THE PATHOLOGIC EFFECTS OF STREPTOCOCCI FROM CASES OF POLIOMYELITIS AND OTHER SOURCES.

By CARROLL G. BULL, M.D.

(From the Laboratories of The Rockefeller Institute for Medical Research.)

PLATES 47 TO 53.

(Received for publication, February 11, 1917.)

The etiology of epidemic poliomyelitis has again become one of the debated questions in pathology. While it is not denied that the filterable virus[1] is the inciting cause of the disease in man and the monkey, the particular question which has now arisen is the relation of the streptococcus to the filterable organism.

Several authors[2] have recently reported almost simultaneously on the streptococcus as capable of setting up, on inoculation into animals, clinical and pathologic states which they identify with poliomyelitis in man and the experimental poliomyelitis induced in monkeys by inoculation of the filtered virus. A variety of animals has been given as subject to inoculation with streptococci; namely, rabbits, guinea pigs, dogs, cats, sheep, and monkeys. The streptococci reputed to produce these effects have been cultivated from the throat, tonsils, blood, and cerebrospinal fluid[3] from clinical cases of poliomyelitis, and from the tissues of the central nervous system, cerebral ventricular fluid, intervertebral ganglia, and lymph nodes of cases at autopsy. The most characteristic effect noted in the inoculated animals was paralysis of a flaccid type. This condition is stated to follow various modes of inoculation, but to result most frequently when the cultures are injected into veins.

[1] Flexner, S., and Lewis, P. A., *J. Am. Med. Assn.*, 1909, liii, 2095. Landsteiner, K., and Levaditi, C., *Compt. rend. Soc. biol.*, 1909, lxvii, 787.

[2] Mathers, G., *J. Am. Med. Assn.*, 1916, lxvii, 1019; *J. Infect. Dis.*, 1917, xx 113. Rosenow, E. C., Towne, E. B., and Wheeler, G. W., *J. Am. Med. Assn.* 1916, lxvii, 1202; *Science*, 1916, xliv, 614; *J. Am. Med. Assn.*, 1917, lxviii, 280 Nuzum, J. W., and Herzog, M., *J. Am. Med. Assn.*, 1916, lxvii, 1205. Nuzum J. W., *ibid.*, 1916, lxvii, 1437; 1917, lxviii, 24.

[3] Abramson, H. L., *J. Am. Med. Assn.*, 1917, lxviii, 546. The recent report of Abramson on approximately 1,200 fluids from acute cases is pertinent: "Except for a few evident contaminations, our cultures remained sterile."

The findings of these authors conflict in several points with those of previous investigators in this country and abroad. In the first place, the extensive investigations of Flexner and his coworkers, Levaditi and Landsteiner,[4] Leiner and von Wiesner,[5] and Kling, Pettersson, and Wernstedt[6] seemed to exclude ordinary bacteria as the inciting agents of epidemic poliomyelitis. The bacteriological studies carried out at various times and in different countries previously resulted, with one exception to be mentioned, either in obtaining no growths whatever or, as in the case of Geirsvold,[7] of obtaining from the cerebrospinal fluid an ordinary micrococcus which was believed to be a contaminant derived probably from the skin. This does not mean that occasionally bacteria were not cultivated from tissues removed post mortem from human beings and even from inoculated and paralyzed monkeys; but the stricter the conditions of bacteriological asepsis, the less frequently were the cultures obtained. The exception alluded to is the report of the cultivation of a minute, strictly anaerobic organism, globoid bodies so called, by Flexner and Noguchi[8] both from human and monkey tissues.

In the next place, the findings differ essentially with regard to the classes of animals subject to infection with the filterable virus. The only species of animals found to be regularly subject to inoculation are monkeys. Experimental poliomyelitis in the monkey is identical with the disease appearing in man. The clinical symptoms are the same, and the essential lesions in the nervous system indistinguishable. In one unmistakable instance[9] and in an instance not as certainly proven,[10] young rabbits have succumbed to inoculation of nervous tissues carrying the poliomyelitic virus. Neither the symptoms nor the lesions of the nervous system in these animals corresponded with poliomyelitis in man and the monkey.

In still another way are the older and these newer findings in conflict. The recent authors have found streptococci cultivated from a wide variety of tissues and fluids effective on inoculation. Of all the older investigators, only Krause and Meinicke[11] claim to have succeeded in producing infection with blood, cerebrospinal fluid, and various extranervous organs taken from human poliomyelitis.

[4] Levaditi and Landsteiner, *Compt. rend. Soc. biol.*, 1910, lxviii, 311.

[5] Leiner, K., and von Wiesner, R. R., in Zappert, J., von Wiesner, R. R., and Leiner, K., Studien über die Heine-Medinsche Krankheit (Poliomyelitis acuta), Leipsic and Vienna, 1911, 137.

[6] Kling, C., Pettersson, A., and Wernstedt, W., *Communications Inst. méd. État à Stockholm*, 1912, iii, 5.

[7] Geirsvold, M., *Norsk Mag. Lægevidensk.*, 1905, iii, 1280.

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

[9] Marks, H. K., *J. Exp. Med.*, 1911, xiv, 116.

[10] Rosenau, M. J., and Havens, L. C., *J. Exp. Med.*, 1916, xxiii, 461.

[11] Krause, P., and Meinicke, E., *Deutsch. med. Woch.*, 1909, xxxv, 1825

It is noteworthy that they also employed rabbits, which they believed to be susceptible to the infection. This work has not been confirmed, except to the extent which the relatively few successful experiments in rabbits by Marks and possibly by Rosenau may be regarded as providing confirmation. But the conditions under which the rabbits succumb are so different from those observed in poliomyelitis that Marks felt obliged to determine the presence of the virus in rabbits by inoculating their nervous tissues into monkeys. The characteristic symptoms and lesions developed. He was able to transmit the virus of poliomyelitis through six series of rabbits. It was then lost.

Finally, the published reports of Mathers, Herzog, and Nuzum, and Rosenow and his coworkers contain descriptions and pictures of lesions occurring in the rabbit and monkey succumbing to the streptococcus infection which they identify with the typical lesions present in poliomyelitis in the central nervous tissues of man and the monkey. This is quite a delicate point to pass upon. Nevertheless it is essential, since the lesions of the nervous tissues in poliomyelitis are particularly characteristic. The descriptions are more convincing than the pictures, which are indeed disappointing. As a matter of fact, on the basis of the published illustrations dealing with lesions in the nervous organs, the suspicion is inevitably aroused that the pathologic processes induced by the streptococci are of another order from those occurring in human cases of poliomyelitis or arising in monkeys from the inoculation of the filtered virus.

We have therefore been led, by the intrinsic importance of the subject, to study streptococci in the manner described by these authors. But we have extended this experimental study to include with streptococci obtained from poliomyelitic patients, streptococci from a variety of other sources.

Origin and Properties of the Streptococci.

Poliomyelitic.—Cultures were made from the tonsils of twenty-seven cases of poliomyelitis, and from four pairs of tonsils extirpated during life and one pair removed at autopsy. The cultures were made both from the surface and the interior of the crypts in living cases and from the surface and the interior of the excised organs. The period of the disease at which the cultures were made varied from 3 days after onset to several weeks of convalescence.

Non-Poliomyelitic.—The cultures for comparison with the preceding were obtained (*a*) from the tonsils of children in an institution in which no case of poliomyelitis occurred during the New York epi-

demic (1916), (*b*) from tonsils extirpated at one of the large clinics of the city,[12] (*c*) from tonsils excised in private hospitals from adults and children suffering from cardiac or renal disease, and (*d*) from carious teeth removed because of root infection.

Cultural Properties.—The cultures were made on ascitic-fluid-glucose-agar and in ascitic-fluid-glucose-broth. In almost every instance the streptococcus was the predominating organism, in several instances the only organism obtained. No distinction in this respect was observed between poliomyelitic and non-poliomyelitic materials. In every instance subcultures of the second or at most of the third generation yielded pure growths.

The streptococci grew luxuriantly on the media mentioned, and on rabbit blood agar and in rabbit blood broth. The ascitic fluid cultures, after 24 hours' incubation at 37°C., usually contained a sediment at the bottom and upon the sides of the tube, the supernatant fluid being cloudy. The colonies in solid media resemble both those of pneumococcus and of streptococcus. About 1 in 20 of the cultures produced definite zones of hemolysis on the blood agar medium. The rest produced either no, or very slight hemolysis. But they caused a green discoloration of the medium indistinguishable from that produced by typical pneumococci.

The grouping of the organisms was chiefly as diplococci, but short chains were not infrequent. None could be classed as long-chain streptococci. Thirty-nine separate cultures were tested for fermentation. 54 per cent fermented inulin, 90 per cent raffinose, 89 per cent salicin, and 17 per cent mannite. Of these, 8 per cent caused definite, and 13 per cent slight hemolysis.

The morphology was affected by the conditions of cultivation. In rabbit blood broth the forms were quite regular and like streptococci. They became more irregular in the ascitic fluid media. Individuals, often in the same chain, were larger or smaller and more or less elongated. Under strictly anaerobic conditions many individuals appeared of smaller size than in the aerobic cultures.

[12] We are greatly indebted to Dr. Cornelius G. Coakley for many specimens which were removed at his clinic at Bellevue Hospital.

EXPERIMENTAL.

Practically the same varieties of animals were inoculated as those employed by other recent workers in this field. Intravenous injection was the usual manner of inoculation; but in special instances intracerebral injection, under ether anesthesia, was resorted to. The plan was to inoculate the earliest possible generations. Hence, in 10 per cent of the experiments the first cultivation generation was inoculated, in 70 per cent the second, and in 20 per cent the third generation. Usually the cultures inoculated had been grown in ascitic-fluid-glucose-broth or upon the corresponding agar; in some instances the blood broth or agar cultures were injected.

Guinea Pigs.—Twelve guinea pigs ranging in weight from 400 to 500 gm. were injected intravenously with from one-half to two cultures derived from cases of poliomyelitis. In no instance did any signs of infection appear. Hence this species of animals was not employed further.

Cats.—Four young animals ranging in weight from 500 to 700 gm. received intravenous injections of one to two cultures. One cat died on the 4th day. No paralysis or other severe symptoms had been detected. The surface of the brain yielded pure cultures of the injected streptococci. Neither brain nor spinal cord showed visible lesions, and sections prepared from both contained no pathologic changes. The remaining three animals remained well.

Dogs.—Sixteen young dogs were each given intravenously one-half to one culture per kilo of body weight from cases of poliomyelitis. Of these, one animal developed paralysis, four developed meningitis, and one an arthritis. The other dogs developed no symptoms except vomiting and diarrhea from 1 to 2 hours after inoculation. Two protocols follow.

Dog 1.—Weight 2.75 kilos.

Nov. 17, 1916. The animal received intravenously three slant cultures from an acute case of poliomyelitis in the second generation. Nov. 18. Animal weak; does not stand; respiration rapid and shallow. Nov. 19. Left hind leg flaccid; left fore leg weak. Nov. 20. Animal stronger. Paralysis continues. Nov. 21. No change. Nov. 22. Died during night.

Autopsy.—The jejunal portion of the intestine was intussuscepted and gangrenous. The meninges of the brain and spinal cord were injected and contained a gelatinous exudate. Stained films showed many streptococci being phagocyted and many free pus cells. A pure culture of streptococci was obtained from the exudate; the heart's blood and other organs gave no growth. Sections of the central nervous organs showed multiple abscesses in the cerebrum, pons, medulla, and white matter of the spinal cord. No lesions characteristic of poliomyelitis were present.

Dog 2.—Weight 2.5 kilos.

Nov. 24, 1916. The animal received three slant cultures of a streptococcus in the second generation derived from the tonsil of a poliomyelitic patient on the 3rd day of illness. Nov. 26. Drowsy; does not eat. Nov. 27. Weak; no paralysis. Died at 12 noon.

Autopsy.—Except for the central nervous system the organs were normal. The meninges contained an excess of slightly purulent fluid. Films showed pus cells and Gram-negative bacilli but no streptococci. The bacilli were being phagocyted. Only the bacillus was obtained in culture; the heart's blood and meninges yielded no streptococci. The cultural properties of the bacillus placed it in the group of *B. coli.* Sections of the brain and cord showed the existence of acute meningitis, but no lesions of the substance of either.

Rabbits.—A much larger number of rabbits was used than of any other species. The reason for this is that rabbits were chiefly used in the work recently reported. The rabbit, is, of course, known to be susceptible to experimental streptococcus and pneumococcus infection, and has been studied with reference to the former more than any other animal. Theoretically, therefore, the course and varieties of streptococcus infection in the rabbit should be well understood. In many ways they are; but this study illustrates the manner in which concentrated attention upon a particular feature of a pathologic process is capable of disclosing a number of unexpected facts. What is vastly more difficult, however, than even this detection is the proper interpretation of the new observations.

In the first place, rabbits alone, of all the varieties of animals studied by us, were inoculated with cultures from other than poliomyelitic sources. 78 animals received intravenous injections of cultures derived chiefly from the tonsils of poliomyelitic patients and 76 received intravenous injections of cultures derived from sources other than poliomyelitis. Table I summarizes the cases of paralysis and menin-

gitis and gives a record of the instances of extranervous infections.[13] A small number of individual protocols follows.

TABLE I.

Paralysis and infections.	Streptococci from poliomyelitic patients.	Streptococci from non-poliomyelitic patients.
Paralysis	4	4
Meningitis	14	15
Joint infections	13	14
Abscess in kidney	10	12
Septicemia	11	16
Abscess in skeletal muscles	5	3
Pericarditis	4	3
Endocarditis	5	4
Totals	66	71

Streptococci from Poliomyelitic Cases.

Of the 78 inoculated rabbits, 43 died and 35 still survive. Of the former number 4 developed flaccid paralysis of the limbs (Figs. 1 and 2). 18 other rabbits showed impairment of motion in one or more legs; but in these animals arthritis, tenosynovitis, or general weakness was discovered to be the cause. Daily or twice daily inspection of the animals was found advisable, because a rabbit would frequently show inability to use a leg freely without any apparent lesion, and then a day or two later it would exhibit an acute arthritis in the affected limb, often still present at the autopsy made at a later date.

Distribution of Lesions in the Rabbits Inoculated with Streptococci from Poliomyelitic Cases.[13]

Flaccid paralysis.	Meningitis.	Infection outside nervous organs.
4	14	30

Fourteen rabbits developed clinical meningitis to which they succumbed. The symptoms were, as a rule, well marked during life (Fig. 3), and were clearly determined at autopsy (Figs. 5 and 6) and from

[13] Infections in the central nervous system and other organs often occurred in the same animal.

sections of the nervous tissues (Fig. 8), while cultures from the meninges were usually positive. The clinical appearances include opisthotonos, spasticity, and hyperesthesia. In some instances, obvious clinical symptoms were wanting, but a purulent meningitis was found at autopsy. The sections showed at times focal lesions of the central nervous tissues, usually affecting the cerebrum, cerebellum, and medulla, and pons, associated with the meningitis (Figs. 9 and 10).

Thirty rabbits exhibited infections outside the central nervous organs. These consisted most often of arthritis and periarthritis, but tenosynovitis, myositis, endocarditis, abscess of the kidney, and septicemia were also frequently present (Table I).

A few illustrative protocols will be given. The first is that of a rabbit developing paralysis.

Rabbit 1.—Weight 1.5 kilos.

Oct. 13, 1916. The growth from two ascitic-glucose-agar slants from a recent case of poliomyelitis was injected intravenously. Oct. 15. Animal restless. Oct. 16. Head drawn back and to the right; hind legs paralyzed (Fig. 2). Oct. 17. Left fore leg flaccid. 10 a.m. Died.

Autopsy.—Outside the nervous system the organs appeared normal except for a small abscess in the left kidney. The meninges were hyperemic, and the cerebellum was covered with a gelatinous exudate. Cultures from the meninges gave a pure growth of streptococcus; the heart's blood gave no growth.

Sections of the brain and cord showed purulent meningitis and slight perivascular extension of the cellular exudate along the pial vessels into the substance of the cerebrum and to a less extent of the spinal cord. The gray matter of the spinal cord and medulla and the intervertebral ganglia were free of any focal lesions. A spinal nerve root in one of the sections showed a purulent infiltration (Fig. 11).

Although this animal showed paralysis of the limbs and to that extent symptoms suggestive of poliomyelitis, lesions of the nervous tissues characteristic of that condition were absent. The only histological changes detected were in the meninges and extensions along the pial investments of the blood vessels into the brain and to a less extent into the spinal cord. What other changes might have been detected had still other portions of the brain and cord been sectioned cannot, of course, be predicted; but as segments from several levels of the spinal cord, from the medulla, pons, and cerebellum, and from

different parts of the cerebrum as well as several intervertebral ganglia were studied, it may be asserted that they would in no way have resembled the typical lesions of poliomyelitis, which are not minutely focal in character.

The question arises as to the immediate causes of the paralysis of the limb. In this instance the source can only be inferred; in others it was found in focal abscesses or necroses, associated with emboli of streptococci, to be described below. Lesions similar to these do not occur in man and the monkey in poliomyelitic infection.

The culture recovered from the meninges of this animal was passed through four other rabbits in succession. The first succumbed in 56 hours to meningitis and septicemia. Sections of the nervous organs showed purulent meningitis and focal abscesses of the brain. The second rabbit, inoculated from the previous one, developed arthritis of both wrist joints. Cultures from this animal produced meningitis in a third, and cultures from the third, septicemia in a fourth rabbit.

Three other rabbits in this series developed, as has been stated, some degree of flaccid paralysis of the limbs. The histological studies of the central nervous organs confirmed essentially the findings given in the protocol above. In each instance there was evidence of acute meningitis with extension of the inflammation along the pial membrane into the substance of the nervous organs and chiefly the cerebrum. The perivascular cellular infiltration always coexisted with and was part of the meningitis; and this in turn was sometimes attended by focal abscesses (Figs. 9 and 10). In none were lesions of the nerve tissues *per se* detected, neither diffuse interstitial infiltration nor degeneration with consequent phagocytosis of the nerve cells. The spinal cord and intervertebral ganglia were notably free of lesions. In rare instances small hemorrhages existed in the spinal cord.

Rabbit 2.—Weight 1.55 kilos.

Oct. 10, 1916. The sediment of two tubes of ascitic-glucose-broth containing streptococci in the first generation was injected intravenously. The culture was obtained from the tonsils of a poliomyelitic patient during the first days of illness. Stiffness of the legs was present the following day; that is, in less than 24 hours after inoculation. The chief clinical symptom was spasticity of the hind legs. No further symptoms developed, but death occurred 50 hours after injection.

Autopsy.—The thoracic and abdominal organs showed nothing abnormal except that the right kidney contained a small infarcted area. The meninges were hyperemic, but no changes were detected in the dissected brain and cord. No streptococci were cultivated either from the heart's blood or the central nervous organs.

The histological lesions present in the cerebrum consisted of areas of marked perivascular infiltration (Fig. 14) associated with foci of necrosis (Fig. 15). The meninges were the seat of marked infiltration—an acute meningitis. And the relation between the meningitis, perivascular infiltration, and focal necroses was readily established. But the medulla, spinal cord, and intervertebral ganglia were all free of lesions, although the meninges about them showed some degree of cellular infiltration.

In all, five other rabbits of this series showed histological lesions similar to those described. In two of these a purulent meningitis coexisted. In none were any changes present in the gray matter of the spinal cord or in the intervertebral ganglia.

Rabbit 3.—Weight 1 kilo.

Nov. 18, 1916. The growth from two ascitic agar slants of a streptococcus isolated from a carious tooth of a convalescent poliomyelitic patient was injected intravenously. Nov. 19. No symptoms had developed. Nov. 20. Pronounced opisthotonos (Fig. 3). Nov. 21. No change. Nov. 22. Meningeal symptoms still present. Rabbit weak. Nov. 23. Condition unchanged. Nov. 24, 8 a.m. Rabbit found dead.

Autopsy.—The meninges of the brain and upper portion of the spinal cord were injected. Impression films from the brain and medulla showed many streptococci and pus cells. No lesions were found in the abdominal and thoracic organs. Pure cultures of streptococci were obtained from the surfaces of the brain and medulla.

Sections of the brain and cord showed a purulent meningitis.

The protocols of the rabbits illustrate instances in which (1) flaccid paralysis of the limbs developed, (2) spasticity appeared, and (3) meningitis was the prominent symptom. The question arises whether the conditions described correspond to or resemble those present in poliomyelitis as it is known in man and the monkey.

The decision must rest not only upon the gross symptoms but even more upon the histological appearances and in part also upon still another consideration; namely, the possibility of conveying, by inoculation of the rabbit culture material, poliomyelitis to monkeys. This

last aspect of the case will be considered below in connection with the experiments made on monkeys.

In order that the symptoms and lesions in rabbits may be interpreted, it is desirable to recapitulate briefly the main symptoms and lesions of poliomyelitis in man and the monkey. Aside from indefinite signs such as fever, malaise, etc., they consist of meningitis and flaccid paralysis. In man the meningitic symptoms often precede the paralytic, but death appears never to be caused by the meningitis but always by the paralysis, affecting mainly the function of respiration. In the monkey symptoms of meningitis appear, but they are trifling and overshadowed by the paralysis. Death here, too, invariably is caused by the paralysis affecting respiration.

The lesions of the nervous organs of man and the monkey are essentially identical: first, a certain degree of meningeal infiltration, not diffuse, but confined mainly to the region of the large blood vessels, particularly about the medulla and spinal cord and slightly over the cerebrum; second, a marked degree of intramedullary perivascular infiltration affecting slightly the cerebrum, and practically constantly the medulla, spinal cord, and intervertebral ganglia. This perivascular infiltration is associated with more or less diffuse cellular invasion of the gray matter, chiefly of the medulla and spinal cord and rarely of the cerebrum. Focal cellular accumulations also occur in the ganglia, sometimes between, sometimes involving the nerve cells. Hemorrhages are larger and more frequent in man than in the monkey. The nerve cells of the spinal cord, less often of the medulla, and least often of the cerebrum, suffer degeneration and necrosis and subsequent phagocytic destruction. Similar occurrences are observed in the intervertebral ganglia. The nerve cells chiefly affected in the spinal cord are the motor, but the cells in the posterior gray matter also suffer. The lesions in the nervous tissues are not sharply focalized; the cellular invasion of the meninges is chiefly lymphocytic and never chiefly polymorphonuclear or purulent.

The lesions met with in the rabbits are sharply contrasted with these. They consist of meningitis of polymorphonuclear and usually purulent kind, either focal or general; of focal abscesses in the gray matter, most frequent in the cerebrum (Figs. 8, 9, and 10), next in the cerebellum, and very rarely in the medulla and spinal cord; of focal

areas of necrosis of nerve cells, almost exclusively in the cerebrum, surrounded by infiltration of cells of lymphocytic type (Fig. 15) and adjacent to blood vessels showing perivascular infiltration (Fig. 14), extending usually from the meninges. This last and most striking lesion, which may be called focal meningo-encephalitis, is unlike any condition observed in poliomyelitis in man and the monkey. In some instances the foci were general throughout the brain; they were very infrequent in the spinal cord. The distribution of necrotic foci and perivascular infiltration was identical. According to the plane of the section, varying pictures are obtained. Sections through the center (Fig. 15) show a necrotic mass of gray matter surrounded by a collar of mononuclear cells; sections through the periphery may show only a collection of the latter cells (Fig. 13).

Finally, preparations stained for bacteria disclose a wide difference between the findings in man and the monkey and in the rabbits under consideration. While no bacteria have been detected in the former, by the use of ordinary methods, the reverse is true of the latter. The diffuse meningitis in rabbits shows, as a rule, in impression preparations many streptococci and polynuclear leukocytes (Fig. 5). In instances of quickly developing meningitis and rapid death, the cocci are more, and the leukocytes less numerous (Fig. 6). Phagocytosis of the streptococci is common. The focal areas of meningitis also contain streptococci, although not so readily demonstrable. The focal abscesses contain streptococci in masses in the central part and scattered in the periphery (Fig. 7). Probably the central mass was derived from an embolus. In other words, the lesions present in the rabbits are such as would be produced if streptococci or other suitable microorganisms entered the central nervous tissues in such a way and in sufficient numbers to survive and set up infection and inflammation.

This last consideration led to an examination of several stock rabbits of a lot in which snuffles was prevailing. Among three of this group which succumbed to a bacillary septicemia and of which sections of the nervous organs were prepared, one was found showing an acute meningitis attended by perivascular infiltration within the cerebrum (Figs. 12 and 13) indistinguishable from the corresponding conditions present in the animals inoculated with streptococci. In

view of the common prevalence of spontaneous infections among rabbits, one is led to inquire whether the perivascular lesions may not have preexisted in the animals prior to the injection of the streptococci. This supposition has indeed just received strong support from a publication by Reasoner[14] in which he figures this precise lesion as caused by *Spirochæta pallida* localized in the cerebrum of the rabbit. We believe more than ever, therefore, that lesions of this kind, obviously not specific, may be the result of bacillary infection to which rabbits under laboratory conditions are subject. In any case, they cannot be regarded as in any degree peculiar to, and indicative of poliomyelitis, a conclusion supported also by the experiments with streptococci of other than poliomyelitic origin.

Streptococci from Non-Poliomyelitic Cases.

76 rabbits were inoculated with streptococci of non-poliomyelitic origin. Of these, 58 rabbits died in the course of the experiments. No differences could be detected between the effects of these streptococci and those obtained from cases of poliomyelitis. Even the numerical results are equivalent.

Distribution of Lesions in the Rabbits Inoculated with Streptococci from Non-Poliomyelitic Cases[13]

Flaccid paralysis.	Meningitis.	Infections outside nervous organs.
4	15	37

A few illustrative protocols follow.

Rabbit 4. Spastic, Succeeded by Flaccid Paralysis.—Weight 2 kilos.

Oct. 16, 1916. Two slant cultures were injected intravenously. The streptococcus employed was in the second generation and came from the extirpated tonsils of an adult suffering from chronic nephritis. Oct. 20. Sluggish. Oct. 21. Hind legs spastic. Oct. 22. Hind legs flaccid; animal prostrate; unable to rise. 6 hours later marked opisthotonos appeared, and 1½ hours afterwards death occurred.

Autopsy.—Except for the central nervous system, the internal organs appeared normal. The muscles about the hip joints contained abscesses from which streptococci were recovered in films and cultures. The meninges of the medulla

[14] Reasoner, M. A., *J. Am. Med. Assn.*, 1916, lxvii, 1799.

and cerebellum contained a gelatinous exudate from which, as well as from the fourth ventricle, streptococci were found in films and cultivated.

Sections from all the nervous organs showed (1) in the cerebrum and cerebellum multiple focal abscesses containing large numbers of streptococci; (2) focal purulent meningitis, in which streptococci were difficult to demonstrate; (3) the absence of lesions in the medulla, spinal cord, and intervertebral ganglia.

Rabbit 5. Flaccid Paralysis and Convulsions.—Weight 1.8 kilos.

Nov. 2, 1916. Three slant cultures were injected intravenously. The streptococcus employed was in the second generation and had been obtained from the tonsil of a child in an isolated infant home in which no case of poliomyelitis had developed. Nov. 4. Left fore leg flaccid (Fig. 4); no other symptoms. Nov. 9. No change until today. About 1 p.m. convulsions developed which became more and more violent. 2.20 p.m. Died.

Autopsy.—The abdominal and thoracic organs appeared normal. The meninges of the brain and cord were moderately congested, and an exudate was present in the lumbar region. Dissection revealed nothing. The joints and muscles of the paralyzed leg showed no abnormality. Cultures and films from the blood, nervous organs, and joints showed no streptococci.

Sections prepared from various portions of the nervous organs showed merely punctiform hemorrhages in the cervical cord, medulla, and pons.

Rabbit 6. No Characteristic Symptoms. Perivascular Lesions in the Meninges and Cerebrum.—Weight 1.6 kilos.

Nov. 6, 1916. Two slant cultures in the second generation were injected intravenously. The streptococci were obtained from the extirpated tonsils of an otherwise healthy child. Nov. 7. No symptoms. Nov. 8. Died.

Autopsy.—The surface of the kidneys contained punctiform hemorrhages; spleen enlarged; acute pericarditis. The meninges were highly congested and an exudate covered the cerebellum and medulla. Streptococci and polymorphonuclear leukocytes were present in the exudate in the pericardium and meninges. Cultures were positive.

Sections from the nervous organs showed infiltration of the meninges and about the blood vessels (Fig. 16) in the cerebrum, attended by foci of necrosis (Fig. 17).

A second rabbit inoculated with this strain developed fatal septicemia.

After what has already been said in the discussion of the results of the injection of rabbits with streptococci derived from cases of poliomyelitis, little need be added here. The essential facts seem to be (1) that streptococci derived from conditions other than poliomyelitis are capable of producing lesions and symptoms in rabbits indistinguishable from those produced by streptococci of poliomyelitic

origin; and (2) that with a sufficiently large series of rabbits, the numerical proportion of lesions tends to be identical in both instances. The general conclusion indicated by the two classes of experiments is that when large numbers of streptococci possessing a certain grade of virulence are injected into the veins of rabbits, they tend by preference to localize in the meninges, from which the cerebrum may subsequently be invaded; next to the meninges, they seek the joints, and then the voluntary muscles and other situations. In these several localizations, they set up inflammations and suppurations in which they survive for a time, but they may also die. The conditions are analogous to experimental pneumococcus infection in the dog. This animal, relatively resistant to pneumococci, will respond to very large doses by the development of a purulent meningitis and associated encephalitis.[15] In the rabbit, too virulent cultures quickly kill through septicemia unassociated with particular localizations, while avirulent cultures are quickly destroyed, and hence do not localize at all.

In other words, it is not evident that the nervous lesions of streptococcus infection in the rabbit can be identified with the specific disease poliomyelitis as we know its manifestations in man and the monkey.

Experiments on Monkeys.

In this paper the monkey has frequently been referred to as the one experimental animal in which thus far the typical symptoms and pathologic lesions of poliomyelitis in man have been reproduced. Hitherto, infection of the monkey has been accomplished by means of the filterable virus contained within the nervous and some other organs of human beings suffering from poliomyelitis, and in a few instances besides through inoculation of the anaerobic cultivated globoid bodies of Flexner and Noguchi.[3] The recent workers alluded to[2] claim to have produced typical poliomyelitis in the monkey with streptococci. Since this species supplies the decisive test, we carried out crucial experiments with it.

The method of inoculation recommended by these authors departs considerably from the one usually employed with the filterable virus.

[15] Bull, C. G., *J. Exp. Med.*, 1916, xxiv, 7.

With the latter, infection by way of the blood is difficult or impossible to achieve;[16] with the streptococci, intravenous inoculation has been preferred.

Monkey 1.—Nov. 18, 1916. A *Macacus rhesus* was given intravenously a suspended sediment from 60 cc. of ascitic-fluid-glucose-broth culture in the second generation of streptococcus obtained from the tonsils from a poliomyelitic patient. Nov. 19. Animal moves about more slowly; generally weak; no paralysis. Nov. 20, 8 a.m. Voice high pitched. 10 a.m. Legs spastic. 11 a.m. Retraction of head; marked hyperesthesia. 1 p.m. Died.

Autopsy.—The organs outside the nervous system showed nothing of importance. The meninges were congested and edematous. The substance of the brain and cord appeared normal. Films from the meninges of the brain and cord showed streptococci, pus cells, and mononuclear cells; pure cultures of streptococcus were obtained.

Sections of the brain and cord showed only a purulent meningitis. No lesions were detected in the substance of the spinal cord, medulla, pons, cerebrum, or intervertebral ganglia.

Monkey 2.—Dec. 11, 1916. A *Macacus rhesus* was given intravenously the suspended sediment from 60 cc. of ascitic-glucose-broth culture. The culture of streptococcus used in this experiment came from the tonsil of a poliomyelitic patient and had been passed through two rabbits, of which one had developed paralysis of the legs, and both at autopsy had shown purulent meningitis and focal cerebral abscesses. Dec. 12. No general effect; double panophthalmitis appearing. Dec. 13. No change in the general condition; anterior chamber and vitreous opaque and purulent. The sediment from 30 cc. of a similar culture was injected intravenously. Within 2 hours the animal became ill and weak; no paralysis was detected. Dec. 14. Died.

Autopsy.—The lungs were edematous and hemorrhagic; spleen swollen, dark, and friable; mesenteric nodes hemorrhagic. The meninges were congested; there was no visible lesion of the spinal cord or brain aside from punctate hemorrhages in the latter. Films and cultures prepared from the interior of the eyes and the fourth ventricle gave streptococci. Cultures from the heart's blood and surface of the brain were negative.

Sections of the brain, medulla, spinal cord, and intervertebral ganglia showed no lesions aside from small extravasations of blood in the cerebrum.

Since this is the type of experiment which is said by Rosenow and his coworkers to have given the most constant result, it was repeated on four other *Macacus rhesus* monkeys. In each instance some degree of depression followed the massive injections; in one instance a

[16] Flexner, S., and Amoss, H. L., *J. Exp. Med.*, 1914, xx, 249.

purulent panophthalmitis again developed. Paralysis never appeared, and all four animals recovered.

In other words, our experiments were uniform failures as far as producing in monkeys paralysis or any condition resembling poliomyelitis was concerned, by means of cultures of streptococci derived from cases of poliomyelitis and injected either directly into monkeys or first into rabbits and then into monkeys. As will be shown below, the monkeys which received massive doses of the streptococci showed no undue resistance to inoculation with the filtered virus of poliomyelitis.

Streptococci from the Poliomyelitic Spinal Cord.

The attention which has been directed to streptococci in poliomyelitis has led to the discovery that streptococci can be cultivated from specimens of the brain and spinal cord apparently of both persons and monkeys succumbing to the disease. By employing considerable portions of the spinal cord of monkeys, we have in a few instances obtained growths of streptococci from them. Only a part of the cultures made yield the cocci; many tubes remain sterile. The following protocol illustrates this point.

When moribund, a poliomyelitic monkey was etherized and portions of the brain and spinal cord and two lymph nodes were removed aseptically. The brain and cord were inoculated (*a*) in fragments and (*b*) in emulsions into ascitic-glucose-broth and agar respectively. The lymph nodes were inoculated into the fluid medium. The agar medium was inoculated while fluid and then allowed to congeal. Twenty tubes in all were inoculated. One-half the tubes were covered with paraffin oil. The incubation was at 37°C. After 48 hours two broth tubes containing emulsified brain tissue contained growths of streptococci. After 72 hours four more tubes of broth showed streptococci, of which two had been inoculated with brain emulsion, one with a brain fragment, and one with a lymph node. 14 tubes remained sterile. It is worth noting that the comminuted brain tissue alone, except for one brain fragment, yielded streptococci; the spinal cord cultures yielded none. The distribution of the streptococci in the central nervous system must at best have been quite irregular. Moreover, none of the solid cultures yielded detectable growths. A single surviving streptococcus in a fluid culture would multiply abundantly; in a solid culture it would probably be lost. The streptococci from these sources were injected intravenously into six medium sized rabbits. One rabbit died in 48 hours. The

autopsy showed a purulent meningitis. Another developed arthritis 72 hours after inoculation. The remaining four showed no symptoms.

The original streptococcus from this source was transferred to 60 cc. of ascitic-glucose-broth of which, after incubation at 37°C., the sediment was injected intravenously into a *Macacus rhesus*. No symptoms developed, and hence 48 hours later, the sediment from 30 cc. of a broth culture was also injected. Within 24 hours the animal was somewhat slow and irritable; the condition quickly passed away. No paralysis or other severe symptoms developed.

At the same time that the ascitic broth culture was prepared, another was made from the original growth in blood agar. The entire growth was washed off and injected intracerebrally, under ether anesthesia, into a *Macacus rhesus*. No symptoms of any kind developed.

The streptococcus derived from the brain tissue produced a green color in blood and otherwise resembled closely *Streptococcus viridans*, so called.

Before considering the significance of this experiment, the following additional note should be made. Several stock specimens of recently glycerolated spinal cord from monkeys which had succumbed to poliomyelitis were incubated in ascitic-glucose-broth and a specimen was obtained yielding streptococci corresponding to *Streptococcus viridans*. Recent cultures from this source were inoculated (*a*) intravenously into two rabbits, (*b*) intracerebrally, under ether anesthesia, into a *Macacus rhesus*, and (*c*) the latter test was controlled by inoculating a filtrate of the spinal cord itself into another *Macacus rhesus*. The results are significant. The rabbits each developed meningitis and kidney abscesses, without paralysis. Sections of the spinal cord and brain showed only lesions of meningitis. The monkey inoculated with the streptococcus remained well; the other inoculated with the filtrate developed paralysis and died.

The conditions presented by the foregoing experiments on monkeys should theoretically, assuming that streptococci have an etiologic relation to poliomyelitis, have been favorable to the production of paralysis and the characteristic nervous lesions in at least part of the monkeys inoculated. That in no single instance any definite infection resulted except purulent ophthalmitis, and that the streptococci derived immediately from the nervous tissues of poliomyelitic monkeys should have been inert is difficult to harmonize with the view that the streptococcus is capable of producing poliomyelitis in monkeys, especially as the filtrate from the nervous tissue of the paralyzed monkey was readily effective under circumstances in which the streptococcus obtained from the same source was entirely inactive.

Does Streptococcus Infection Protect against Infection with the Filtered Virus?

Recovery from poliomyelitis induced by the filtered virus leaves a state of resistance in monkeys indicated by insusceptibility to reinoculation[17] and by the power of the blood serum to neutralize the filtered virus.[18] Tests were made to determine whether monkeys having received several injections of cultures of streptococci from poliomyelitic sources exhibited protection by either of the foregoing reactions.

Neutralization in Vitro.—For this test two monkeys which had resisted streptococcus inoculation were taken and their immunity to the organism was increased by the following injections: to each was given intravenously the sediment from 100 cc. of broth culture of the original strain employed and then on 2 successive days sediment from 10 cc. of a broth culture of the streptococcus cultivated from the glycerolated spinal cord of a poliomyelitic monkey. 10 days after the last injection the sera of the monkeys agglutinated the streptococci in dilutions up to 1:1,500. Normal monkey serum agglutinated the same cultures up to 1:300.

The tests for neutralization were made by mixing 0.1 cc. of a Berkefeld filtrate prepared from a 5 per cent emulsion of active poliomyelitic virus with 1 cc. of monkey serum, incubating for 2 hours at 37°C., and keeping for 16 hours at 4°C. The injections were made intracerebrally, under ether anesthesia, into two *Macacus rhesus* monkeys.

Monkey 3.—Jan. 3, 1917. A *Macacus rhesus* was inoculated. No symptoms appeared until Jan. 10 when the right arm became flaccid. Jan. 11. The left arm was flaccid. Jan. 12. The muscles of the trunk were paralyzed. Jan. 13. The animal was etherized.

Autopsy.—The gross poliomyelitic lesions of the medulla were typical, and the sections of the spinal cord, medulla, and intervertebral ganglia were characteristic of the disease.

Monkey 4.—Jan. 3, 1917. A *Macacus rhesus* was inoculated. No symptoms developed until Jan. 7 when the animal protected the right arm. Jan. 11. Ataxia

[17] Flexner and Lewis, *J. Am. Med. Assn.*, 1910, liv, 45.

[18] Landsteiner and Levaditi, *Compt. rend. Soc. biol.*, 1910, lxviii, 311.

and pronounced tremor of head. Jan. 12. Legs flaccid; animal prostrate. Jan. 13. Etherized.

The spinal cord and medulla showed typical gross lesions and the sections were characteristic of poliomyelitis.

Protection Tests.—Three monkeys which had received large injections of streptococci from cases of poliomyelitis were tested for protection against the filtered virus.

Monkey 5.—Nov. 22, 1916. A *Macacus rhesus* received intravenously 50 cc. of ascitic-glucose-broth culture of poliomyelitic streptococci which had been passed through one rabbit. A period of depression followed, but on Nov. 24 the animal appeared normal. The streptococci from 40 cc. of a similar broth culture were injected. No marked symptoms developed. Dec. 12. 1 cc. of the filterable virus from fresh brain and spinal cord of a paralyzed monkey was injected intracerebrally under ether anesthesia. Dec. 17. Ataxia. Dec. 18. Prostrate. Dec. 19. Died.

The autopsy showed marked poliomyelitic lesions.

Monkey 6.—Nov. 26, 1916. A *Macacus rhesus* received intravenously 45 cc. of a broth culture of streptococcus of poliomyelitic origin. Nov. 28. No symptoms had appeared. The sediment from 50 cc. of culture was injected. Dec. 1. Animal weak, moves about slowly. Dec. 3. Recovered. Dec. 12. Very active. 1 cc. of a suspension of brain and spinal cord of poliomyelitic monkeys was injected intracerebrally under ether anesthesia. Dec. 15. Ataxia; tremor; double ptosis. Dec. 17. Prostrate. Dec. 21. Very weak. Etherized.

The autopsy showed typical lesions of poliomyelitis.

Monkey 7.—Dec. 6, 1916. A *Macacus rhesus* received intravenously the sediment from 60 cc. of an ascitic-glucose-broth culture of poliomyelitic streptococci, Dec. 7. Animal somewhat ill; eyes cloudy. Dec. 8. The sediment from 30 cc. of a culture was injected intravenously. Dec. 9. Moves slowly; not well. Dec. 12. Well. Jan. 23, 1917. The sediment from 100 cc. of a broth culture was injected. No effect. Jan. 24. 10 cc. of a broth culture of streptococcus obtained from the nervous tissues of a poliomyelitic monkey were injected. Jan. 25. 10 cc. of the same culture were injected as on the day before. No effect produced. Feb. 3. 0.5 cc. of a suspension of poliomyelitic spinal cord of a monkey was injected intracerebrally under ether anesthesia. Feb. 7. Both arms paralyzed. Feb. 8. Prostrate. Etherized.

The spinal cord showed marked lesions of poliomyelitis.

DISCUSSION.

The results obtained by us from the inoculation of several species of laboratory animals with cultures of streptococci from poliomyelitic patients and other sources fail to establish an etiologic relationship

between streptococci and epidemic poliomyelitis. In this respect our experiments are in sharp contrast to those of Mathers, Herzog, and Nuzum, and Rosenow and his coworkers. Not only were we unsuccessful in inciting poliomyelitis with cultures of streptococci, but we found that these organisms produce chiefly in rabbits various conditions and lesions that are absent from man and the monkey, the subjects of poliomyelitis, and the conditions and lesions result equally well from streptococci originating in persons not suffering from or exposed to the disease. In other words, they are referable not to poliomyelitis, but to streptococcus infection. That they may also be present accidentally in stock rabbits has also been shown. Finally, we have failed to detect any immunological relationship between streptococci and the filterable virus of poliomyelitis.

It may be pointed out that the mere appearance of paralysis in animals is no sufficient criterion for concluding that poliomyelitis exists or has been produced. The toxin of the Shiga bacillus, for example, frequently causes flaccid paralysis in rabbits. It may be considered as established that streptococci do sometimes cause paralysis in rabbits and possibly other animals, but our experiments show that this is determined not by the source but by the degree of virulence of the cultures. The final test indeed is the monkey, not the rabbit or other domestic animals, and we not only failed with streptococci to induce paralysis in monkeys, but we failed to render them more resistant or their blood serum neutralizing to the filtered virus of poliomyelitis.

Our failures cannot be ascribed to minor variations in technique. About 10 per cent of the streptococcus cultures from poliomyelitic cases were obtained pure in the first generation and at once inoculated; and no distinction was discovered between the lesions thus produced and those set up by the cultures in the second and third generations. There is one advantage which the earlier generations possess over the later ones; they are somewhat more virulent. Yet even the first generations possessed little infective power and enormous doses were required to produce any pathologic effect. The rapid loss of power of streptococci to produce paralysis even when passed from animal to animal is in sharp contrast with the increase occurring under similar conditions in the filtered virus. No two sets

of pathologic actions and effects could be more diverse than those observed by us to be caused by streptococci and by the filtered virus of poliomyelitis.

SUMMARY.

Streptococci cultivated from the tonsils of thirty-two cases of poliomyelitis were used to inoculate various laboratory animals.

In no case was a condition induced resembling poliomyelitis clinically or pathologically in guinea pigs, dogs, cats, rabbits, or monkeys.

On the other hand, a considerable percentage of the rabbits and a smaller percentage of some of the other animals developed lesions due to streptococci. These lesions consisted of meningitis, meningo-encephalitis, abscess of the brain, arthritis, tenosynovitis, myositis, abscess of the kidney, endocarditis, pericarditis, and neuritis.[19]

No distinction in the character or frequency of the lesions could be determined between the streptococci derived from poliomyelitic patients and from other sources.

Streptococci isolated from the poliomyelitic brain and spinal cord of monkeys which succumbed to inoculation with the filtered virus failed to induce in monkeys any paralysis or the characteristic histological changes of poliomyelitis. These streptococci are regarded as secondary bacterial invaders of the nervous organs.

Monkeys which have recovered from infection with streptococci derived from cases of poliomyelitis are not protected from infection with the filtered virus, and their blood does not neutralize the filtered virus *in vitro*.

We have failed to detect any etiologic or pathologic relationship between streptococci and epidemic poliomyelitis in man or true experimental poliomyelitis in the monkey.

[19] Many thousand cases of epidemic poliomyelitis occurred in New York and elsewhere during the past summer (1916) and many cases were subjected to autopsy. But no instance of metastatic infection and inflammation such as occur usually in streptococcus infection has been reported.

EXPLANATION OF PLATES.

PLATE 47.

FIG. 1. Rabbit. Flaccid paralysis of fore leg appearing 48 hours after an intravenous inoculation of streptococci from a poliomyelitic tonsil.

FIG. 2. Rabbit. Flaccid paralysis of both hind legs and meningitis appearing 72 hours after an intravenous inoculation of poliomyelitic streptococci.

PLATE 48.

FIG. 3. Rabbit. Meningitis and opisthotonos appearing 48 hours after an intravenous inoculation of poliomyelitic streptococci.

FIG. 4. Rabbit. Flaccid paralysis of the left fore leg appearing 72 hours after an intravenous inoculation of non-poliomyelitic streptococci.

PLATE 49.

FIG. 5. Contact film from the cerebrum of the rabbit shown in Fig. 2. × 1,000.

FIG. 6. Contact film from the cerebrum of a rabbit which survived 72 hours after an intravenous inoculation of non-poliomyelitic streptococci. × 1,000.

FIG. 7. Streptococci in brain abscesses from a rabbit which survived 72 hours after an intravenous inoculation of poliomyelitic streptococci. Gram-Weigert stain. × 1,000.

PLATE 50.

FIG. 8. Purulent meningitis. Section from the rabbit shown in Fig. 2. × 250.

FIG. 9. Cerebral abscess. Section from a rabbit which survived 72 hours after an intravenous inoculation of non-poliomyelitic streptococci from the tonsil of an adult. × 250.

FIG. 10. Cerebellar abscesses from the same rabbit as Fig. 9. × 40.

PLATE 51.

FIG. 11. Purulent neuritis. Section from a spinal nerve root of a rabbit which survived 72 hours after an intravenous inoculation of poliomyelitic streptococci. × 250.

FIG. 12. Perivascular infiltration. Cerebrum of a stock rabbit which succumbed to bacillary infection. × 250.

FIG. 13. Round cell infiltration from the same rabbit as Fig. 12. × 250.

PLATE 52.

FIG. 14. Perivascular infiltration. Cerebral cortex of a rabbit which survived 4 days after an intravenous inoculation of poliomyelitic streptococci. × 250.

FIG. 15. Focal necrosis and round cell infiltration. Cerebrum of a rabbit which survived 7 days after an intravenous inoculation of poliomyelitic streptococci. × 180.

PLATE 53.

FIG. 16. Perivascular infiltration. Cerebrum of a rabbit which survived 48 hours after an intravenous inoculation of non-poliomyelitic streptococci. × 105.

FIG. 17. Focal necrosis and round cell infiltration. Cerebrum of the same rabbit as Fig. 16. × 105.

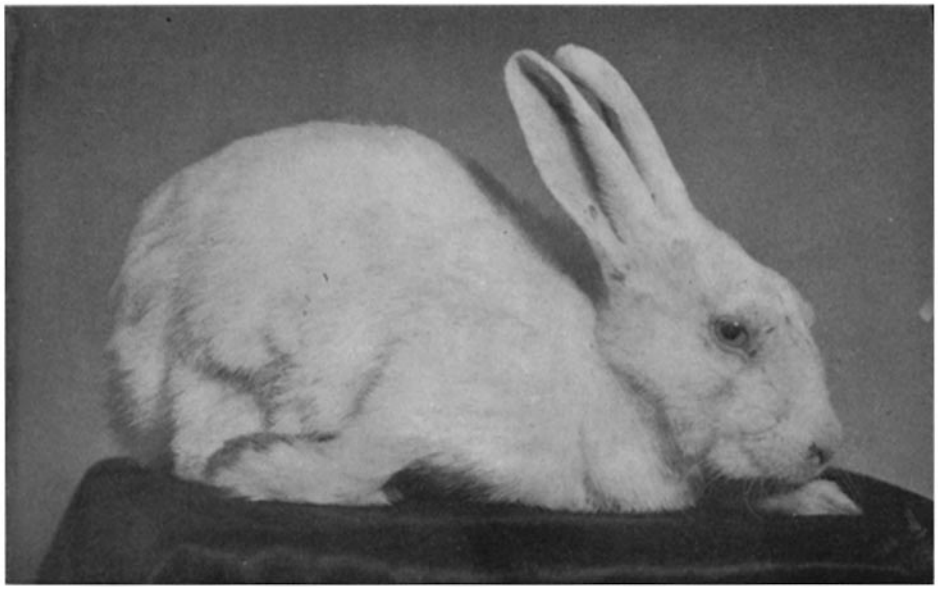

FIG. 1.

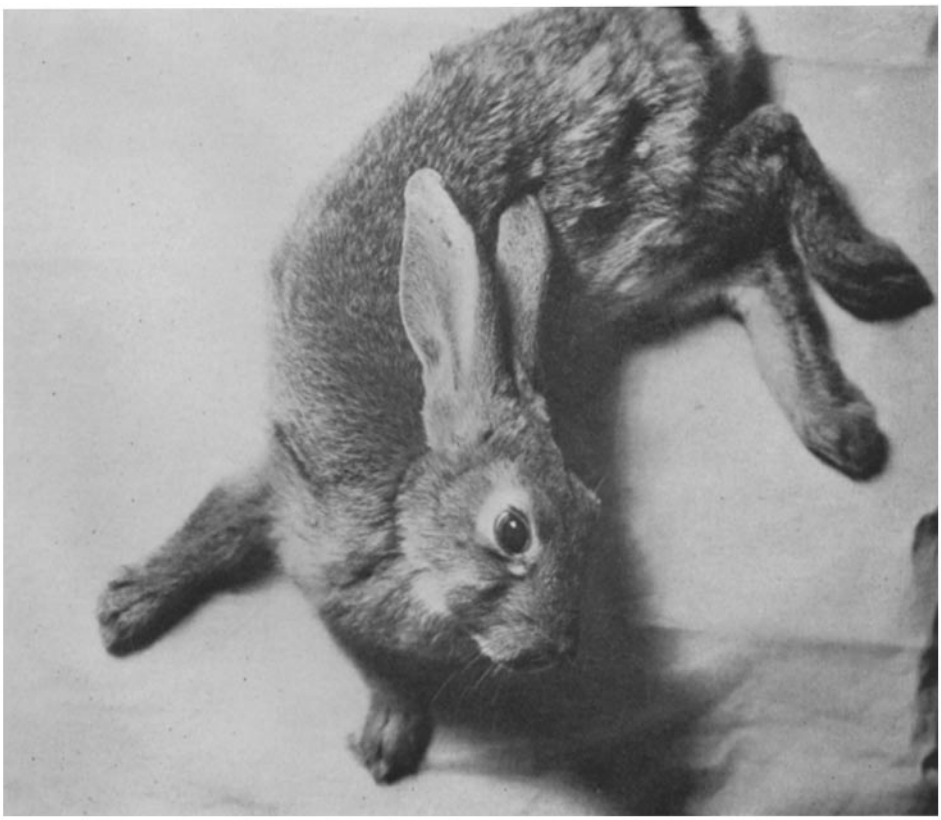

FIG. 2.

(Bull: Streptococci from Poliomyelitis.)

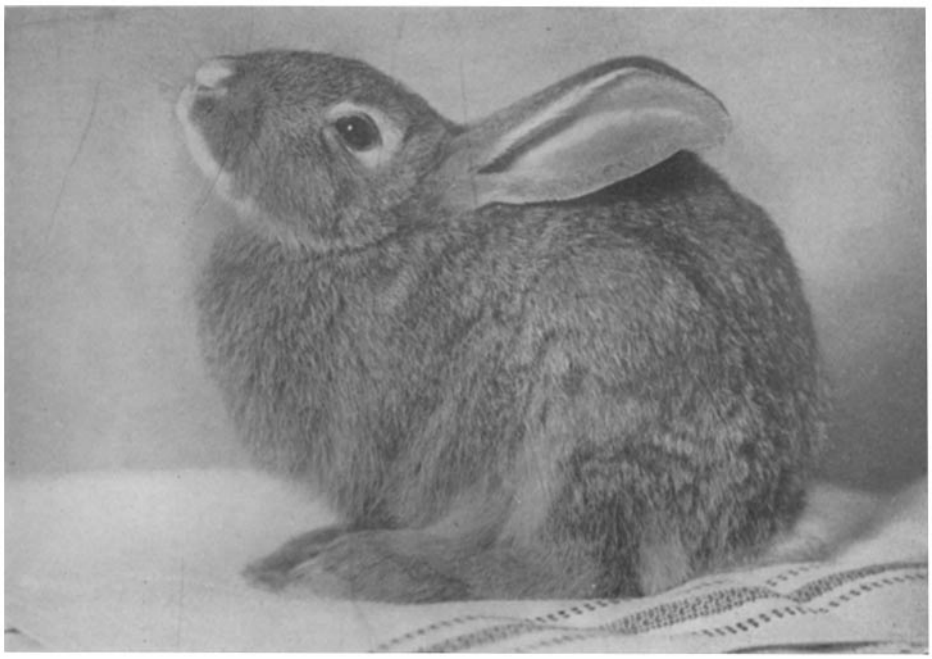

FIG. 3.

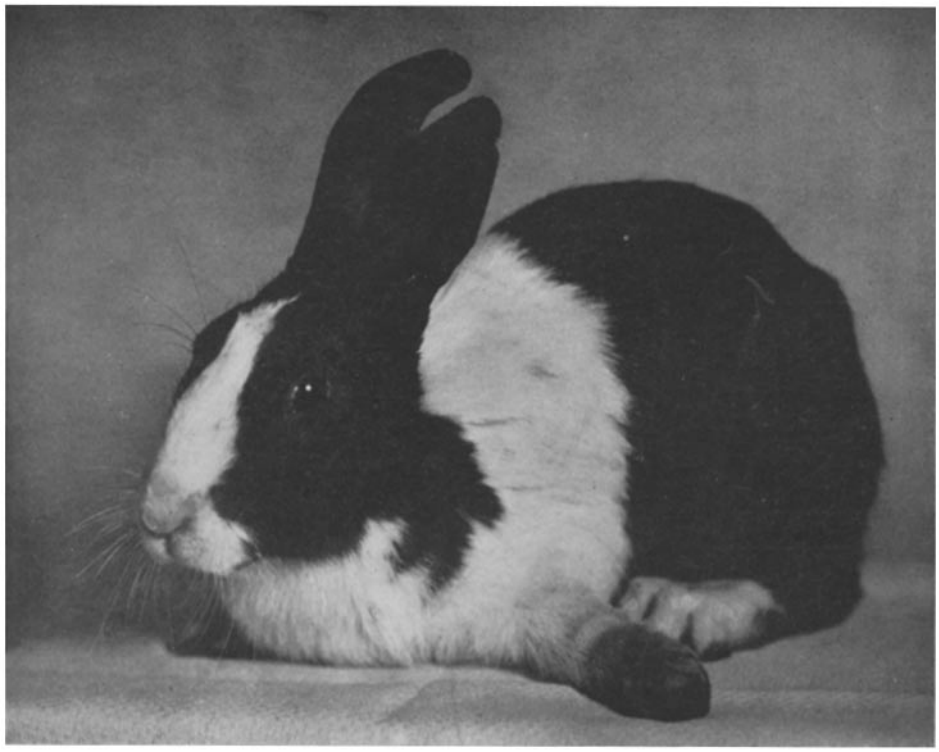

FIG. 4.

(Bull: Streptococci from Poliomyelitis.)

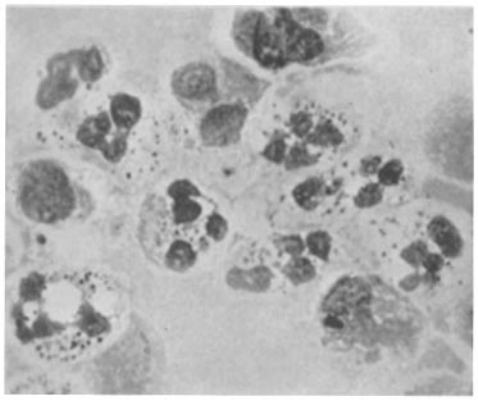

FIG. 5.

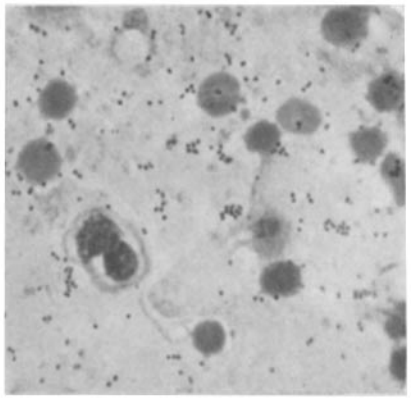

FIG. 6.

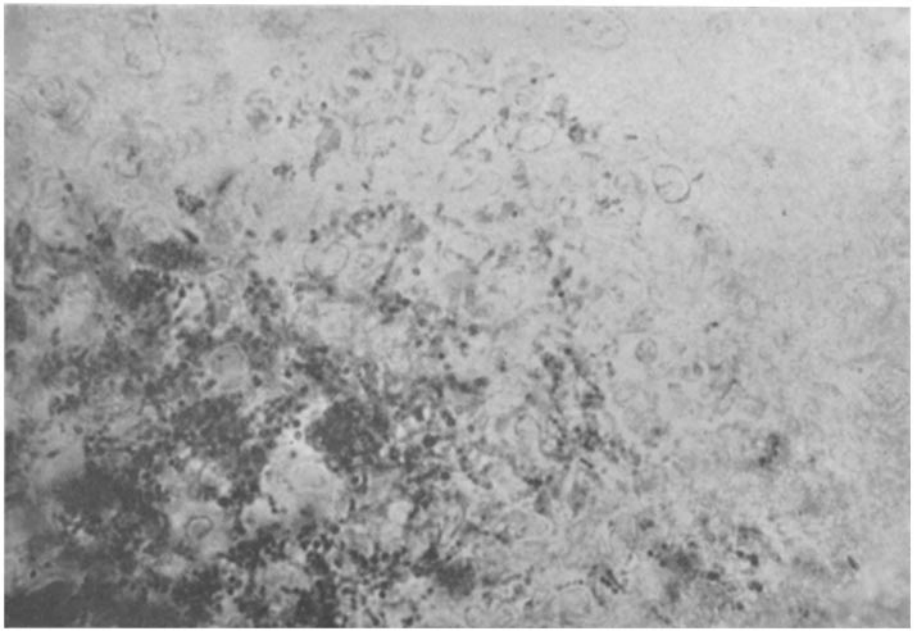

FIG. 7.

(Bull: Streptococci from Poliomyelitis.)

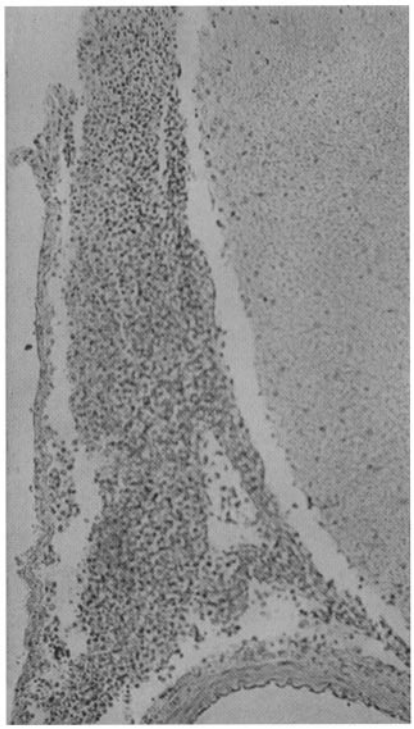

FIG. 8.

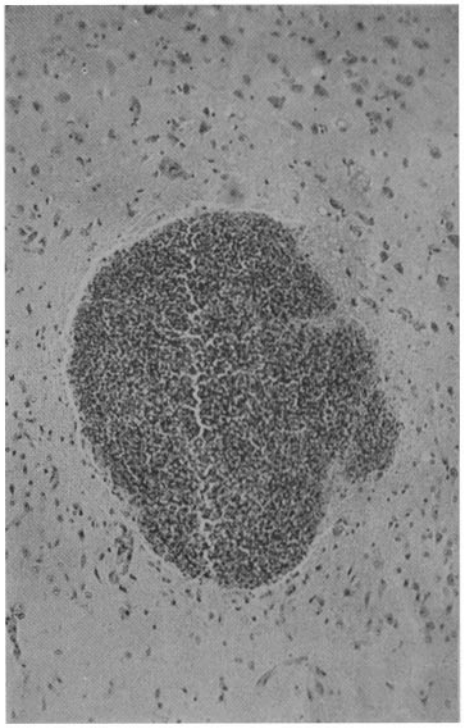

FIG. 9.

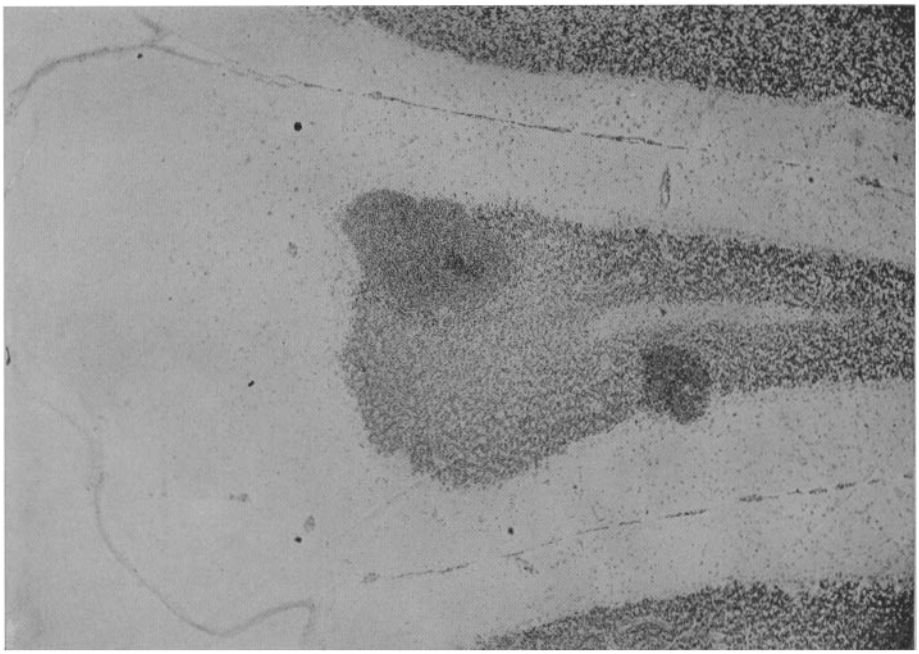

FIG. 10.

(Bull: Streptococci from Poliomyelitis.)

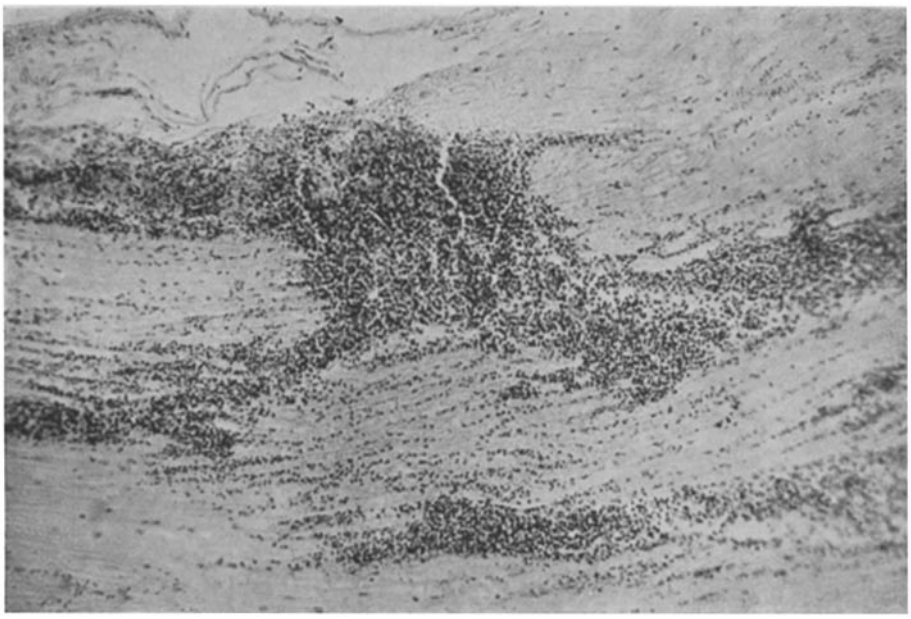

FIG. 11.

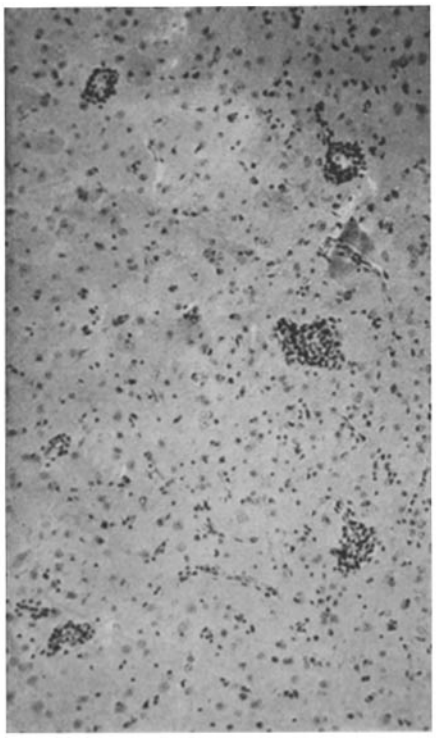

FIG. 12.

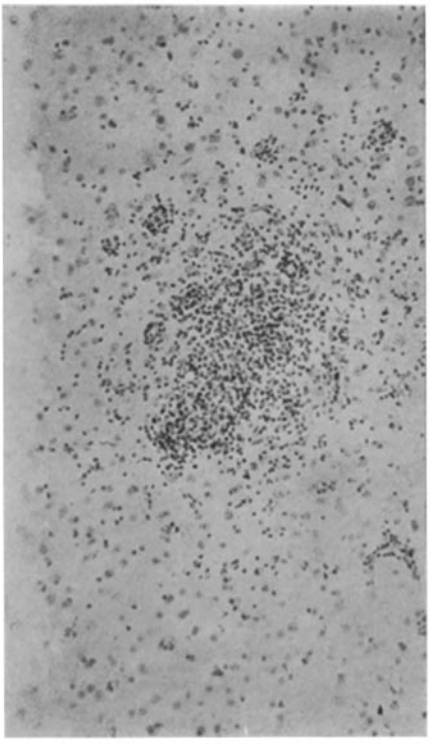

FIG. 13.
(Bull: Streptococci from Poliomyelitis.)

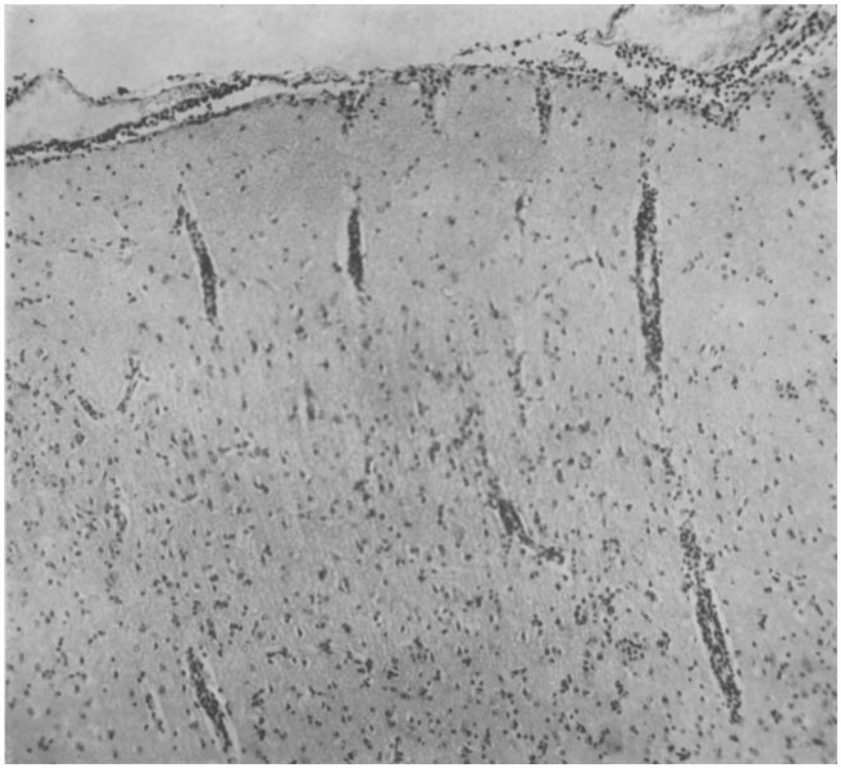

FIG. 14.

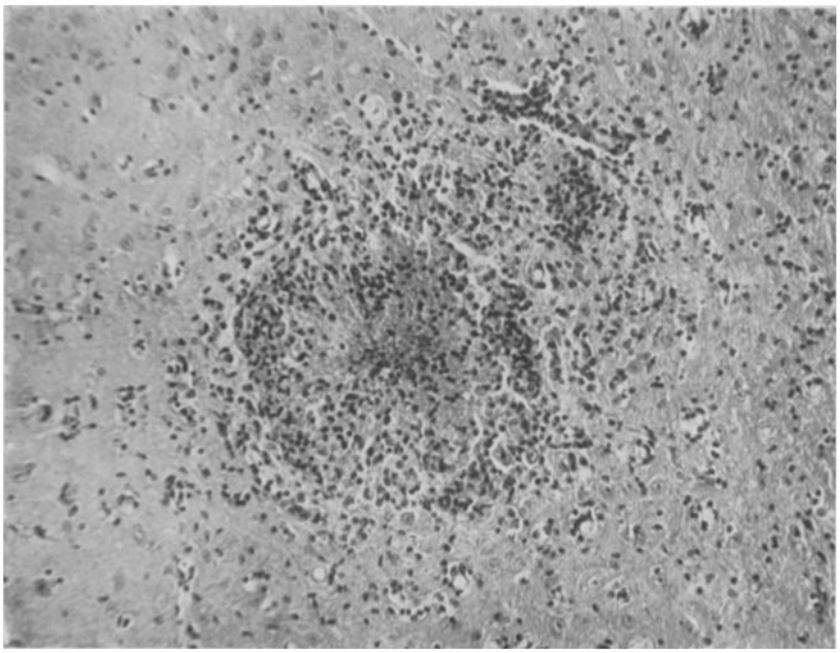

FIG. 15.

(Bull: Streptococci from Poliomyelitis.)

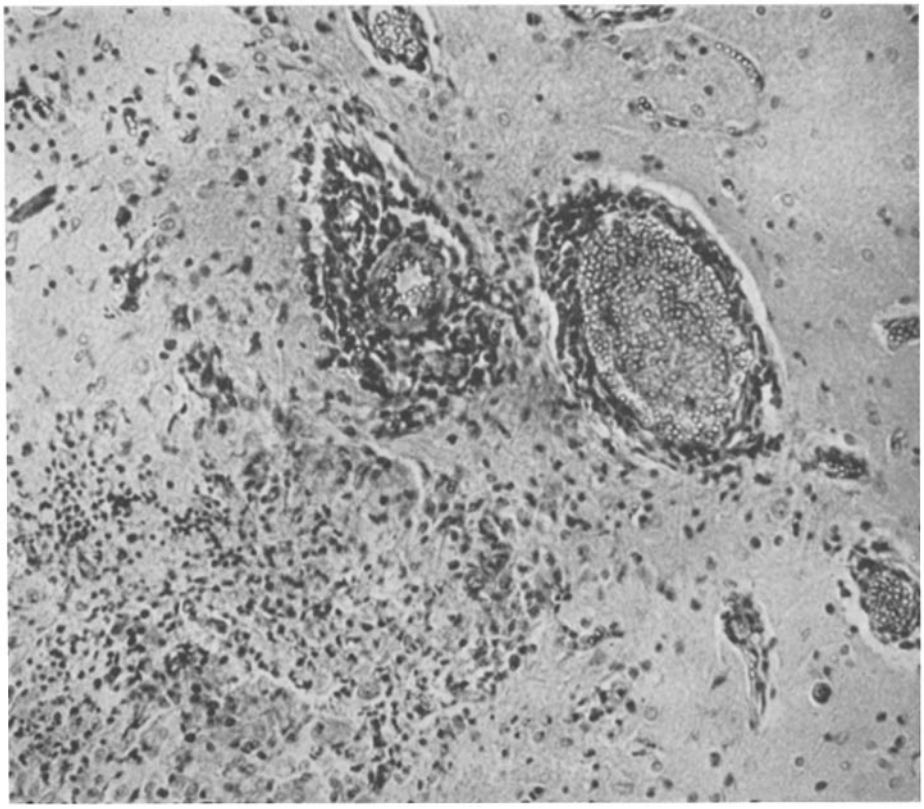

FIG. 16.

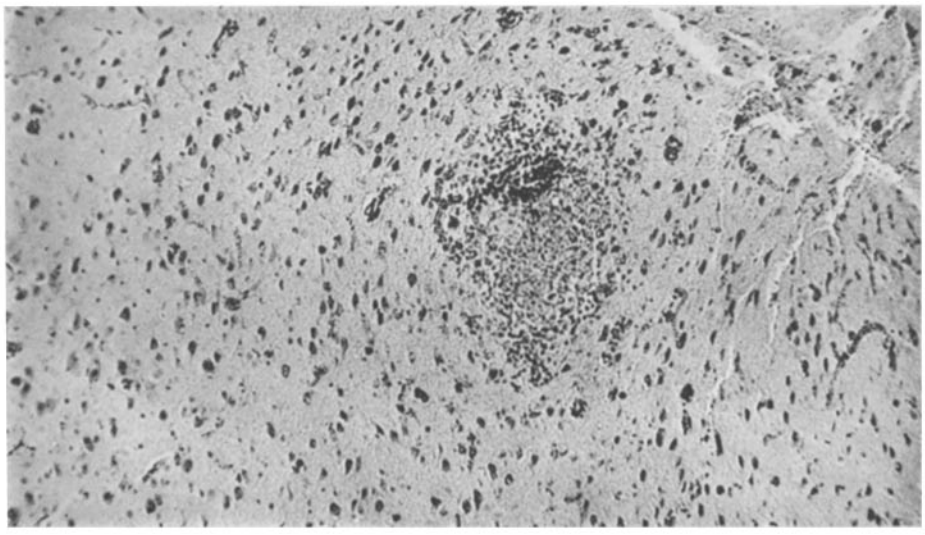

FIG. 17.

(Bull: Streptococci from Poliomyelitis.)