# EXPERIMENTAL EPIDEMIC POLIOMYELITIS IN MONKEYS.[1]

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Plates XVIII and XIX.

## INTRODUCTION.

Epidemic poliomyelitis has become, in the past decade, a world-wide disease. The present state of our knowledge of the epidemic spread of poliomyelitis, up to the outbreaks in Europe and America since 1907, is well given in Wickman's[2] monograph. That epidemic poliomyelitis is an infectious disease is clearly pointed out by Medin,[3] although, at an earlier date, Cordier[4] gave it as his belief that it is a contagious disease. The most convincing evidence of the contagiousness of epidemic poliomyelitis is supplied by Wickman's[5] studies of several Swedish epidemics.

Up to the present time there has existed no convincing knowledge of the nature of the agent causing epidemic poliomyelitis. Various bacteria and especially certain cocci[6] have from time to time been isolated in cultures from fluids obtained by lumbar puncture from patients suffering from epidemic poliomyelitis, or from specimens of the central nervous system removed at autopsy. These bacteria did not conform to one species or group of microörganisms and did not suffice to set up poliomyelitis in animals. They can be accounted for more satisfactorily as contaminations or secondarily invading bacteria than as the cause of the disease.

[1] Received for publication January 3, 1910.

[2] Wickman, Beiträge zur Kenntniss der Heine-Medinschen Krankheit, Berlin, 1907.

[3] Medin, Verhand. des x Internat. Med. Congresses, Berlin, 1890, ii, 37.

[4] Cordier, cited by Medin, *Lyon médical*, 1888, lvii, 5, 48.

[5] Wickman, *op. cit.*

[6] Geirsvold, *Norsk Magazin f. Laegevid*, 1905, iii, 1280 (cited by Harbitz and Scheel).

Since 1907, poliomyelitis has prevailed, during the summer months, as an epidemic in some parts of the United States. In 1907, the disease spread widely in the Eastern States;[7] in 1908, it appeared in the middle Western States, and in 1909, it attacked a large number of people in the Northwest (Minnesota, etc.) and reappeared in the East.[8] During this period, a similar epidemic appeared in Austria and Germany and by 1909 it had invaded France.

Our studies are limited to the two epidemics which affected New York and vicinity in 1907 and 1909. In 1907, one of us (Flexner) studied in various ways a number of fluids, obtained by lumbar puncture, from cases in different stages of the disease. The cellular nature, proteid strength, bacteriological content, and infectivity were investigated. The lymphocytes were increased in number, the proteid was not markedly increased, and aerobic and anaerobic cultivations were, as a rule, unsuccessful. Rarely, bacteria were present in the fluid; but the bacteria were not of uniform species. The fluids, obtained by lumbar puncture, were injected into laboratory animals, including monkeys, but without setting up any recognizable pathological condition. During the epidemic of 1907, we did not secure organs from a case of pure infantile paralysis and we failed, therefore, in our intention to inoculate monkeys from the spinal cord.[9]

In September, 1909, we secured the spinal cord from two cases of infantile paralysis in human beings. For these valuable specimens we are indebted to Dr. Ridner, of Lake Hopatcong, N. J., and Dr. Le Grand Kerr, of Brooklyn, N. Y. Dr. Ridner's patient died on the fifth or sixth day following the appearance of the paralysis, which affected the legs. The lumbar cord was obtained in a sterile condition, twenty-six hours after death, and employed for inoculation of animals. Dr. Kerr's patient had been widely paralyzed and died on the fourth day of the disease. The lesions in the cord were wide-spread and severe and affected gray matter and white. The entire spinal cord was obtained twelve hours after death and inoculated into animals four hours later.

[7] Committee Reports, *Jour. of Nerv. and Ment. Dis.*, 1909, xxxvi, 619.

[8] Kerr, *New York State Jour. of Med.*, 1909, ix, 484.

[9] Wollstein, *Jour. of Exper. Med.*, 1908, x, 476.

EXPERIMENTAL POLIOMYELITIS.

In order to favor the transmission of the disease to monkeys, we chose the brain as the site of inoculation, which was made under ether anesthesia, through a small trephine opening. After the operations, the animals were at once lively and normal. The injected material consisted, first, of emulsions in salt solution of the spinal cord from the children and, later, of emulsions and filtrates from the spinal cord and other organs from the monkeys developing paralysis.

Before proceeding to a detailed account of our experiments and results, it is desirable to review two publications, which immediately preceded ours, dealing with the experimental production of poliomyelitis. The first and more important is the report of Landsteiner and Popper,[10] which appeared in the summer of 1909, and which marks the beginning of the new era in the study of epidemic poliomyelitis.

The material employed for inoculation of two monkeys consisted of the emulsified spinal cord in salt solution obtained from a child nine years old, who died on the fourth day of attack from infantile paralysis. The emulsion was injected into the peritoneal cavity of the monkeys. One of the latter became severely sick on the sixth day and died on the eighth day after inoculation. The other monkey became paralyzed on the seventeenth day and was killed on the nineteenth day after inoculation. The spinal cord of the first monkey was not used for further inoculation, while that from the second monkey was used to inoculate, probably by intraperitoneal injection, two other monkeys that did not, however, develop the symptoms of the disease. Apparently they were unaffected by the injection.

The anatomical examination of the first monkey showed normal internal organs, except the spinal cord which presented severe lesions. The microscopical examination of the spinal cord gave the following result: the pia was infiltrated with small, round, deeply-staining cells and the infiltration extended chiefly along the median anterior fissure, and less along the median posterior fissure into the cord and medulla, and into the brain. The substance of the cord showed throughout areas of inflammation, which were most marked at the cervical enlargement. These areas appeared either in the form of heavy perivascular infiltrations or as more diffuse infiltrations in the substance of the cord. An edema was also present. The diffuse infiltrations occurred chiefly in the anterior gray matter, and to a less extent in the posterior gray matter. Hemorrhages were also present in the gray matter. Similar lesions, but of less intensity, were found in the region of the medulla, pons and brain stem. The ganglion cells of the anterior horns especially showed severe degeneration and were often surrounded by, or actually invaded with, mononuclear and polynuclear cells.

[10] Landsteiner and Popper, *Zeit. f. Immunitätsforsch., Orig.,* 1909, ii, 377.

The anatomical findings of the second monkey were similar to, but less intense than, those in the first. The lesions were most pronounced in the lumbar region, while the thoracic and cervical portions were little affected. The microscopical appearances were similar to those of the first monkey. Hemorrhages were, however, absent and the infiltration of the substance of the cord showed fewer polynuclear cells.

Owing to their inability to transmit the disease from one monkey to another, Landsteiner and Popper discuss the three following possible explanations of the failure, namely: (1) whether the disease in the first monkey was caused by a transferred poison or by the infectious agent; (2) whether a successful transfer from monkey to monkey might not have been secured had the cord of the monkey, dying on the sixth day of the disease, been used; and (3) whether the infectious agent may not have become attenuated in virulence in its passage through the monkey and thus have lost its power of further transmission. They inclined to the last explanation.

At the time our studies of 1909 were begun, we were familiar with the foregoing publication; but before our first report[11] appeared in print, a second account of a successful transfer of poliomyelitis to a monkey was published from Vienna, whence the first appeared, by Knoepfelmacher.[12]

On December 22, 1908, 14 hours post-mortem, the spinal cord was removed from a child of one year, who had died on the sixth day of the disease, and 10 centimeters of the cervical portion, after emulsification in salt solution, were injected into the peritoneal cavity of a *Macacus rhesus*. The first evidence of sickness was noted on the eighth day, and the first evidence of paralysis on the twelfth day, after the inoculation. On the fourteenth day the animal was killed with chloroform and 10 centimeters of the spinal cord were rubbed up in salt solution and the emulsion was injected into a second monkey (presumably by way of the peritoneum) which was unaffected. The anatomical and histological findings agreed with those described by Landsteiner and Popper.

### CLINICAL FEATURES.

We have observed with care the monkeys inoculated successfully with the virus of epidemic poliomyelitis and have classified them into groups according to the symptoms recognized. The descrip-

[11] Flexner and Lewis, *Jour. of the American Med. Assn.,* 1909, liii, 1639.

[12] Knoepfelmacher, *Med. Klin.,* 1909, v, 1671. Strauss and Huntoon (*New York Med. Jour.,* 1910, xci, 64) have since reported a successful intraperitoneal inoculation of a monkey and failure to transmit the disease further.

tion to be given is based upon a study of 81 monkeys which became infected with the virus.

*Incubation Period.*—The incubation period has been taken to be the interval elapsing between the time of inoculation and the appearance of the first definite paralysis. During this interval certain other clinical manifestations of illness may be noted and these will be considered separately as prodromal symptoms. The latter besides being inconstant call frequently for the exercise of personal judgment for their detection and hence they can be defined less accurately than the paralysis. In several instances the monkeys fell ill and died without paralysis having occurred or been noted. These animals were included among the series of successful inoculation only when the typical lesions were present in the nervous system. The incubation period in them was calculated from the inoculation to the onset of definite symptoms of illness.

The shortest period thus far noted as elapsing between the inoculation and the onset of paralysis has been 4 days and the longest period, 33 days. The average period has been 9.82 days. The number of animals developing paralysis after the twelfth day was 16, and the number developing paralysis before the eighth day was 18. Hence the extremes of the incubation period are probably simple variations from a mean which represents the activity of the virus for monkeys. We possess some evidence that the virus may suffer, in certain strains, an enduring alteration through which its activity is diminished. When the site chosen for inoculation is some other than the brain, or the material used some other than an emulsion of the spinal cord, the incubation period has not usually been at either extreme period.

*Prodromal Signs.*—The inoculation of the virus into the brain or other parts produces no immediate effects. As soon as the effects of the anesthetic disappear, the monkeys appear normal. This condition persists until a period of from six to forty-eight hours before the onset of paralysis when certain abnormal signs may be noted. The animals become nervous and excitable; on being disturbed and made to move about the cages, they tire quickly; a tremor of the head, face or limbs develops; when the attention can be attracted the gaze is shifting, rather than fixed as in the normal monkey, and

the face is wrinkled and mobile rather than smooth and placid; the hairs are erected somewhat, and the animals prefer to remain quiet. All these symptoms are almost never noted in a given animal and they occur in varying combinations. The temperature does not rise constantly during the incubation period and gastro-intestinal symptoms rarely occur. A few animals have shown diarrhœa but this condition may well have been a coincidence.

*Onset of Paralysis.*—Either with or without the premonitory signs, the paralysis develops suddenly. In general it may be stated that any of the larger groups of voluntary muscles may be first involved. The table shows the frequency with which the chief types of paralysis developed in our series at the onset.

TABLE I.

*Frequency of Chief Paralyses at Onset.*

| Location of Paralysis. | No. of Animals Affected. | Total. |
|---|---|---|
| Both legs | 20 | |
| Right leg | 8 | |
| Left leg | 12 | 40 |
| Both arms | 3 | |
| Right arm[13] | 9 | |
| Left arm | 9 | 21 |
| Four limbs | 10 | 10 |
| Right leg and left arm | 1 | 1 |
| Neck[14] | 1 | 1 |
| Bulbar or cerebral[15] | 8 | 8 |
| Total | 81 | 81 |

Concomitantly with the appearance of paralysis of any large muscle group, other muscle groups are found either weak or partially paralyzed. The exceptions to this rule are present among the cases of slight affection. The onset of symptoms in the bulbar or cerebral group has not been the same in any two cases,[16] and death

[13] One animal showing paralysis of right arm was chiefly a case of bulbar affection.

[14] In six instances the back or neck was involved as soon as any limb.

[15] The eye muscles were paralyzed once in a case of cerebral affection. Facial paralysis has occasionally been seen but never at the first manifestation up to the present time.

[16] Illustrative protocols are given at the end of the article.

has occurred before the developments of flaccid paralysis. The striking features of the cases have been spasticity of the limbs, marked incoördination resulting in violent pseudo-convulsions; epileptiform convulsions with tonic and clonic muscle-spasms; and, finally, sudden death of apoplectiform type. Death has occurred within thirty minutes of the first appearance of cerebral symptoms.

*Usual Type of Paralysis.*—The rule is for the paralysis to extend quite rapidly to other muscle groups. In mild examples of the affection, the extension may be slight or pass unnoticed. In examples of moderate severity, the extension soon ceases. In the usual examples, which are severer than these, the paralysis extends, with greater or less celerity, from one limb to another, to the back and the neck, until death supervenes. The paralysis, when developed, is of the flaccid variety. The severely affected animal lies recumbent, the respiration being feeble and shallow and the temperature subnormal. When the condition is one of moderate severity, the progress of paralysis has reached its limit in about twenty-four hours, after which it remains stationary or recedes, or death occurs. In the severer conditions, all the muscles, possibly excepting those of the neck, are completely paralyzed in twenty-four, or at most forty-eight hours. The progress of the paralysis is usually, if not always, outlined in advance, by weakness of the muscles next to be affected. There is no rule observed of ascent or descent, but the extension occurs in both directions. The period elapsing between the onset of paralysis and death ranges from one to six days.

In several instances, the weakness of certain muscle groups, accompanying the paralysis of others, has been replaced by a spastic state producing contractions of the limbs or retraction of the head. This condition usually has not been intense, since the muscles would be relaxed when the animal attempted to use them.

The investigation of the sensory disturbances has been far less satisfactory. Both what appeared to be areas of anesthesia in some animals, and hyperesthesia in others, were noted. The paralyzed limbs are usually colder than the non-paralyzed, and the circulation is sluggish in them. One monkey, which became affected, gave evidence of pain in the feet for forty-eight hours preceding the onset of the paralysis. When the paralysis was developed, the pain had disappeared.

The mental condition of the animals remains unaffected even in the severe forms of the disease. The temperature, in severe cases, is definitely subnormal.

*Termination.*—This division of our subject cannot be discussed with the completeness of the other sections. Since the main object of our study required that we maintain an active virus, we have been obliged to sacrifice a considerable number of the affected animals within twenty-four to forty-eight hours of the onset of the paralysis.

The affected monkeys may recover. When this happens, the paralysis reaches a maximum, becomes stationary, and then recedes more or less. When the affection has been severe, the animals appear sick for several days, but when recovery commences the general symptoms of sickness quickly disappear. The muscles which were weak, but not definitely paralyzed, regain strength. Hence, the actual paralyses are more sharply demarcated. Although the variations are considerable, it happens that within a week of a severe or critical state the animal has regained health and general strength, except for the actually paralyzed muscles. In other instances, two or three weeks have not sufficed for the restoration of strength to muscles apparently intact. In some instances in which the paralysis affecting a single limb appeared to be complete, it has entirely disappeared within a few weeks.

When paralysis has been marked it usually persists. There has not yet been sufficient time to show what the end result of these cases will be; but present indications, based on several animals paralyzed from three to ten weeks, are that further recovery will be very slow and incomplete. Within a month or so, the paralyzed limb tends to stiffen and to contract, the kind of contracture depending somewhat upon the position assumed by the paralyzed limb in the meantime.

The animals may die. When the first force of the affection is exerted upon the medulla, death may occur within a short time of the first appearance of symptoms. When the limbs are first affected, the progress and extension of the paralysis may be very rapid and death be caused quickly through involvement of the medulla. Again, the progress may be slow and the prostrated

animal may gradually grow weak and die after one or several days. When the period of disease is prolonged, then other factors, such as secondary infections and general nutritive and trophic disturbances, must be considered.

The accompanying table exhibits the mortality in the cases considered in this paper. It requires a few words of explanation, since, while a part of the recoveries and deaths noted are facts, another part are surmises. Each set is clearly indicated. The necessity for this division has been explained already and was caused by the need of maintaining an active virus for inoculating other animals. We are of the opinion that we have put the number of probable recoveries rather too high, but this was done to avoid over-statement of the probable fatality of experimental poliomyelitis in monkeys. When the probable outcome of certain sacrificed animals could not be predicted, the animals were excluded from the tabulation.

TABLE II.

*Showing Probable Mortality of Experimental Poliomyelitis.*

| Termination of Disease. | No. of Animals. | Total. |
|---|---|---|
| Actual death | 23 | |
| Probable death | 21 | 44 |
| Actual recovery | 9 | |
| Probable recovery | 7 | 16 |
| Uncertain | 21 | 21 |
| Total | 81 | 81 |

From the figures given it follows that in 54.3 per cent. of the monkeys, in this series, which developed poliomyelitis, the issue would in all probability have been fatal. Hence, the experimental disease is more highly fatal than is the spontaneous disease in human beings.

PATHOLOGICAL ANATOMY.

The spinal cord and its membranes show, in fatal cases, visible lesions of varying intensity. The pia mater is usually congested, the hyperemia being most apparent at the lumbar and cervical enlargements. An edema is not perceptible and the cerebrospinal fluid is not appreciably increased. The consistence of the cord is rarely altered except in autopsies made some hours after death, when it is

softer than normal. The latter condition usually is of post-mortem development and autolytic in nature. Definite lesions of the white matter were not made out, while it is common to find the gray matter altered. The chief lesions consist of an edema (excessive moisture of surface), diffuse vivid injection of the blood-vessels and punctiform or larger (pin-head) hemorrhages. The hemorrhages are disclosed less well by transverse than by median incision. In the latter preparations, in which the gray matter is exposed, the hemorrhages are seen to follow the course of small vessels. The lesions are not diffuse or uniform but they tend to be most marked in those regions of the spinal cord corresponding with the paralyzed groups of muscles. And yet, it would be wrong to suppose that the lesions were strictly limited to these regions, for, as a matter of fact, they are much more widely distributed.

The macroscopic lesions are far less regularly present in the monkeys killed soon after paralysis has set in. In some of this class of animals, no lesions were visible; but the greater number show lesions not unlike those present in the animals dying spontaneously.

The lesions of the medulla, when present, resemble those of the spinal cord. The congestion is, however, more apparent than the hemorrhages, although the latter, of small size, have been seen.

Gross lesions of the brain have been observed rarely. In a few instances, hemorrhages along the needle-track have been present, but they were of traumatic origin and are not to be regarded as part of the pathological anatomy of the experimental disease. In one or two instances, small cysts with clear contents were found at the inoculation-site long after the operation. They gave rise to no symptoms. The substance of the brain has been excessively moist in some instances; and in a few cases yellowish dots, not clearly distinguishable, have been noted which, on microscopic study, proved to be areas of necrosis. There occurred, a few times, small subdural hemorrhages, present on one or both sides.

The appearance of the cord is somewhat different at a later stage, when healing has set in. Then the lesions, when easily visible, are firmer, paler, more sharply circumscribed, and raised somewhat above the surrounding gray and white matter. No

instance has yet been examined in which the lesion was more than a couple of weeks old, so that the ultimate appearance of the scar cannot be described.

To resume, therefore, the lesions in the spinal cord and medulla of the monkeys, visible to the naked eye, consist of congestion and hemorrhage into the gray matter chiefly, but not exclusively confined to the anterior horns. The general appearances of the spinal cord, medulla and brain are not greatly altered, and the visible effects are no proper measure of the damage inflicted by the virus.

### PATHOLOGICAL HISTOLOGY.

The general descriptions of the lesions revealed by the microscope will be discussed in respect to the spinal cord and brain and as they relate to the membranes, white and gray matter, and to the intervertebral ganglia.

*Spinal Cord.*—The lesions are more severe and widespread in the spinal cord than in the brain. In point of severity the lesions are most pronounced in the gray matter and membranes and least marked in the white matter. As a rule, no part of the spinal cord, including the medulla, is entirely free from lesions; but in respect to severity there is considerable difference at various levels. As could be predicted, the severest lesions tend to occur at the levels corresponding to the most marked paralysis of muscle groups; but there is room for discrepancy here since the paralysis of certain muscle-groups (leg or arm) is more apparent than of others (back or neck). In this way, we would account for the severe lesions, sometimes present in the thoracic cord although the effects of the lesions were not very apparent.

*The meninges* usually showed a more or less diffuse infiltration with round cells. The layer immediately next to the white matter of the cord tended to show more cells than the layer next to the dura mater. The greatest accumulations of the cells were about the blood-vessels (arteries and veins), the sheaths of which were surrounded by a thick collar of the cells. The muscular coats and the intima remained intact, although the lumina were often encroached upon by reason of compression of the vessels. When the vessels were small the effects on the lumina and hence upon the permeability were considerable.

The infiltration of the adventitial and perivascular lymph sheaths and adjacent pial membrane continued as the membranes and contained vessels entered the fissures and extended into the substance of the cord; and often the degree of infiltration was greater in the intramedullary parts of the membranes and vessels (Plate XVIII, Fig. 1). The height of the infiltration, now of the lymph sheath, was reached in the gray cornua.

In the early stages of the paralysis all the infiltrating cells were round, showed dark, nearly solid nuclei and scant protoplasm, and resembled lymphocytes. Later on, when the reparative process had set in, the lymphoid type of cell was replaced by spindle cells, possessing processes resembling fibroblasts. The affected vessels, moreover, now showed still greater diminution of caliber and excess of spindle nuclei in the muscular coat.

Nothing remained in the membranes to indicate either the quality or the quantity of the cerebrospinal fluid; but specimens removed by lumbar puncture at different periods taught that at the onset of paralysis the fluid was sometimes under slightly increased pressure and sometimes under very low pressure, was faintly opalescent, contained an excess of proteid and was spontaneously coagulable, and further contained a large excess of lymphocytes. This condition is of brief duration and is succeeded by one in which the fluid is clear and non-coagulable, the proteid not increased,[17] only the lymphocytes being more numerous than normally. In course of the healing process the lymphoid cells of the pia-arachnoid are also substituted by spindle cells.

The meningeal cellular invasion is always interstitial and does not give rise to an exudate upon the surface of the cord or brain such as occurs in acute exudative inflammations. There is, indeed, a notable absence of polymorphonuclear leucocytes. On the other hand, the cellular infiltration does extend along the extramedullary nerve roots, affecting their pial investment and penetrating into the connective tissue between the nerve fibers. The impression is gained that the posterior roots are infiltrated to a greater degree and more frequently than the anterior roots. The change from round to

[17] This determination is made with Noguchi's butyric acid test. *Jour. of Exper. Med.,* 1909, xi, 604.

spindle cells, in course of reparation, also takes place in the nerve roots.

*The white matter* of the cord is not spared, but in point of frequency and severity of affection it occupies an inferior position. The lesions observed were the following: edema; perivascular cellular infiltration which was sometimes of a high degree and penetrated beyond the lymph sheath into the nervous tissue; hemorrhages; degeneration or necrosis of nervous tissue, associated with cellular invasion and fragmentation of nuclei or hemorrhage; and focal accumulations of lymphoid cells, independent of vessels or necrosis. The lesions in the white matter were always focal and occurred indifferently in the various columns. Often no connection was discovered with lesions of the gray matter and sometimes a continuous cellular infiltration, with or without degeneration, extended from gray into white matter. The lesions tended to occur near by rather than at a distance from the gray cornua.

*The gray matter* was invariably affected. The lesions involved the anterior and posterior horns and the commissure, and they affected the blood and lymphatic vessels, the ground substance and nerve cells.

The anterior horns were, as a rule, more severely and widely injured than the posterior horns. The vessels and arteries, especially in the anterior gray matter, showed a high degree of cellular infiltration of the perivascular spaces together with edema of the spaces, which often spread them widely apart and destroyed the fibrillar meshwork, and hemorrhage into the spaces. From the vessels, the cells often passed into the ground substance. But independent foci of small cells, edema or hemorrhage also existed in the nervous tissue. The nerve cells often showed degenerations which consisted of hyaline transformation and necrosis, leading to loss of tigroid substance, cell-processes, nuclei, etc. Sometimes merely a contracted nucleolus remained stainable. Often the cell was surrounded by lymphocytes or invaded with polynuclear leucocytes. It happened that the nerve cells had disappeared and leucocytes occupied their places. Not all the nerve cells, in a segment, would show equal degeneration, and in rare instances only was the entire width of the anterior horn degenerated. Ultimately, a

part or all the nervous elements would be removed and replaced by an indefinite cellular tissue, containing many compound granular corpuscles (Plate XIX, Fig. 3).

The commissures, Clarke's columns and posterior horns and the blood vessels entering them, showed similar if less profound lesions.

The infiltration of the perivascular sheaths of the vessels was continuous with that of the pia-arachnoid and especially the pial extensions into the spinal cord. The number of polymorphonuclear leucocytes in different specimens varied considerably, but they rarely if ever were as numerous as the lymphocytic cells.

The lesions in the spinal cord were more abundant and of severest grade in the lumbar and cervical enlargements.

*The medulla oblongata* exhibited lesions similar to those of the cord and affecting the membranes and substance. The blood vessels, nuclei and ground matter were all involved. The foci were, as a rule, smaller than those in the spinal cord.

*The brain* showed lesions which were, however, more sparsely scattered than in the cord. The meninges showed infiltrations extending between the sulci and along the vascular sheaths into the brain tissue. Focal necrosis of cells, lymphocytic emigration, and hemorrhages occurred in the cortex and white matter, in the basal ganglia and in Ammon's horn. The ventricles showed no lesions.

When the virus was confined mechanically to a given part of the brain, the vascular infiltration was intensified. This occurred in connection with the inoculation of the glycerinated virus into the brain substance. When degeneration and necrosis of cells were produced by the injection of the virus into the brain (as rarely happened) the perivascular infiltration was intensified, possibly because of a freer development of the virus in the necrotic tissue.

The blood-vessels and arteries, especially, *in the subcutaneous tissue,* at the site of the nodule caused by a subcutaneous inoculation, showed infiltration with round and spindle cells similar to those present in partly healed lesions in the spinal cord. Other parts of the subcutis were quite free from infiltration.

*The intervertebral ganglia* are regularly involved in the process. The involvement consists of a diffuse and nodular infiltration with

lymphocytic cells which collect between the nerve cells and about the nerve fibers, and degeneration and necrosis of single ganglion cells. These cells either become completely necrotic and hyaline and appear pink and homogeneous and devoid of nuclei, or they show contracted nuclei and altered eosin staining with loss of tigroid markings. The degenerated cells become invaded with or replaced by leucocytes and lymphocytes (Plate XVIII, Fig. *2*).

### AVENUES AND SOURCES OF INFECTION.

The intracerebral mode of infection is not the only successful one. It has been determined that the virus of epidemic poliomyelitis when introduced into the body by means of the blood, subcutis, peritoneum, spinal canal and large nerves, tends to localize in the spinal cord and brain and set up the specific lesions, in the same manner as when injected into the brain. It remains to be determined whether these several routes give uniformly as good results as the intracerebral route. Our impression is that infection is readily accomplished by way of the subcutis and less readily by way of the peritoneal cavity. We have made several unsuccessful attempts to produce infection through intratracheal inoculation and by feeding. But the number of experiments will have to be considerably greater before a final conclusion can be ventured on these points. On the other hand, it has been shown that the cerebrospinal fluid, at the beginning of the paralysis, is capable when injected into the brain, of setting up paralysis in other animals. Hence at this period, at least, this fluid contains the virus.

The blood contains the virus at the beginning of the infection, but how richly has not been accurately determined. As little as two cubic centimeters may fail to cause infection while as much as twenty has caused typical paralysis. After a successful subcutaneous inoculation with an emulsion of the spinal cord, the regional lymphatic glands (axillary and inguinal) and the subcutaneous nodule resulting proved infectious; and the paralysis developed earlier from the gland than the nodule.

The naso-pharyngeal muscosa also contains the virus. The excised membrane, including the tonsils and turbinate mucosa, on

being rubbed up with quartz sand, macerated and filtered, yielded a fluid which, on being injected into the brain, caused paralysis. Thus far infection has not been secured with the spleen, bone-marrow, liver or retro-vertebral glands.

## NATURE AND RESISTANCE OF THE VIRUS.

Film preparations and sections prepared from the spinal cord of human beings and monkeys examined, prepared and stained in the most various ways, showed no parasites, either bacterial or protozoal, that could account for the infection. The readiness with which epidemic poliomyelitis could be transmitted to monkeys and the absence of visible and stainable parasites, early suggested the possibility that the infectious agent belonged to the minute filterable viruses. To determine this point, the spinal cords, removed from monkeys just paralyzed, were triturated with sterile quartz sand, thoroughly shaken, and pressed through a Berkefeld filter which had previously been tested and found bacteria-tight. These clear and bacteriologically clear filtrates have been used repeatedly to inoculate monkeys, both by the intracerebral and subcutaneous routes, and have regularly caused paralysis. From these paralyzed animals, the virus has been transferred to other monkeys, so that it can be asserted that the effects it produces are caused not by a filterable toxic substance but by a true virus or living microörganism.

It was because of the fact of the filterability of the virus that we succeeded in detecting the presence of the organism in the muscosa of the mouth and nose.

The clear fluids obtained by filtration when examined under the dark field microscope show innumerable bright, dancing points devoid of definite size and form and not truly motile. This fluid prepared and stained by means of Loeffler's flagella stain shows minute particles of roundish or oval form which were absent from a similar filtrate prepared with the nervous system of a rabbit. That the particles represent the microörganism of poliomyelitis cannot be affirmed at present.

The filterable viruses of other diseases are resistant to injurious agencies. In common with some of them, we have ascertained

that the virus of poliomyelitis, as contained in the comminuted spinal cord, withstands glycerination for at least seven days and resists drying over caustic potash in a desiccator for the same period.

Moreover, it has been found that the virus retains its virulence apparently unimpaired on being kept constantly frozen at $-2°$ to $-4°$ C. in the Frigo apparatus for a period of at least forty days, and also when kept for at least fifty days at a temperature of about $+4°$ C., in course of which time the latter specimen of spinal cord became slowly softened through autolysis and overgrown superficially with mould.

The virus appears to be readily injured by heating. Using the filtrate to avoid the effects of coagulation of the proteid, we have found that a temperature of $45°$ to $50°$ C., maintained for half an hour, sufficed to render the filtrate incapable of causing paralysis when injected into the brain.

The parasite of epidemic poliomyelitis is, therefore, very minute and cannot, for the moment, be further classified, since the precise position among living things held by the filterable viruses has not been determined. At least one of these viruses, that of pleuropneumonia of cattle, has been cultivated artificially. An attempt has been made to cultivate the virus of poliomyelitis in test tubes.

The result has seemed to be successful, but it is still in doubt. What has thus far been determined is that the filtrates when added to bouillon prepared with human blood serum or ascitic fluid become clouded and retain their virulence for four days in the thermostat. The transfer of a fraction of a cubic centimeter of such turbid fluids to a second set of tubes may cause a turbidity to develop in them. The staining of these turbid fluids with Loeffler's flagella stain brings out the oval or roundish particles in numbers. But thus far we have not induced paralysis by inoculating the culture fluid of the second or third transfer of the original virus.

## IMMUNITY.

Experiments have been and are still being conducted to determine the kinds and degrees of immunity which are produced by the inoculations of the virus. Since the literature on epidemic

poliomyelitis is silent on this subject it can be inferred that a second attack of the disease is rarely if ever suffered by one individual. Two possible reasons can be assigned for this: the first and most probable is that one attack of this disease, as with other acute general infections, tends to afford an enduring immunity; and the other, that as epidemics occur infrequently and reappear after long intervals, the children once affected have passed beyond the susceptible age period at the time of the next epidemic.

We have attempted, and up to the present unsuccessfully, to reinfect monkeys which had recovered or were recovering from the disease. Thus far ten monkeys have been subjected to reinoculation, into the brain, at periods varying from eight days to two months after the first paralysis appeared. The larger number of reinoculations were made at the end of the first month following the paralysis.

In point of severity the first attack varied between mere tremor of the head, a partial paralysis of one limb and complete paralysis of legs and arms. The paralysis had in some instances nearly or completely disappeared and in others it had become reduced but still affected all the muscles of one or two limbs. In no instance did the second inoculation produce a frank renewal of the disease or appear to retard the progress toward recovery. Two animals seemed somewhat sick a few days after the second injection and soon recovered; it is not at all certain that the indisposition was the effect of the injection. The virus employed for reinoculation always produced infection in control animals.

There should be contrasted with this result the number of failures occurring after intracerebral inoculation in normal monkeys. Of eighty-three such animals, inoculated with emulsions of the spinal cord, all but six developed paralysis. Of the six failures, two animals are suspected of having suffered a mild attack unaccompanied by definite paralysis. Hence it can be concluded that in the period immediately following an attack of poliomyelitis and for a time thereafter, the monkeys are refractory to reinoculation with active virus. How enduring the immunity may be is still to be determined.

Of the six animals which failed to become paralyzed, three were

reinoculated. Two remained well and the third developed a severe paralysis. The last animal was reinoculated three weeks after the first injection, which period, we now know, is shorter than the longest limit of the incubation period, so that the result cannot be interpreted accurately. However, it appears that rare individual monkeys are highly refractory to infection.

Efforts have been and are still being made to induce active immunity through the employment of gradually increasing doses of active virus or of a virus modified by heat and other physical and various chemical agents. A certain degree of success has been achieved already and a greater success may be looked for. These results are reserved for a later report.

Experimental poliomyelitis develops, as a rule, quickly and after an incubation period of only a few days. It is not probable, therefore, that protective inoculation can be accomplished, by way of the subcutis, that shall prevent the onset of paralysis after an intracerebral inoculation. We have made an unsuccessful effort to achieve such protection. Emulsions of active spinal cord, warmed to 55° to 57° C. for one hour or to 60° C. for one half hour were injected beneath the skin at the same time that a usual intracerebral injection of virus was given. The two monkeys employed in the experiment developed paralysis in the usual manner.

### VARIATION IN VIRULENCE OF THE VIRUS.

There is at present no reliable way of estimating the degree of activity of the virus since the number of organisms inoculated is not subject to control. The inoculated materials consisted of heavy suspensions in salt solution of the spinal cord, for preparing which portions from several levels were employed, or filtrates obtained from the suspensions, of which from two to four cubic centimeters were injected. Each of the two viruses has thus been passed through fifteen generations, in the course of which transfers it was observed that with each virus there occurred a sharp decrease of virulence at one point, namely, at the second generation of virus K. and the fourth generation of virus M. A. The change was indicated by feeble effects during several passages and final loss of power to transmit the infection. The strains of

virus, however, in parallel inoculations, retained their activity undiminished and serve, at the present time, to transmit the infection regularly and, we are inclined to believe, even more constantly than at first. Moreover, virus K. has, in the last generations, tended to produce the paralysis on the sixth or seventh day and virus M. A. on the eighth or ninth day.

## MODES OF SPONTANEOUS INFECTION.

We have been successful in transmitting the virus through several avenues to the central nervous system of monkeys. The important question that arises relates to the avenue through which spontaneous infection in man takes place. In considering this phase of the subject, we have paid attention to the path of elimination of the virus into external nature, since such elimination must occur in order to maintain the virus alive and transmissible, and because of the possibility that the path of elimination may also be the portal of infection. In designing experiments to cover this point, we have had in mind the relation proven to exist between the infiltrations of the meninges and the meningeal vessels and the lesions in the cord and brain, and the fact that infection can be induced by intralumbar inoculation of the virus and the cerebrospinal fluid of paralyzed monkeys contains active virus. We also consider that the effectiveness of the intracerebral injections is in some way bound up with successful infection of the meninges. The brain matter, at the site of inoculation, does not show a marked specific reaction to the immediate presence of the virus.

The most direct connection existing between the meninges and the external world is by way of the lymphatics of the nasal and pharyngeal mucosa, through the cribriform plate. On account of its filterability, through which circumstance all bacteria can be eliminated, it has been possible to determine that the virus escapes from the meninges into the naso-pharyngeal mucosa. There still remains to be accomplished infection by the reverse route. The difficulty that presents itself in respect to this experiment is a common one in determining paths of infection in relatively non-susceptible species that shall reproduce those occurring naturally in man. The contributing factors, which act to promote spon-

taneous infection, are at present unknown. The observation on the infectivity of the naso-pharyngeal mucosa does not actually establish it as the site of infection in man or exclude the possibility of other modes of elimination of and infection with the virus.

## VARIETIES OF ANIMALS SUSCEPTIBLE.

We have employed only the lower species of monkeys for our experiments. The greater number were *Macacus rhesus,* but all other species of old world monkeys that we used seemed equally susceptible. They included *M. cynomolgus, M. nemestrinus, Cercocebus fulignosus, Cercopithecus callitrichus* and *Papio babuin.* We also employed two species of new world monkeys, one belonging to the genus *Cebus* and including *Capuchinus.* The larger ring tail proved susceptible and the smaller did not, although five individuals of the latter were inoculated. The question whether the catarrhine are more uniformly susceptible than the platyrrhine species is an interesting one.

In this connection brief reference should be made to other species of animals employed for inoculation. Besides many guinea-pigs and rabbits, one horse, two calves, three goats, three pigs, three sheep, six rats, six mice, six dogs, and four cats have had active virus introduced in the brain but without causing any appreciable effect whatever. These animals have been under observation for many weeks. It remains to be discovered whether some of these species of animals may not after all be made to develop poliomyelitis by using directly spinal cord from many human cases, on the supposition that a virus of greater virulence may be found. We inoculated rabbits and guinea-pigs directly with each of the two specimens of human virus, and additional rabbits and guinea-pigs and the other animals with virus derived from monkeys.

## DISCUSSION.

The studies[18] which we have made and presented here bring epidemic poliomyelitis among the well-defined infectious diseases and serve to explain some of its obscure features.

[18] Preliminary notes of these studies have been published in the *Journal of the American Medical Association* as follows: *Jour. of the American Med. Assn.,* 1909, liii, 1639; 1909, liii, 1913; 1909, liii, 2095; 1910, liv, 45; 1910, liv, 535.

It has now been shown that the cause of the disease is a minute organism that readily passes through Berkefeld filters and is demonstrated with difficulty under the microscope. Since no example of this class of pathogenic organisms is known to maintain a saprophytic existence, it can be assumed, for the present, that this virus does not lead a prolonged existence apart from its host.

The virus becomes implanted upon the leptomeninges, especially in the region of the spinal cord and medulla, where it sets up cellular infiltrative changes that are most marked in the perivascular lymph spaces of the arteries entering the nervous tissues. The vascular lesions constitute the primary causes of the lesions of the nervous system, the severity of which is determined by the particular vessels affected and the intensity of the involvement. The infiltrative lesions are confined to the perivascular lymph sheath and adventitia, but still other lesions must occur in the intima of the vessels from which the edema and hemorrhage arise.

The central arteries, entering the anterior median fissure and supplying the anterior gray matter of the cord, invariably show the infiltration and other changes described, through which the preponderance of affection of the anterior horns is accounted for (Plate XVIII, Fig. 1). Since the arteries supplying the posterior gray matter are less important, the lesions in the posterior cornua are slighter in spite of rich infiltration of the posterior spinal vessels. The gray commissure and Clarke's columns are affected to the extent to which their blood vessels are involved in the infiltration.

No part of the spinal cord is, as a rule, spared and the medulla probably never entirely escapes injury. But the degree of affection is determined by the richness of the arterial blood supply, whence is explained the liability of the lumbar and cervical enlargements to severe lesions. The irregularity in the branching of the central arteries probably explains the common variations observed in respect to affection of the two lateral halves of the body.[19]

The brain is far less commonly the seat of lesions but it is not

[19] Harbitz and Scheel, Path. Anat. Untersuchen über Akute Poliomyelitis und verwandte Krankheiten, Christiania, 1907, p. 194.

spared. Paralysis of the cranial nerves and especially of the facial nerve (Plate XIX, Fig. 4) follows upon them, but lesions also occur in parts of the brain which do not respond by paralysis. The brain injuries, like those of the cord, depend upon vascular lesions. Herpes was never observed in the monkeys, although the intervertebral ganglia were frequently infiltrated. The condition has been observed rarely and lesions have been described in the spinal ganglia in human beings.[20]

There are good grounds for believing that a considerable part of the paralyses, especially those that are not permanent, are the effects of temporary vascular impediments. The impediments are all outside the lumina of the vessels which are merely reduced in caliber through pressure; thrombi do not occur. Some of the functional disturbances are, possibly, thus anemic in origin, others are probably caused by slight degenerations and still others are undoubtedly caused by focal hemorrhages and edema. All these effects may, possibly, be recovered from: part by resolution of the cellular vascular infiltrate and reëstablishment of the lumen, part by absorption of edema and hemorrhage; and part by restoration of the mildly degenerated nerve cells. The severer degenerative and other lesions, through which actual necrosis is produced, do not become restored.

The lesions of the nervous tissue are highly variable in respect to the degree of degeneration, but remarkably uniform in respect to the character of the changes. Both the ground substance and the nerve cells in the gray matter are the seat of alterations: in the one there are edema, hemorrhage and cellular, lymphocytic chiefly, infiltrations; and in the other necrosis with disintegration of the nerve processes and cells. Polymorphonuclear leucocytes serve to disintegrate these and other degenerated cells, but play, on the whole, a very subordinate part. The white matter shows similar infiltrative and degenerative changes. Nuclear fragments occur in both places but not numerously. When the lesions are older, large numbers of vacuolated compound granular corpuscles and proliferated cells are present, and the proper elements of the nervous matter have disappeared.

[20] Harbitz and Scheel, *op. cit.,* p. 207.

A well-defined incubation period precedes the onset of the paralysis, but once the monkeys show symptoms of illness, the paralysis tends to appear abruptly. The prodromal symptoms and the concomitant symptoms are slight as compared with the spontaneous disease in man. The average incubation period in the monkey is about nine days, which is somewhat greater than that estimated for human beings. Wickman[21] believes that in human beings the incubation period ranges from one to four days, but he records examples in which it appeared to be as long as twenty-two and twenty-seven days.

The incubation period has been worked out on the supposition that the spontaneous disease in man is contagious. We observed no instance among our monkeys of a spontaneous transfer of the infection. However, we made no purposive experiments to test this point, and yet the inoculated and uninoculated animals were not kept carefully separate.

We have shown that the naso-pharyngeal mucosa contains the virus and yields a filtrate capable of setting up poliomyelitis. It can be assumed that the monkey is relatively insusceptible and hence difficult to infect through the natural channels, a fact already established for other infectious diseases. Spontaneous infection would, therefore, not be expected and more especially as monkeys rarely cough. Were the virus eliminated with the excreta, which has not yet been shown to occur, and were infection readily accomplished by way of the gastro-intestinal tract,[22] examples of spontaneous infection would conceivably occur more readily because of the usual soiling of the hands and food of the animals.

All the definite clinical types of poliomyelitis described in human beings have been observed in monkeys. Just what the significance of the abortive type so-called is may be ascertainable more readily in the monkeys than in man. The end results appear also to be identical: recovery may be complete or partial or death may result. Experimental poliomyelitis in the monkey is a severe and highly fatal disease and exceeds in the latter respect the spontaneous disease in man.

[21] Wickman, *op. cit.*

[22] Harbitz and Scheel, *op. cit.*

The literature on epidemic poliomyelitis in man is silent on the subject of reinfection and it does not appear that an attack of the disease has been noted to afford immunity from subsequent attack. We have made a number of attempts to reinfect, by intracerebral inoculation, animals which have recovered partially or completely from the first infection, but thus far without success. Hence it appears that immunity is afforded by an attack of epidemic poliomyelitis, with which observation we are inclined to believe experience with the spontaneous disease in man will be found to agree.

When our studies were begun, the only publication on experimental poliomyelitis in monkeys was that of Landsteiner and Popper.[23] Since our first results were published, the reports of other successful experiments have appeared. Thus Leiner and Wiesner,[24] Landsteiner and Levaditi,[25] and Römer[26] have employed successfully the intracerebral mode of inoculation of the virus. We shall not set down here a full transcription of their results but merely mention those which are of special interest in conjunction with our own. Thus Landsteiner and Levaditi independently ascertained that the virus can be filtered, and that the salivary glands are sometimes infectious; and Leiner and Wiesner found that the virus when injected into the small intestine, peristalsis being prevented by means of opium, or when introduced into the stomach by means of a catheter, sufficed to cause paralysis.

CONCLUSION.

The experimental study of poliomyelitis has yielded a large number of important facts relating to the spontaneous disease in man. The nature of the virus has been discovered, many of its properties have been ascertained, some of its immunity effects have been established, the clinical and pathological peculiarities of the disease have been elucidated, and a basis has been secured on which to develop measures of prevention.

[23] Landsteiner and Popper, *op. cit.*

[24] Leiner and Wiesner, *Wiener. klin. Woch.,* 1909, xxii, 1698; 1910, xxiii, 91.

[25] Landsteiner and Levaditi, *Compt. rend. Soc. de biol.,* 1909, lxvii, 592; 1910, lxvii, 787.

[26] Römer, *Münchener med. Woch.,* 1909, lxi, 2505.

ILLUSTRATIVE PROTOCOLS.

M. A. VIRUS.

*Protocol A. Sudden onset, rapid progress and fatal termination of paralysis.* —*Macacus rhesus* inoculated intracerebrally,[27] October 21, 1909, with emulsion of cerebral cortex. Well until 8 a. m., October 30, when it was noted that the animal got to his perch with some difficulty. The disability was rather indefinite. By 11 a. m. the four limbs were spastic; at 12 m. he could no longer get to the perch and a tremor of the body and nystagmus had developed. Attempts to move brought on marked incoördinated muscular seizures. 9 p. m. the condition appeared unaltered, but the handling required to take the temperature (which was 37° C.) induced collapse. Edema of the lungs set in and death occurred at 10 p. m.

*Protocol B. Sudden onset and rapid progress of paralysis from subcutaneous inoculation.*—*Macacus rhesus* inoculated with suspension of medulla December 20, 1909. The medulla had been kept for four days at room temperature. January 2, 1910, 8 a. m., paralysis of the neck, arms and legs present. The paralysis is not complete and the animal can stand, but unsteadily. 5 p. m. flaccid paralysis complete. January 3, animal weaker. Etherized.

*Protocol C. Paralysis of cranial nerves, neck and limbs. Descending progress.*—*Macacus rhesus.* November 18, 1909, inoculated intracerebrally. Until November 29, no symptoms. Then a tremor of the head appeared and the face and mouth were drawn to one side (facial palsy). The arms appeared weak, especially the right arm. November 30, the neck muscles are completely, the arms partly, paralyzed and the animal cannot stand upright. Etherized.

*Protocol D. Paralysis of legs, weakness of arms, sudden onset and death.*— *Macacus cynomolgus.* Intracerebral inoculation, October 28. Well but perhaps quieter than usual, November 5. November 6, 11 a. m., complete paralysis of both legs and weakness of both arms; sick. November 7, arms very weak but not paralyzed; animal very sick. Died 7.30 a. m., November 8.

*Protocol E. Sudden onset of violent symptoms and death.*—Baboon (*Papio babuin*). Inoculated intracerebrally with spinal cord November 16, 1909. No symptoms of illness until December 7, 9 a. m., when it was noted that the muscular movements were weak; no definite paralysis. About 2 p. m., the animal suddenly fell from the perch after which he was unable to stand. The sensorium remained clear, but attempts to move brought out marked involuntary muscular incoördinative movements. Once started, these movements were not readily controlled, apparently, voluntarily. Death occurred suddenly at 2.30 p. m.

*Protocol F. Gradual onset of paralysis; partial recovery, resists second inoculation.*—*Macacus rhesus* inoculated intracerebrally with spinal cord October 28, 1909. Well until November 9, when a tremor of body was noticed. Next day, when descending from perch the animal fell and it was then noticed that both legs were weak, the left more than the right. November 11, left leg paralyzed, right weaker. Arms remain strong. Animal only slightly sick. November 20. Left leg completely paralyzed, right weak. November 24, right leg becoming stronger. November 30. The right leg has recovered much of its strength and is

[27] All inoculations were made under ether anesthesia.

used in climbing; left leg limp. December 14, left leg has recovered so far that the residue of paralysis is noticeable only when the animal moves about quickly. Right leg normal. On reinoculation intracerebrally, no effect was produced.

*Protocol G. Partial recovery; death from pneumonia.*—Green monkey (*Arcopithecus callitrichus*). November 30, 1909, inoculated intracerebrally with spinal cord. Well until 8 a. m., December 10, when complete paralysis of legs was present. The paralysis did not extend, but pneumonia set in and death occurred, December 22. The lumbar enlargement of the cord showed large gray foci in the anterior horn.

*Protocol H. Inoculation with blood.*—*Macacus cynomolgus.* October 28, 1909, injected 2 c.c. citrated blood from paralyzed monkey intracerebrally. No effects followed. December 14, injected intravenously 25 c.c. defibrinated blood from monkey at onset of paralysis. December 31, paralysis of back, neck and left arm appeared. Etherized.

*Protocol I. Failure of first followed by successful second inoculation.*—*Macacus cynomolgus.* December 24, 1909, subcutaneous inoculation with spinal cord. No effect. January 3, 1910, repeated subcutaneous inoculation. 9 a. m., January 6, animal appeared well. 12.15 extensive paralysis present; cannot stand. Died at 12.30 p. m.

## VIRUS K.

*Protocol J. Intraperitoneal inoculation of human cord.*—*Macacus nemestrinus.* Inoculated October 22, 1909. Well until October 30, when paralysis of left arm and right leg appeared. Paralysis rapidly increased hour by hour and involved both legs. Etherized.

*Protocol K. Successful intraperitoneal inoculation of monkey's cord.*—*Macacus nemestrinus.* Inoculated October 30, 1909, with 2 c.c. cord suspension from Monkey J. November 8, animal somewhat sick; weak in legs and arms. November 9, right leg and left arm completely paralyzed. Animal very sick. Cannot raise head. Etherized. Typical lesions in cord.

*Protocol L. Successful subcutaneous inoculation.*—*Macacus rhesus.* November 24, 1909, inoculated subcutaneously suspension of monkey's spinal cord. December 3, 9 a. m., animal appeared well and strong. 5 p. m. legs weak. December 4, 8 a. m. complete paralysis of both legs; weakness of both arms. Etherized.

*Protocol M. Successful intraneural inoculation.*—*Macacus rhesus.* November 14, 1909, injected 5 c.c. suspension spinal cord into sheath of left sciatic nerve. Next day no effect. November 21, well. November 23, 8 a.m., left leg completely paralyzed; right leg weak. 5 p. m., right leg paralyzed. November 24, arms very weak.

*Protocol N. Successful intravenous inoculation.*— Bonnet monkey. November 10, 1909, injected 2 c.c. suspension of spinal cord intravenously. November 18, 9 a.m., sick. Paralysis left leg; weakness right leg. 5 p.m. both legs almost completely paralyzed. November 19, arms weak. November 20, arms and right leg stronger. No change in left leg. November 29, recovery complete except for paralysis of left leg. Etherized.

*Protocol O. Inoculation of filtrate.*[28]—*Macacus rhesus.* December 1, 1909,

[28] The filtrates employed for inoculation were prepared from the original human spinal cord and from the cords of monkeys which were paralyzed. The filtrate is active also by way of the subcutaneous tissues.

intracerebral inoculation of filtrate from monkey's cord pressed through Berke-feld filter. December 5, 5 p. m., tremor of head and weakness of arms. December 9, 9 a. m., left arm paralyzed; 11 a. m., both legs completely and right arm partially paralyzed; neck muscles weak. Etherized. With the spinal cord obtained from this animal another monkey was infected.

*Protocol P. Inoculation of glycerinated cord.—Macacus cynomolgus.* An emulsion of monkey's cord kept in pure glycerine for 7 days was, after washing away the glycerine with salt solution, injected intracerebrally on November 16, 1909. November 24, animal quiet; next day legs weak. November 26, both legs paralyzed; arms weak. Etherized.

*Protocol Q. Inoculation of frozen human cord.—Macacus rhesus.* The cord was kept in the Frigo apparatus at — 2° to — 4° C. for 40 days and inoculated intracerebrally on December 1, 1909. December 8, animal not well. December 9, legs completely and arms almost completely paralyzed. Next day the arms and neck were completely paralyzed. Etherized.

*Protocol R. Inoculation of human cord kept just above freezing temperature. —Macacus rhesus.* The cord had been kept at about + 4° C. for 50 days, during which time it has softened and mould had overgrown its surface. Portions of the interior were inoculated intracerebrally on December 10, 1909. On December 23, the animal appeared sick and on the twenty-fourth, both legs were completely, and the left arm partly, paralyzed. Both arms and the neck finally became paralyzed. Death occurred on December 29.

*Protocol S. Inoculation of cord dried over KOH.—Macacus rhesus.* The cord had been suspended in a desiccator over KOH for 7 days. December 11, 1909, a subcutaneous inoculation was made. December 21, animal sick; left arm and right leg incompletely paralyzed. Next day paralysis of legs, arms and neck complete. Death at 11 a. m.

*Protocol T. Inoculation into spinal canal.—Macacus rhesus.* A suspension of the cord was injected into the spinal canal on January 18, 1910. Animal well until January 26 when both legs were first incompletely, then completely paralyzed. Etherized.

*Protocol U. Inoculation with cerebrospinal fluid.—Macacus rhesus.* This fluid, drawn by lumbar puncture at the onset of paralysis, was injected intracerebrally on January 23, 1910. Animal well until February 1, when paralysis, at first partial and later complete, of the legs and arms occurred. Etherized.

*Protocol V. Inoculation with filtrate prepared from the naso-pharyngeal mucosa.—Macacus rhesus.* The mucosa of the nose and the pharynx, including the tonsils, was dissected, rubbed up with quartz sand, macerated in salt solution, and pressed through a Berkefeld filter. The filtrate was injected intracerebrally on January 23, 1910. On January 31, the animal was sick and showed tremor of head and arms. The next day the legs and back were weak. February 2, the legs were paralyzed and the arms were weak. February 5, the arms had regained strength but the legs were still completely paralyzed.

*Protocol W. Recovery of strength but persistence of paralysis.—Macacus rhesus.* November 26, 1909, intracerebral injection of spinal cord from monkey succumbing to glycerinated virus. December 6, 9 a. m., left leg partially paralyzed. 6 p. m., both legs completely paralyzed. Next day arms weak and
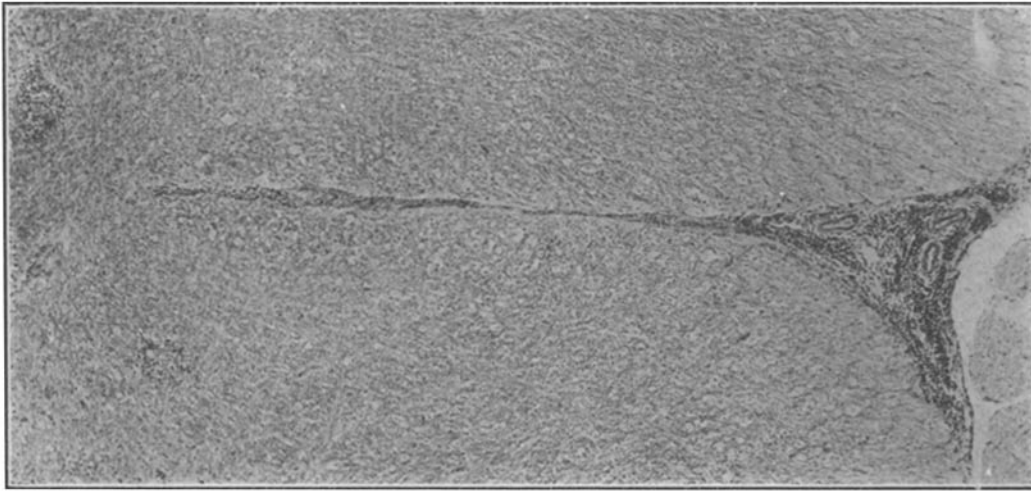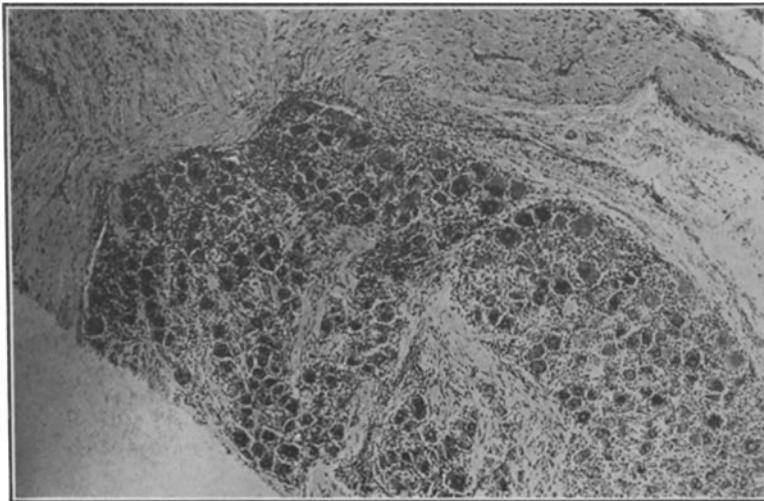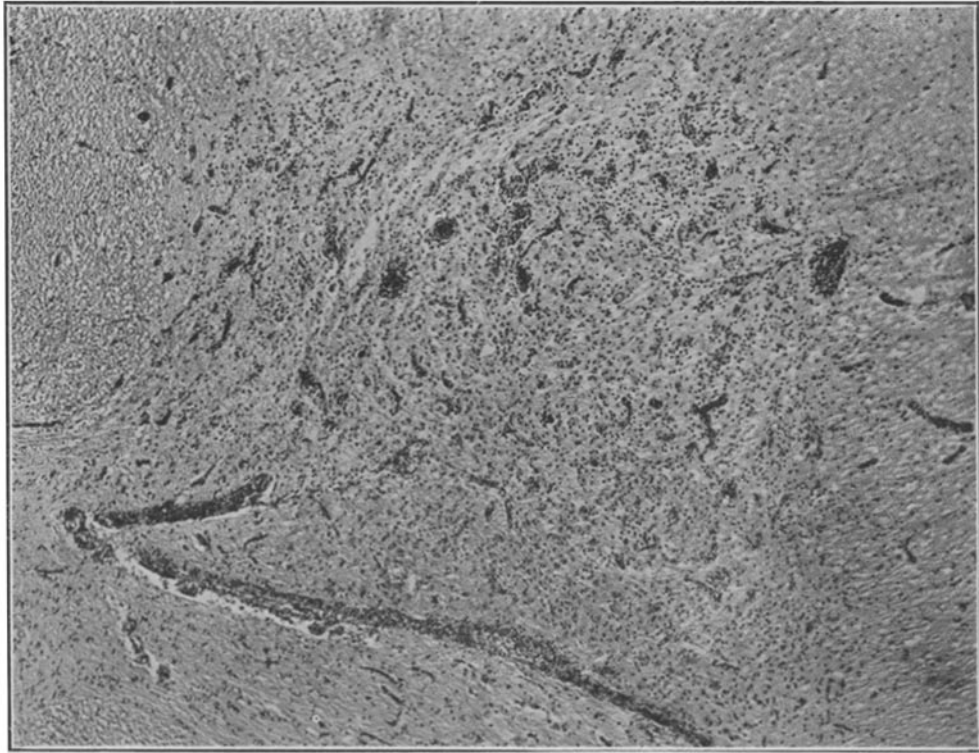
Fig. 1.



Fig. 2.

FIG. 3.



FIG. 4.

partially paralyzed. The animal was quite sick. Gradually the general health returned; the arms partly recovered strength; the body muscles remained strong; but the legs remained paralyzed and showed contractures.

## EXPLANATION OF PLATES.

### PLATE XVIII.

FIG. 1. Low power, showing pial and perivascular cellular infiltration.

FIG. 2. Moderate magnification. Showing diffuse and nodular round cell infiltration of intervertebral ganglion and adjacent nerve roots.

### PLATE XIX.

FIG. 3. Medium magnification. Showing entire anterior horn degenerated and infiltrated with round cells; and extensive perivascular infiltration.

FIG. 4. Facial paralysis and deflection of the tongue.