination.

Rabbit 11.—Weight 605 gm. Feb. 15, 1917. Injected with 2 cc. of a 24 hour broth culture of pleomorphic streptococci isolated from Monkey 7. Feb. 27. Died.

Histological Examination.—Vascular congestion in the cerebrum and cerebellum, otherwise there was no change in the nervous system.

Rabbit 12.—Weight 850 gm. Feb. 15, 1917. Injected with 2 cc. of a 24 hour broth culture of pleomorphic streptococci isolated from Monkey 7. Feb. 22. Died.

Histological Examination.—With the exception of hemorrhage in the central canal of the cord, the cerebrum, cerebellum, and cord were normal.

Series IV.

Rabbits Injected Intravenously with Streptococci Isolated from Other Rabbits That Died after Injections with Poliomyelitic Virus or Rabbit Brain Emulsion.—Altogether fourteen rabbits were injected—ten with streptococci from rabbits dead after injections with poliomyelitic virus with one death (no autopsy); and four with streptococci

from rabbits dead after injections with rabbit brain or cord emulsions, and all of these died (two autopsies performed).

Rabbit 13.—Weight 580 gm. Feb. 8, 1917. Injected with 4 cc. of a 48 hour broth culture of streptococci isolated from Rabbit 10. Feb. 22. Died.

Histological Examination.—Cerebrum and cord normal. Cerebellum showed slight cellular infiltration of the pia.

Rabbit 14.—Weight 1,075 gm. Feb. 8, 1917. Injected with 4 cc. of a 48 hour broth culture of streptococci isolated from Rabbit 10. Feb. 14. Died.

Histological Examination.—Cerebrum, cord, and posterior root ganglia normal.

Series V.

Rabbits Injected Intravenously with Streptococci Isolated from Other Rabbits That Died after Injections with Streptococci.—Three rabbits were injected, with only one death.

Rabbit 15.—Weight 1,180 gm. Feb. 17, 1917. Injected with 2 cc. of a 48 hour broth culture of streptococci isolated from Rabbit 14. Mar. 1. Died.

Histological Examination.—Macroscopically and microscopically the cerebrum, cerebellum, and cord were normal.

Control Series.

We have shown before that streptococci could be isolated from monkeys that died of poliomyelitis as well as from other causes. The next question was whether or not we could also isolate streptococci from normal rabbits and those that died from causes other than poliomyelitis. For this purpose we have used two syphilitic rabbits and eleven normal rabbits.

Syphilitic Rabbits.

Rabbit 16.—Dec. 13, 1916. Both testes were injected each with 1 to 2 cc. of an emulsion of testes containing *Treponema pallidum* in ascitic fluid. From Dec. 14 to Jan. 4 there was no change. Jan. 11. Diffuse lesions in both testes containing *Treponema pallidum*. The right testis was removed for another experiment, while the left scrotum and the back were injected for the luetin reaction. Jan. 12. No reaction. Jan. 16. Left testis was removed for another experiment. Feb. 23. All the extremities were found to be paralyzed. Spinal fluid was negative for spirochetes. Feb. 24. Legs were still paralyzed. The rabbit was etherized and the brain taken for cultivation according to the technique already described.

Bacteriological Findings.—Feb. 26. Small diplococci were found in all the tubes inoculated 2 days previously. The growth was transferred to glucose broth.

Animal Injection.—Feb. 27. Four small rabbits were injected intravenously with the isolated diplococci. Rabbits 17 (weight 631 gm.) and 18 (weight 645 gm.) each received the growth from 30 cc. of glucose broth suspended in 2 cc. of sterile normal salt solution, and Rabbits 19 (weight 695 gm.) and 20 (weight 655 gm.) each received the growth from 45 cc. of glucose broth suspended in 3 cc. of sterile normal salt solution. Feb. 28. Rabbits 17, 18, and 19 were found dead in the morning. Death occurred within 16 hours after injection. Autopsies were done on Rabbits 17 and 18. No macroscopic changes of the brains and cords were seen. Cultures of the brains were made in several media. Mar. 2. Diplococci and also streptococci were found to be present in all the inoculated tubes. Mar. 3. Rabbit 20 was found dead but no cultures were made.

Histological Examination of the Brain of Rabbit 17.—Cerebrum: normal. Cerebellum: one or two areas of necrosis in the granular layer, without changes in the vessels or increase of cells around them. (These may be artifacts.) Cord: there had been a large hemorrhage destroying most of the right posterior horn; there was no cellular infiltration around it, and the rest of the section appeared normal.

Rabbit 21.—Oct. 11, 1916. Back, shoulder, hips, and right testis were injected with an emulsion of testis in ascitic fluid containing *Treponema pallidum*. Oct. 25. Skin showed a few slight elevations. Both front legs were turned inward and stiff. Oct. 30 to Nov. 16. There was no change. Nov. 21. There was a lesion in the right testis. Dec. 5. The right testis was found to contain spirochetes. From this date up to Mar. 6, 1917 there was no change. Mar. 8. With the exception of the front legs being turned inward, no signs of illness. Animal etherized and cultures of the brain were made. Mar. 10. Streptococci were found in all the tubes inoculated.

Normal Rabbits.

Cultures of the brains of eleven rabbits were made. Ten brains were removed under ether and one 20 minutes after death. This last rabbit was purposely killed for another experiment. Streptococci and, sometimes, other Gram-positive diplococci have been isolated from the brains of seven of the eleven animals.

Globoid Bodies.

In 1909 Flexner and Lewis (40) and Landsteiner and Levaditi (41) independently discovered that the causative agent of poliomyelitis was filterable. A few years later Flexner and Noguchi (26) by the use of Noguchi's technique of cultivating *Treponema pallidum* (42) succeeded in isolating and cultivating a minute

organism to which they gave the name globoid bodies. These bodies are filterable and grow only under anaerobic conditions. They have been found in the lesions of poliomyelitis of man and the monkey; they have not been detected in lesions or conditions other than poliomyelitis; they have sufficed in several instances to reproduce the experimental disease in monkeys; and they have been recovered in cultures from the lesions thus produced (43). Thus they fulfilled in several instances Koch's postulates. But with the possible exception of the work of Heist, Solis-Cohen, and Kolmer (29), no one outside of The Rockefeller Institute has so far been able to confirm this finding. The latter investigators report that they have successfully isolated and cultivated an organism which corresponds with the globoid bodies of Flexner and Noguchi, but the pathogenicity seems not to have been tested.

We have attempted to repeat this work. Using the Noguchi (26) technique and making many trials we have succeeded in finding organisms similar to the globoid bodies culturally, morphologically, and in staining reactions, but, owing to some cause which we still cannot explain, we could not carry the culture for more than three generations. We have not, therefore, injected monkeys with these organisms—an essential step for the establishment of an etiological relation between any organism and this disease—and cannot at present draw any conclusion from our culture work.

DISCUSSION.

Streptococci.

It is evident from our findings as well as from those obtained by others that the presence of streptococci, diplococci, or staphylococci in the brains, cords, or cerebrospinal fluids of human beings or monkeys that have been infected with poliomyelitis, does not mean that they are the specific causative agents of the disease. Even if the monkey should show typical changes of poliomyelitis after injections with poliomyelitic brain or cord emulsions which aerobic cultivation proved to contain streptococci, we cannot conclude that these microorganisms, and not some others which we do not yet know and which might be present in the emulsions, are responsible for the disease.

It is known that streptococci can be easily isolated from many parts of the normal body such as the skin, mouth, throat, gastrointestinal and the upper respiratory tracts, etc., and that these microorganisms

under certain abnormal conditions enter the blood and lymph streams and then the organs.

Thus they have been found in these places in smallpox and scarlet fever by Hektoen (44, 45); in tuberculosis by Brown, Heise, and Petroff (46), Pettit (47), White (48), and Petruschky (49); in typhoid fever by von Wassermann and Keysser (50), and Senger (51); in congenital syphilis by Kassowitz and Hochsinger (52); in chronic nephritis, cancer of the epiglottis, and compound fracture of the skull by White (48); in measles by Babes (53) and Tunnicliff (54); in yellow fever by Babes (53); in diphtheria by Loeffler (55); and in many other diseases (56). In short, there is practically no type of disease with which streptococci have not been associated.

It has been shown further by a number of investigators that, even in perfect health, living bacteria may enter the blood and lymph streams; but under these conditions they are continually being destroyed after their entrance. In 1899 Adami (57) disproved the belief that was generally held that in health blood and tissues were sterile and bacteria could only be found there under abnormal conditions; he showed that tissues were not actually, but only potentially sterile. Many years before this Ruffer (58) had demonstrated the existence of an enormous number of microorganisms in Peyer's patches of healthy rabbits, and Desoubry and Porcher (59) and Nocard (60) found micrococci in the chyle and blood during digestion. In 1900 Ford (61) reported that he had succeeded in growing micrococci from the livers and kidneys of normal rabbits, and also found that the negative results obtained by Neisser were due to the use of a solid medium, the low temperature at which the cultures were kept, and the short incubation time.

We have, in connection with our present investigation, isolated both aerobic and anaerobic diphtheroids and also small staphylococci from the kidneys of normal rabbits. It thus seems to be proved that the blood and tissues of healthy individuals are not always sterile in the strict sense of the word.

The fact that streptococci, and also diplococci and staphylococci have been found more frequently in the body than other bacteria may be due to the greater invasive power possessed by these pyogenic cocci, and their isolation from the brain of the sick and normal rabbits may not mean anything more or less than their wide distribution in the body.

The lack of reports on the isolation of pyogenic cocci from normal brains does not prove that they do not at times invade this part of the body. It only means that they have not been searched for. In diseased conditions where cultures have been made, the presence of these cocci in the brains and cords, spinal fluids, and blood has been

reported more than once. In 1912, Donath (62) found staphylococci in these places in many cases of Sydenham's chorea. In 1916, Rosenow (63) reported the finding of streptococci and staphylococci in the nervous system in many nervous diseases other than poliomyelitis. We have found streptococci and small diplococci in the brains of syphilitic rabbits. All these seem to be mere instances where bacteria, after their entrance into the body, are not completely destroyed as a result of the lowered body resistance. Indeed, in 1896 Flexner (64) said, after testing the bactericidal action of normal human blood serum on *Staphylococcus aureus*, that the former did appear to possess distinct bactericidal properties for the latter, and also that this power was absent, or diminished, in at least some cases of advanced chronic disease.

From the evidence given above we can safely say that ordinary streptococci, diplococci, and staphylococci, or, as they have recently been described in literature, cocci in chains, pairs, and clumps, are not the specific causative agents of poliomyelitis, but rather that the latter permits of a greater invasion of the nervous system by the former.

Nuzum (19) and Gauss (21) claim to have cultivated aerobic streptococci from 90 per cent of the spinal fluids taken from cases of poliomyelitis. Previous and coincident workers have usually had contrary results. Thus Abramson (65) concluded, from the study of about 1,200 fluids from patients with acute poliomyelitis in all stages of the disease, that "there is present little, if any, virus demonstrable either by culture or animal inoculation in the fluids of human poliomyelitis in the early stages." We have examined one sample of spinal fluid which was negative for bacteria. Furthermore, the early accumulation of a large number of negative results soon led to the opinion that the original discoveries might be due to errors in technique. In 1909, Leiner and von Wiesner (37) found that if the first part of the fluid was used, growth of cocci could be frequently obtained, but never from the rest of the fluid. They tested cases of poliomyelitis as well as of hydrocephalus, tuberculous meningitis, and pneumonia, and obtained similar results in all.

Rosenow and others reported that an intravenous injection into the rabbit of a pure culture of streptococci isolated from a case of poliomyelitis would produce flaccid paralysis, but paralysis does not necessarily signify poliomyelitis. Very large doses were given in these experiments, and it is possible that the paralysis may have been due to special localizations of the organism. In 1908 Strauss (66) reported a case "of streptococcemia with septic endocarditis and infarcts. This patient developed a flaccid paralysis of all extremities, and died in a state of coma two hours after the onset of the hemorrhage" of the brain. Moreover, other in-

investigators, such as Gilbert and Lion (67), Thoinot and Masselin (68), and many others have produced paralysis in rabbits by the injection of colon bacilli. Römer (69) says: "Rabbits are peculiarly liable to suffer from paralysis when any molecular substance is injected into the blood stream."³ Expressing this point in another way he says:⁴ ". . . the introduction of morbid material of various kinds (streptococci, *B. coli*), in fact of any foreign substance in a molecular state directly into the blood stream will cause paralysis or symptoms similar to paralysis at a time considerably later than the date of injection."

It has been stated that cultures of the pleomorphic streptococci isolated from cases of poliomyelitis, when injected into rabbits, had produced paralysis of various groups of muscles and lesions "similar in every detail to the changes considered characteristic of acute poliomyelitis in man" (Mathers, 20). This is in sharp contrast to our findings as well as those obtained by others. We have not even been able to produce typical lesions in rabbits with the actual virus, while with streptococci Bull (70) has only produced in rabbits various conditions and lesions referable not to poliomyelitis, but to streptococcus infection.

Finally, our isolation of streptococci and diplococci cannot be ascribed to air contamination, as we have found them constantly in the brains of so many animals. Further, we have found growth more frequently or, to express it more accurately, growth in about two-thirds and sometimes even all the tubes inoculated with brains from sick monkeys and rabbits, while from normal brains we have found growth in only one or, at most, two out of sixteen tubes of aerobic cultures. Smillie (31) has had a similar experience. Moreover, such cocci have also been found by Flexner and Noguchi, and by Rosenow; but they interpreted their findings in different ways. Flexner and Noguchi in their work on poliomyelitis regarded these cocci as accidental contaminations. Rosenow, on the contrary, regarded them as the specific causative agents of many diseases. In poliomyelitis we believe that these cocci are neither the extracorporeal contaminations nor the specific causative agents of the disease. We regard them as organisms present in the normal body which, under conditions of lowered resistance in the course of disease from other causes, acquire the power of more extensively invading the tissues.

More recently, Rosenow and his associates (71, 72, 73) have reported (1) that the streptococci could produce immunity in monkeys against poliomyelitis and (2) that the serum of horses immunized with their streptococci could neutralize

³ Römer (69), p. 31.

⁴ Römer (69), p. 78.

the active poliomyelitic virus. In our work we have not been able to confirm the first part of this statement (experiment with Monkey 6), while Amoss and Ebersson (74) have failed to find in Rosenow's antipoliomyelitic horse serum any greater neutralizing power against the poliomyelitic virus than that which was present in the control normal horse serum.

It has been claimed still further by Rosenow (15, 75), Nuzum and Herzog (18), Mathers (20), and Hektoen, Mathers, and Jackson (76) that they have found cocci—large and small, arranged either singly, in pairs, or in groups—in the sections of the central nervous system in epidemic poliomyelitis. The last three investigators have examined fifty-seven cases and found cocci in sections of the central nervous systems of the fifty-three that showed positive changes of poliomyelitis. In the sections from the other four cases which showed no changes of poliomyelitis they found no bacteria. On the other hand, Norris, Shatara, and Dean (77) reported that they have found Gram-positive cocci not only in 50 per cent of the cases of poliomyelitis but also in 50 per cent of the normal spinal cords. After an anatomical study of fifteen cases of acute poliomyelitis Blanton (78) says: "It was extremely difficult to feel convinced of having found any organisms in any of the cords we studied." We have found cocci in only one section, located outside the lesion, which Rosenow explains as due to the low virulence of the organisms, but we think a better explanation can be given. Negative results have also been obtained by Wickman (9), Harbitz and Scheel (79), Robertson and Chesley (27), and many others. It seems to us, therefore, that the cocci have no etiological relation to the lesions of poliomyelitis.

Globoid Bodies.

Although the findings of Flexner and Noguchi and Smillie have received but little confirmation, yet they have not been positively disproved. At least two links in the chain of proofs for the establishment of an etiological relation between the globoid bodies and poliomyelitis have so far not been supplied. First, the globoid bodies have not yet been proved to be able to produce immunity against poliomyelitis, and, second, the serum of monkeys immunized with the globoid bodies has failed to show neutralizing power against poliomyelitic virus (43)—two points which seem to be against the globoid bodies being the causative agents of poliomyelitis. The acquisition of saprophytic properties by long cultivation outside the body does not seem to explain the failure of these bodies to fulfill these two conditions, because they have been found in one instance to be virulent 18 months after isolation (80).

The chief differences and similarities between the poliomyelitic virus, and the globoid bodies and the streptococci are tabulated below.

Globoid bodies.	Poliomyelitic virus.	Streptococci.
<p>“They have been found repeatedly in the lesions of poliomyelitis in man and the monkey; they have not been detected in lesions or conditions other than poliomyelitis” (Amoss, 43).</p>	<p>It has been found only in cases of poliomyelitis and in poliomyelitis carriers.</p>	<p>They have been found in the lesions of poliomyelitis, in the lesions of other diseases, and in normal brains and cords.</p>
<p>“ . . . they have sufficed to reproduce in several instances the experimental disease in monkeys” (Amoss, 43).</p>	<p>It is capable of producing typical lesions of poliomyelitis only in monkeys.</p>	<p>They can produce gross and microscopic changes similar to those found in acute poliomyelitis in man, not only in monkeys but also in rabbits, guinea pigs, and many other animals.</p>
<p>They have not been shown to produce immunity against poliomyelitis.</p>	<p>It can render monkeys immune against poliomyelitis.</p>	<p>Rosenow, Towne, and Wheeler and others have reported successful immunization of monkeys with these organisms against poliomyelitis.</p>
<p>Sera of monkeys immunized with these organisms cannot neutralize poliomyelitic virus (Amoss, 43).</p>	<p>Serum of monkeys immunized with this can neutralize the active virus.</p>	<p>“ . . . serum of the horse immunized with recently isolated strains from experimental poliomyelitis in the monkey appears to have developed neutralizing, protective and curative power against the virus of poliomyelitis” (Rosenow, 72). But this serum has failed to show more neutralizing power against poliomyelitis virus than normal horse serum (Amoss and Ebersson, 74).</p>
<p>The serum obtained from monkeys recovered from experimental poliomyelitic shows very little agglutination power (Amoss, 43).</p>		<p>“The serum of patients and monkeys recovered from poliomyelitis cross-agglutinates specifically many, but not all, of the strains in the lower dilutions” (Rosenow, Towne, and Wheeler, 71).</p>

CONCLUSIONS.

Streptococci have been isolated from the central nervous system of monkeys dead of poliomyelitis.

Streptococci have also been isolated from the central nervous system of monkeys dead of other causes as well as from the brains of normal rabbits.

Streptococci isolated from poliomyelitic monkeys do not differ from those isolated from monkeys and rabbits dead from other causes.

An etiological relation has not been established between streptococci and poliomyelitis.

We have at several times isolated an organism that was similar to the globoid bodies culturally, morphologically, and in staining reaction, but have not been able to carry it along for more than three generations. The pathogenicity of these organisms has therefore not been tested on monkeys.

We have not been able to produce typical lesions of poliomyelitis in rabbits by the injection of either the poliomyelitic virus or streptococci.

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