A DISSERTATION

ON

"THE ANATOMICAL DISTRIBUTION AND CHARACTER OF THE LESIONS OF THE CENTRAL NERVOUS SYSTEM IN POLIOMYELITIS, WITH SPECIAL REFERENCE TO THE TYPE OF CELL AFFECTED AND TO THE PORTAL OF ENTRY OF THE VIRUS".

by Charles Swan. M.B., B.S. (Adelaide).

TOGETHER WITH

THE FOLLOWING UNPUBLISHED PAPERS

ENTITLED,

A."STUDIES IN EXPERIMENTAL POLIOMYELITIS":

1."THE EFFECT OF THE INOCULATION OF POLIOMYELITIS VIRUS INTO DENERVATED AREAS OF SKIN, AND THE MODE OF PROPAGATION OF THE VIRUS FROM SUCH AREAS TO THE CENTRAL NERVOUS SYSTEM";

2."THE ALLEGED DEVELOPMENT OF IMMUNITY FOLLOWING INTRANASAL INOCULATION OF POLIOMYELITIS VIRUS INTO MONKEYS SUBJECTED TO PRELIMINARY BILATERAL SECTION OF THE OLFACTORY TRACTS";

3."THE EFFECT OF FARADIC STIMULATION ON THE LOCALISATION OF PARALYSIS INDUCED BY THE INTRANASAL INOCULATION OF POLIOMYELITIS VIRUS".

B."THE METHOD OF TRANSMISSION OF THE VIRUS OF INFECTIOUS MYXOMATOSIS OF RABBITS TO THE CENTRAL NERVOUS SYSTEM".

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THE ANATOMICAL DISTRIBUTION and CHARACTER of the LESIONS of the CENTRAL NERVOUS SYSTEM in POLIOMYELITIS, with SPECIAL REFERENCE to the TYPE of CELL AFFECTED and to the PORTAL of ENTRY of the VIRUS.

By Charles Swan, M.B., B.S., (Adelaide).

"The war against disease in all its forms presents a stirring challenge and maintains a keen and never-failing interest. One of its satisfactions is that the battle line never retreats and, while progress may prove slow, each gain is held and forms the base for further advance. The more difficult the objective, the greater is the incentive for attack. Infantile paralysis, and the possibility of its prevention and cure, holds a peculiar appeal, especially to those who love children. Other similar problems, no less difficult, have in the end yielded to persistent and devoted research and the solution has been found."

Jeremiah Milbank.

A. INTRODUCTION.

In recent years no acute infectious disease has been studied with greater intensity than poliomyelitis. The facility with which the disease can be reproduced in monkeys has resulted in many valuable contributions to its pathology, yet despite them, a considerable degree of confusion still exists in regard to the portal of entry of the virus, its method of spread within the organism, and the reason for its predilection for the motor neurones of the anterior horns.

The thesis submitted is based on a pathological examination of eight cases of poliomyelitis, undertaken in the hope of throwing

light on the relative susceptibility of different cells in the central nervous system and on the portal of entry of the virus.

It is intended to trace the development of the pathology and pathogenesis of poliomyelitis up to the present time; to submit the results of the research undertaken; and finally, to indicate wherein it is considered the thesis advances medical knowledge.

B. THE DEVELOPMENT of the MODERN CONCEPTION of the PATHOLOGY OF POLIOMYELITIS.

1. HISTORICAL REVIEW.

Heine to Charcot.

One hundred years ago when Heine (1840) published his first monograph, he not only placed the disease on a sound clinical basis, but he also submitted a theory concerning its pathological anatomy, and thus paved the way for all subsequent research. Heine from a logical analysis of the signs and symptoms of the disease postulated a lesion, "of the cord, of an irritative and congestive sort," the congestion leading to an exudate which caused the paralysis. He called attention to the fact that "the gradual reduction of the paralysis, both in extent and in intensity, gives ground for the belief that it is due to the gradual re-absorption of exudate around nervous elements, whereby the latter are partially relieved from pressure." In support of his hypothesis he pointed to the grey matter of the spinal cord as being "the chief channel of motor activity," and with prophetic insight went on to say," ----- knowing that the gray matter is so well supplied with blood vessels that

accidental plethora may easily cause extravasations, while the gray matter is exceedingly liable to suffer from variations in its nutriment, knowing that the sections show atrophy of the gray matter to be the cause of the paralysis, what is to hinder us from deducing the cause of the diminution of motility from the same factors? Why should we not suppose some acute process to take place, which causes an alteration in the gray matter, impairs its conductivity, produces the paralysis in the lower limbs, and, by affecting the nutrition of the gray matter, causes secondary atrophy in it?"

It was a matter of regret to Heine that through lack of post-mortem material of his own he was unable to confirm his assumption. There seems little doubt that had it been available he would have made the discovery credited to Charcot and Joffroy (1870) thirty years later. Nevertheless he remained convinced that the seat of the pathological process was in the spinal cord, as is shown in his reply to Rilliet and Barthez (1861), who opposed his views on the ground of negative macroscopic findings at autopsy. Heine wrote, "If such people, who are only too prone to give the name 'essential infantile paralysis' to our disease, because it avoids the difficulty of specifying the nature of the illness, bring to me sections which show no lesions of the nerve centres, I would advise them to use the microscope; if this also fails to reveal a lesion I would suggest that, if at the present time changes are not evident to our eye, it is the latter which is at fault."

Among the autopsies cited by Heine were those performed by Longet and by Hutin (1827). In the former autopsy there was marked atrophy of

the anterior roots and the corresponding lumbo-sacral nerves, but no lesions were detected in the substance of the spinal cord. In the latter autopsy there was an extraordinary atrophy of the spinal cord. These must be looked upon as the first positive findings at post-mortem, even though they were based only on macroscopic examination.

In 1863 Cornil published the first report of a microscopic examination of the spinal cord of a case of poliomyelitis (a woman, dying of cancer at the age of 49, who had contracted infantile paralysis 47 years before). He found that there was well-marked atrophy of the anterior and lateral columns especially in the thoraco-lumbar region, together with amyloid bodies in the anterior horns, most numerous in the vicinity of the blood vessels. Furthermore he noted the absence of anterior horn cells, but completely failed to realise the significance of this important fact. Thus,"On voit une cellule nerveuse, qui est du reste la seule qui montrait cette préparation; mais sur les coupes plus épaisses nous avons vu que les cellules nerveuses étaient intactes et avaient conserve leurs rapports normaux."

In the following year Laborde (1864) reported two cases in which there was considerable proliferation of connective tissue in the anterior and lateral columns, but in which the anterior horn cells were apparently normal.

Prévost and Vulpian (1865) went distinctly further; they observed atrophy in the anterior and lateral columns and, in addition, distortion and diminution in the number of anterior horn cells in the lumbar region of the spinal cord.

Their report was confirmed by Clarke (1868).

Charcot to Rissler.

It was left to Charcot and Joffroy (1870) to realise the fundamental significance of the loss of the ganglion cells, and their work must therefore be regarded as laying the foundation of the micropathology of the disease. Their observations were based on the examination of the spinal cord of a woman aged 40, who had suffered from an attack of poliomyelitis at the age of seven years. It was noted that the lesions, especially in the cervical and lumbar enlargements, were more severe on the left side of the cord than on the right, and that loss of motor power and atrophy of the muscles were also more pronounced on the left side. Because of the correlation between the localisation of the changes in the spinal cord and the distribution of the paralyses, Charcot and Joffroy concluded that the degeneration of the anterior horn cells resulted in trophic changes in the muscles. Moreover, "in view of the absence of any true inflammatory or haemorrhagic changes they inferred a primary affection of the ganglion cells with the production of a certain degree of secondary reaction in the neighbouring interstitial tissues."

The conclusions of these authors were supported by Parrot and Joffroy (1870), who examined the spinal cord of a child who died one year after the onset of the disease. They found vascular congestion and perivascular infiltration in regions where the motor cells of the anterior horns appeared normal, while in regions where these cells were affected, there was no apparent change in the blood vessels. They therefore agreed with

Charcot and Joffroy that the pathological process was primarily parenchymatous.

The contrary view was suggested for the first time by Roger and Damaschino (1871), who examined three less chronic cases, dying 2½, 6 and 13 months after the onset of the disease. On account of the marked vascular changes and cell proliferation, especially in the anterior horns they raised the question as to whether the change might not be primarily interstitial.

During this period, it was becoming increasingly evident to clinicians that the disease was not confined to children, but that occasionally adults were affected also. The first to confirm their observations pathologically was Gombault (1873). Examination of the nervous system of a woman aged 67, who had suddenly developed a flaccid paralysis of the extremities at the age of 60, revealed atrophy of the anterior horn cells and the corresponding motor roots, together with degeneration of nerve cells in the hypoglossal nucleus. This was the first report of a bulbar lesion in poliomyelitis.

Confirmation of Gombault's results came with the reports of Petitfils (1873), Erb (1875), Leyden (1875) and Schultze (1878).

In 1873 Roth reported the results of the pathological examination of a child of two years, who died of diphtheria 11 months after an attack of poliomyelitis. He noted atrophy of nerve fibres and loss of ganglion cells in the lumbar region of the spinal cord. In addition there was connective tissue hypertrophy and granular cells were present in the

adventitia of the vessels. Roth concluded that the pathological process was primarily interstitial.

Eisenlohr (1878) contributed the first account of the pathological changes in the acute stage. His paper was also of importance because its summary of earlier work emphasized the confusion existing at that time between Landry's paralysis and the ascending form of poliomyelitis. Thus Leyden and Westphal believed that Landry's paralysis was due to an isolated lesion in the medulla oblongata, while van de Velde believed that this lesion was associated with inflammatory foci in the spinal cord. On the other hand Déjerine and Gotz maintained that the central nervous system in Landry's paralysis was free from lesions, and that cases such as Kiever's (reported in Chalvet's thesis, 1871) were cases of central myelitis and not of Landry's paralysis. Eisenlohr's case was that of a 42 year old bricklayer who died 10 days after the onset of the disease with paralysis of all four extremities and symptoms of involvement of the brain-stem. Microscopic examination revealed congestion of the pial vessels and exudation and extravasation in the spinal cord and brain-stem. In the thoracic region of the spinal cord the ganglion cells were swollen, while in the brain-stem there were minute haemorrhages and collections of round cells. Eisenlohr concluded that further work was necessary before negative pathological findings could be assumed for the central nervous system in Landry's paralysis.

A clearer description of the acute stage was given by Drummond (1885). His case was that of a five year old girl who died from respiratory

paralysis 6-7 hours after the onset of the disease. In the cervical region of the spinal cord vascular congestion was marked and the ganglion cells were granular, swollen and ill-defined. In most of the ganglion cells the nucleus had disappeared, and the cell processes were no longer visible. In addition there were minute haemorrhages and leucocytic infiltration. Roth, Money (1883), and Putnam (1883) had drawn attention earlier to the fact that the pathological changes were not confined exclusively to the anterior horns, but that the posterior horns and white matter were involved as well. Drummond confirmed their findings and pointed out, like Putnam, that the term "anterior poliomyelitis", introduced by Kussmaul, was therefore not appropriate.

During this period the question was raised as to whether the pathological process was primarily parenchymatous as Charcot contended, or primarily interstitial as had been suggested by Roger and Damaschino. From this discussion three main views developed.

Déjerine (1878), Stadelmann (1883), Rissler (1888), von Kahlden (1893, 1894), Leegard (1889), Gowers (1892), Mönckeberg (1903) and Bruining (1904) supported Charcot's theory. In one case Rissler found that the nerve cells showed granular clouding of the cytoplasm or complete chromatolysis, while the vessels were only at an early stage of exudation. In other cases where the interstitial tissues were strongly affected, the degree of degeneration of the ganglion cells was extremely variable. He concluded, therefore, that the ganglion cells were primarily involved by the causal agent, and that the interstitial tissues, including the blood vessels, were secondarily affected either by the same agent or by the

products of degeneration of the ganglion cells. Von Kahlden (1893) pointed out that there was disparity between the degeneration of anterior horn cells and the myelinated nerve fibres passing through the region of the anterior horns, and argued that if the pathological process were interstitial the effect on the ganglion cells and nerve fibres should be parallel. In a later paper (1894) he drew attention to the rapid onset of the paralysis and considered that this could not be explained if the process were primarily interstitial. Moreover, he attached overwhelming importance to the disappearance of ganglion cells in groups, and maintained that any lesion affecting these cells secondarily through their blood supply must be diffuse. Mönckeberg thought that the products of degeneration of the damaged parenchyma led to an increased permeability of the walls of the blood vessels and, in addition, to the chemotaxis of leucocytes and adventitial cells.

A second group of investigators including Roth (1873), Leyden (1875, 1880), Schultze (1876, 1878), Eisenlohr (1878), Turner (1879), Taylor (1879), Archambault and Damaschino (1883) and Matthes (1898) upheld the theory of primary involvement of the interstitial tissues originally suggested by Roger and Damaschino. These observers pointed out that perivascular infiltration was frequently present, and considered that the process was a diffuse myelitis which tended to be more intense in particular regions. Schultze noted that often only isolated groups of cells were affected, and thought that this could not be explained if the process were primarily parenchymatous.

A third group of students of the disease including Petitfils (1873), Drummond (1885), Déjerine and Huet (1888), Dauber (1893), Hedlich (1894), Schwalbe (1902) and Schmaus (1905) preferred to leave the question an open one, and to suppose a simultaneous involvement of the parenchymatous and interstitial tissues.

Rissler to Bülow-Hansen and Harbitz.

The first great Swedish epidemic (1887), enabled Medin (1890) to amplify Heine's classical description by the recognition of cerebral, bulbar, ataxic and neuritic types of the disease. Rissler (1888) based his important contribution to the pathology of poliomyelitis on the fatal cases of this epidemic. In all there were five cases, three of which were in the acute stage. Lesions were found not only in the spinal cord, but also in the medulla and pons. There was increased vascularity of the pia mater in one case and of the dura mater in two cases. Throughout the spinal cord and brain-stem vascular congestion was severe and was associated with the exudation of fluid. The perivascular spaces were crammed with leucocytes. Although diapedesis of red blood corpuscles was frequent, actual haemorrhage was rare. The degree of degeneration of ganglion cells was variable, ranging from granular clouding of the cytoplasm to complete disintegration. Rissler was the first to describe the phenomenon known as neuronophagocytosis. Furthermore he was the first to draw attention to the involvement of the lymphatic system in poliomyelitis; in which he found softening of the spleen, and enlargement of the lymph follicles of the intestine and of the mesenteric lymph glands. Rissler's classical description forms the

basis of the modern conception of the pathology of the disease.

Dauber (1893) was the first to describe infiltration of the pia mater. His report was confirmed in the same year by Goldscheider, and later by Wickman and by Harbitz and Scheel.

The possibility of a vascular origin of the disease was suggested first by Petitfils (1873), and later by Money (1883) and Putnam (1883). In 1889 Kawka showed, by means of serial sections, that there was a connection between the localisation of poliomyelitis lesions and the changes in the vessels. In the same year Kadyi published his work on the distribution of blood vessels within the spinal cord, which led Marie (1892) to suggest that the lesions were due to embolism or thrombosis of the central artery. Goldscheider (1893) using Kadyi's technique, came to the conclusion that the localisation of the disease was due to vascular changes, and postulated that the vessels in the central region of the spinal cord had certain peculiarities in their walls and that the conditions of pressure in the adjacent stroma were different from elsewhere. He regarded the degeneration of ganglion cells as secondary, and due to nutritional disturbance. His views were strongly supported by Siemerling (1894), Trevelyan (1895), Matthes (1898), Wing (1899) and Bülow-Hansen and Harbitz (1899).

Bülow-Hansen and Harbitz to Wickman.

The report of Bülow-Hansen and Harbitz (1899) was based on the examination of two brothers, aged four and five years, respectively, who died in the acute stage of the disease. These authors regretted

that the pathological changes in organs other than the nervous system had been largely neglected, for they considered that a more detailed examination might have resulted in clues leading to the portal of entry of the causal agent. In reviewing the findings in eleven acute cases, they noted that changes had been reported in the respiratory system in four cases, and lesions in the gastro-intestinal tract in a similar number. They considered that the changes in the respiratory system, such as bronch--pneumonia, were secondary to paralysis of the respiratory muscles. In the intestine there was swelling and congestion of Peyer's patches, which was sometimes associated with enlargement of the mesenteric lymph glands and softening of the spleen. In view of these findings and of the initial symptoms of gastro-enteritis in their two cases, they suggested that the most probable portal of entry of the causal agent was the alimentary canal. They regarded the respiratory system and oral cavity as other possible atria of infection; the latter on account of Dauber's case, in which there was reddening of the right tonsil and enlargement of the related cervical lymph gland.

Strümpell (1885), Marie (1885), and Medin (1890) maintained that poliomyelitis and polioencephalitis were infectious processes due to the same causal agent and differing only in the localisation of the lesions. In this view they were supported by Möbius (1884) who referred to the case of two sisters who fell ill at the same time with symptoms of gastro-enteritis and with fever. One developed a typical spinal paralysis, the other, cerebral hemiplegia. On account of these observations Bülow-Hansen and Harbitz came to the conclusion that cases

such as their own, with inflammatory foci involving the medulla oblongata, were transitional forms between poliomyelitis and polioencephalitis, In this regard they were influenced particularly by a case reported by Redlich (1894), in which numerous scattered foci were found in the medulla oblongata, the cerebral peduncles, the basal ganglia and adjacent white matter. The cerebral gyri, however, were apparently normal.

Money (1883) and Mott (1899) had each noted thrombosis of the central artery of the spinal cord in one instance. Batten (1904) was able to confirm their findings in one of his three cases and, influenced by the experiments of Prévost and Coutard, considered that the disease was due to a primary thrombosis of the central artery. He suggested that the thrombosis might be brought about by various forms of infection, and that it was more likely to occur in the lumbar region of the spinal cord, owing to the greater distance of its blood supply from the heart. His views were contested by Buzzard (1907) and Wickman (1911). The former criticised the interpretation of the experiments of Prévost and Courtard, in which the injection of sterile particles (fine tobacco seed) into the lumbar arteries of dogs, led to embolic softenings and perivascular infiltration of the white and gray matter of the lumbar cord. As Buzzard in citing the similar experiments of Hoche (1899) pointed out, mere mechanical blockage of the central artery resulted only in infarcts, and it was only when the particles were disseminated through the finer branches and came into intimate contact with the surrounding tissues that perivascular infiltration occurred. Even then the intensity of the infiltration was dependent on the chemical nature of the particles; thus

in Hoche's experiments the infiltration was most marked with kamala glands, less so with Typha japonica, and insignificant with lycopodium granules. Wickman considered that reports of thrombosis in three isolated cases were inadequate to permit of generalisation, and that as the most recent of these cases had already lasted 13 days, the thrombosis might easily have been a secondary phenomenon. Moreover, the areas of necrosis characteristic of embolism were never seen in poliomyelitis, while in diseases such as ulcerative endocarditis in which embolism of the spinal cord has been demonstrated, there was no preferential selection of the gray matter. Finally, in recent cases the most marked changes usually appeared within, but never coincided with, the area of distribution of the central artery, while slighter lesions occurred outside this area.

Wickman and his contemporaries.

The studies of Wickman (1905, 1909, 1911) initiated a new era in the history of poliomyelitis. Römer (1911) regarded his work as "of extreme importance, not only because of its extent...., and its thoroughness and clearness of description, but also because of the expert critical analysis of all the vexed points in the pathology and pathogenesis of the disease. He raised the questions, really for the first time, of the point of entrance, the method of infection and spread of the virus."

Wickman regarded poliomyelitis as an inflammatory process affecting the whole of the central nervous system, including the spinal cord, brain-stem, cerebellum, cerebrum and meninges. He found that although the pathological process was usually most intense in the gray matter

especially in the anterior horns of the cervical and lumbar enlargements, the white matter and pia mater were involved also. Infiltration of the pia mater was usually most apparent in the lumbar and sacral regions, and diminished as it was traced upwards.

The majority of the cells of the infiltrate were lymphocytes, together with a relatively large number of Maximow's polyblasts. In the substance of the spinal cord, changes were most evident in the vessels and interstitial tissues. The veins were affected more than the arteries, but in neither was there evidence of thrombosis. Minute haemorrhages occurred not only in the severely affected areas, but also in regions which otherwise would have been affected only mildly. The haemorrhages were attributed in part to the inflammatory process, and in part to agonal changes following the paralysis of the respiratory muscles. The infiltration mainly followed the distribution of the blood vessels. Changes in the ganglion cells were never found in the absence of interstitial lesions, but the opposite condition sometimes occurred. Lesions in the posterior horns were similar to those in the anterior horns, but usually were less intense. Clinically the oedema was considered to be very important as it afforded a plausible explanation for the rapid disappearance of a paralysis.

Wickman considered that the most probable portal of entry was the gastro-intestinal tract, not only because the paralysis usually began in the lower limbs, but also because of the occasional occurrence of vomiting and diarrhoea in the initial stages of the disease.

Harbitz and Scheel (1907 a) examined autopsy material from 19 cases, 13 of which were in the acute stage. The inflammatory changes were found to be much more extensive than might have been expected from the clinical symptoms, particular stress being laid upon the wide-spread involvement of the gray matter of the brain and brain-stem. In the spinal cord, death of nerve cells was associated with neuronophagocytosis. Despite careful search, lesions in the lymphatic system, similar to those noted by earlier authors, were never observed. The digestive tract was considered to be the most probable portal of entry.

Lesions in the posterior root ganglia were noted for the first time by Forssner and Sjövall (1907), whose findings were confirmed in the human disease by Strauss (1910), and in the experimental disease by Flexner and Lewis (1910) - Forssner and Sjovall also stressed the importance of neuronophagocytosis.

The Transmission of Poliomyelitis to Monkeys.

Although the discovery that poliomyelitis could be transmitted to monkeys was reported first by Landsteiner and Popper in 1908, it was not until the following year that a detailed account of their investigations was published. The material used in their experiments was obtained at the post-mortem on a boy of nine years, who died four days after the onset of the initial symptoms. A considerable quantity of the spinal cord was suspended in sterile saline, and injected intra-peritoneally into two monkeys. In addition two rabbits, two guinea-pigs and two mice were also inoculated, but with negative results. One of the monkeys, a young Cynocephalus hamadryas, became ill six days after the injection

and died on the eighth day, while the other, a small Macacus rhesus, died on the 19th day after the inoculation, two days after paraplegia had first been noted. In both, lesions in the spinal cord similar to those present in the human disease were detected, but they were more intense in the Cynocephalus hamadryas. Landsteiner and Popper considered that the disease in monkeys was brought about by an infectious organism, rather than by a toxin present in the diseased human spinal cord, despite the fact that they were unable to transmit the disease to a second generation. They suggested that as no organisms could be demonstrated by staining or culture, the causal agent was an ultra-microscopic virus.

Knoepfelmacher (1909) also succeeded in producing poliomyelitis in a rhesus monkey, but he had no greater success than the former investigators in his attempts to transmit the disease to a second generation.

However, during the last two months of 1909, a number of authors including Flexner and Lewis (1909 a), Leiner and von Wiesner (1909 a), Landsteiner and Levaditi (1909), Römer (1911), and Landsteiner and Prasek (1909) reported success in serial transmission of the disease to monkeys. Their success was due largely to the use of more effective routes of inoculation, such as the intracerebral. The transmission of the disease to the third generation showed clearly its infectious nature; while the fact that bacteria-free filtrates of emulsions of nervous tissue, obtained with the aid of Berkefeld candles, proved virulent, enabled Landsteiner and Levaditi (1909) and Flexner and

Lewis (1909 b) to confirm the assumption of Landsteiner and Popper that the causal agent was an ultra-microscopic filtrable virus.

(2) RECENT ADVANCES.

The discovery of the monkey as an animal susceptible to poliomyelitis inaugurated the present period of experimental research, during which important investigations have been carried out, not only on the pathological changes in the acute stage, but also on the portal of entry of the virus and its method of spread within the organism.

A. THE PORTAL OF ENTRY OF THE VIRUS.

Logically, before referring to the spread of the virus within the organism, and its effect upon it, attention should be paid to the atrium of infection. It has seemed advisable, however, to postpone discussion of this problem until later in the thesis, when it will be considered in conjuction with the results of the investigation.

B. THE SPREAD OF THE VIRUS WITHIN THE ORGANISM.

(1) THE SPREAD OF THE VIRUS TO THE CENTRAL NERVOUS SYSTEM.

The Vascular Route.

One of the most obvious routes to suggest itself for the conveyance of the virus to the central nervous system, is the blood stream. Although

transmission by this route was favoured strongly by Harbitz and Scheel (1907 a), and later by Draper (1917, 1931), it must be admitted that the available evidence does not lend much support to it.

For instance, the virus has never been demonstrated in human blood, despite the use of large quantities (Leiner and von Wiesner (1909 b), Strauss and Huntoon (1910), Flexner and Clark (1911), Clark, Fraser and Amoss (1914), Flexner (1915), and Amoss (1928)). Hurst (1936) considers that the negative results with human cerebro-spinal fluid and blood should be treated with reserve, in view of the difficulty of establishing human poliomyelitis virus in the monkey.

On the other hand, in monkeys, Flexner and Lewis (1911) were able to demonstrate the virus at the height of the disease, but only when a large volume of blood (25 cc.) was used. Leiner and von Wiesner (1911) and Clark, Fraser and Amoss (1914) could obtain positive results in only 1 out of 5 and 1 out of 10 instances, respectively.

Thompson (1930) endeavoured to ascertain whether the cellular elements of the blood of infected monkeys contained the virus in any greater proportion than whole blood, but his results were negative.

More recently Gordon and Lennette (1939) have attempted to detect the virus in the blood stream, by giving multiple transfusions of blood obtained from infected monkeys, to monkeys whose haemato-encephalic barrier had been damaged by intrathecal or intracerebral injections of starch suspension or horse serum. They were unable, however, to demonstrate virus "....by these means in 14 sample of blood ranging

in volume from 10 cc. to 45 cc., taken from paralysed monkeys, nor in 28 samples, 8 cc. to 30 cc. in volume, from infected monkeys before paralysis appeared."

Attacking the problem from another angle, Flexner and Lewis (1909 c,1910), and Landsteiner and Levaditi (1922) succeeded in producing poliomyelitis by intravascular injection; but as Clark, Fraser and Amoss (1914) pointed out, it was only when overwhelming quantities of an active virus were inoculated into the blood stream, that paralysis resulted. Flexner and Amoss (1914 a, 1914 b, 1917) found that the dosage necessary for infection by the intravascular route, was approximately 1,250 times that for direct nervous routes. Even then the incubation period was much prolonged. Following intravenous inoculation, these authors found that no virus could be detected in the cerebro-spinal fluid after the expiration of 48 hours, and only small amounts at the end of 72 hours. When 96 hours had elapsed, the virus had passed more freely, and was still detectable in the fluid at the onset of the paralysis, 19 days after the inoculation. It was noted, however, that infection was facilitated by the intrathecal injection of serum or saline, the dosage of virus then required being about 50 times that for the direct nervous route. Flexner and Amoss therefore postulated the existence of a mechanism of defence, a so-called "meningeal-choroidal complex", which, when intact, prevented the passage of the virus from the blood to the cerebro-spinal fluid, but which could be rendered more permeable by an aseptic inflammatory reaction.

In monkeys infected by the blood stream, Flexner and Amoss noted extensive vascular involvement, together with infiltrative lesions in

the choroid plexuses, and pointed out that similar lesions had not been reported in human cases. They were therefore of the opinion, that the lesions in human cases of poliomyelitis corresponded with those caused by intraneural, rather than with those following intravenous inoculation.

In this connection, it may be mentioned that Marinesco, Manicatide and State-Draganesco (1929) occasionally noted the occurrence of a mild perivascular infiltration of the choroid plexus of the fourth ventricle in human poliomyelitis.

Pette, Demme and Környey (1932) noted that the histological process after infection by the haematogenous route, differed from that following other modes of infection, in that the mesodermal reaction was more pronounced, the white substance of the spinal cord was affected more strongly by the mesodermo-glial reaction, and certain centres, such as the mamillary bodies, were involved.

The observation of Flexner and Amoss that the posterior root ganglia are capable of removing the virus from the blood stream, after intravenous inoculation, is of interest, in view of the fact that lesions have been found in them in the preparalytic stage of the experimental disease. Moreover, sensory disturbances such as pain and hyperaesthesia, are common initial symptoms.

Lennette and Hudson (1935) found that bilateral section of the olfactory tracts prevented infection by poliomyelitis virus injected intravenously. They explained this observation on the basis that virus administered by this route was excreted on to the nasal mucosa, and was prevented from reaching the central nervous system owing to the interruption

of the olfactory pathway. This result must be accepted with reserve, for the monkeys had been used in a previous experiment in which the virus was instilled intranasally. Although no paralysis had occurred, it cannot be ruled out that some degree of immunisation might possibly have taken place. Furthermore, German and Trask (1938) and Toomey (1939) repeated the experiment in monkeys which had not been submitted to the preliminary administration of virus intranasally, and obtained the opposite result.

In an attempt to explain the mechanism whereby the virus reaches the central nervous system after intravenous inoculation, Flexner (1936) suggested the possibility of some degree of contamination of nerve fibres by the needle in the course of its insertion into a vein.

The Perineural Lymphatic Route.

Wickman (1911) thought that if infection took place by means of the blood stream, it would be,".....difficult to account for the continuity of the changes in the long axis of the cord in fatal cases; for in a vascular condition one would expect the scattered foci of invasion to be separated by normal areas." He considered that it was more probable that the virus reached the central nervous system by travelling along the perineural lymphatics. In this theory he was supported by Leiner and von Wiesner (1910), Römer (1911), Peabody, Draper and Dochez (1912), Flexner and Amoss (1914 b, 1917) and Abramson (1918).

Flexner and Clark (1912) noted that after intranasal inoculation, the virus could be detected in the olfactory bulbs and adjacent parts of the brain before it could be demonstrated in the medulla and spinal cord. They looked upon this as evidence of spread by the direct lymphatic path

and not by the blood stream. Had infection spread by the latter route, they would have expected early localisation in the parts of the spinal cord and medulla which are especially susceptible to the virus.

That the perineural lymphatics accompanying the filaments of the olfactory nerve are in direct communication with the subarachnoid space, has been shown by the experiments of Weed (1914), Le Gros Clark (1928) and Rake (1937), and by the anatomico-pathological investigations of Turner and Reynolds (1926, 1927). Le Gros Clark has postulated the existence of a current running centripetally in these lymphatics.

The position with regard to the perineural and endoneural lymphatics of the peripheral nerves is much less certain. A number of authors including Teale and Embleton (1914, 1919), Yuien (1928), Funoaka and Yamada (1929), Mantell (1932), Horster and Whitman (1932) and Sullivan and Mortensen (1934) believe that there is a definite connection centrally, between the perineural lymphatic spaces and the subarachnoid space. The most interesting work performed in connection with this problem, is that of Yuien. This investigator reported that solutions injected into nerves were conveyed by the perineural spaces to the junction of the anterior and posterior roots. In its further course, the solution passed along the anterior root to the spinal cord, and then along, though apparently not within, the motor fibres to the anterior horn cells. Provided sufficient time was allowed to elapse, the solution passed outwards along the posterior roots, but not along the contralateral anterior root. Yuien concluded, therefore, that in motor nerves the direction of lymph flow is centripetal, while in sensory nerves it is centrifugal.

On the other hand Elman (1923), Iwanow and Romodanowsky (1927 - 28), Abel, Evans, Hampil and Lee (1935) and Hurst (1936) deny the existence of any normal communication between the perineural spaces and the subarachnoid space, and consider that the pressure of injection can lead to rupture of the existing barriers, demonstrated histologically by Elman. Moreover, Hurst (1936) has repeated the experiments of Yuien and has been unable to confirm them.

Further work is necessary before it will be possible to decide between these two views. So far as poliomyelitis is concerned, however, indirect evidence in support of the contention of the latter group of authors, is furnished by the fact that the virus has never been demonstrated in the cerebro-spinal fluid of humans (Flexner and Lewis (1909 a), Flexner and Clark (1911), Leiner and von Wiesner (1909 b), Strauss and Huntoon (1910), Potpeschnigg (1910), Römer (1911), Abramson (1917), Amoss (1922, 1928), Levaditi (1922), Olitsky, Rhoads and Long (1929), Fairbrother and Hurst (1930) and Brodie (1932)), and only exceptionally in the cerebro-spinal fluid in the experimental disease (Olitsky, Rhoads and Long (1929), Fairbrother and Hurst (1930) and Hurst (1930, 1936)).

The Axonic Route.

Recent experimental work has led to the conclusion that the virus of poliomyelitis spreads, not only in the peripheral nervous system, but also in the central nervous system, by travelling along the axis cylinders. This theory will be considered in detail when discussing the spread of the virus within the central nervous system.

(2) THE SPREAD OF THE VIRUS WITHIN THE CENTRAL NERVOUS SYSTEM.

The first theory to be submitted with regard to the spread of the virus within the central nervous system, was that of Wickman (1905, 1911), who believed that the virus was distributed by means of the perivascular "lymphatics". In this view he was supported by Römer (1911), von Wiesner (1911) and Abramson (1918). By the term "lymphatics" these authors apparently meant the spaces of Virchow-Robin.

Harbitz and Scheel (1907 b) found it difficult to accept Wickman's theory, for, as they pointed out, Kadyi (1889) had already shown that long longitudinal "lymph channels" do not exist in the central nervous system. These authors (1907 a) considered that the inflammation commenced in the pia mater, with a probable simultaneous infection of the cerebro-spinal fluid, and that it extended to the cord along the vessels.

The theory postulated originally by Harbitz and Scheel has been strongly supported by Peabody, Draper and Dochez (1912), Flexner and Amoss (1914 a, 1914 b, 1917), Amoss (1928) and Marinesco, Manicatide and State-Draganesco (1929). The last mentioned group of authors believe that there is a second route of transmission of the virus along the vessels of the ependymal cavities. A somewhat similar view has been advocated strongly by Seifried and Spatz (1930), who consider that the viruses of poliomyelitis, Borna disease, epidemic encephalitis and rabies, respectively, are all distributed by means of the cerebro-spinal fluid, and that the localisation of the lesions bears a marked relationship either to the inner (ependymal) or to the outer (pial) surface of the central nervous system.

That experimental infection of the cerebro-spinal fluid with the virus of poliomyelitis leads readily to the disease has been shown by the investigations of Flexner and Lewis (1910), Neustaedtler and Thro (1911), Römer (1911), Clark and Amoss (1914), Flexner and Rhoads (1929) and Hurst (1932). There is little evidence, however, that the cerebro-spinal fluid and the meninges play more than minor roles in the dissemination of the virus under normal conditions. For instance, as has been mentioned already, the virus has rarely been found in the cerebro-spinal fluid. Moreover, recent studies have confirmed the original view of Rissler (1888) that the nerve cells are affected primarily, and that the meningitis occurs secondarily (Landsteiner and Levaditi (1910), Landsteiner, Levaditi and Pastia (1911), Blanton (1917), Howe (1918), André-Thomas and Lhermitte (1929), Hurst (1929), Brodie (1932) and Spielmeyer (1932).

During the last decade, the work of Fairbrother and Hurst (1930), Hurst (1930, 1932), Jungeblut and Spring (1930), Faber and Gebhardt (1933) and others, has resulted in the accumulation of a considerable amount of evidence in support of the theory, that the main route of transmission of the virus of poliomyelitis is the axis cylinder. Fairbrother and Hurst (1930) found that the progressive march of virus and lesions in the nervous system during the incubation period, was similar to that which might have been predicted from knowledge of the neuronal connections of the inoculated area. As an instance, the involvement of the cornu Ammonis following intranasal inoculation,

and its sparing after intracerebral infection, may be cited.

Furthermore, following intracerebral inoculation, a marked predominance of "crossed" initial paralysis was noted. This suggestion of the participation of a decussating mechanism in the spread of the infection had been mentioned previously by Römer (1911), who failed, however, to realise its importance. Similar findings were reported by Pette, Demme and Környey (1932). On the other hand, Brodie (1932) could find "no marked evidence of a crossed paralysis" after infection by the intracerebral route. More precise studies have been made by Howe and Ecke (1937-38). These authors investigated the effect of inoculation of poliomyelitis virus into various cortical areas. Inoculation into the motor area (area 4 of Brodmann) led to "crossed" initial paralysis in 87% of cases. However, when the virus was injected into the pre-motor cortex (area 6 of Brodmann) and the visual cortex (area 17 of Brodmann), "crossed" initial paralysis occurred in 58% and 50% of cases, respectively. In the majority of instances the paralysis resulting from the inoculation of these two areas affected the upper extremities. The results show that within certain limits, it is possible to alter at will the pattern of the paralysis, by varying the point of inoculation of the virus into the central nervous system. Moreover, they offer strong support for the theory of axonic propagation.

Perhaps the most significant series of observations in connection with this theory are those of Hurst (1930). Following inoculation of poliomyelitis virus into the left sciatic nerve, the virus was noted first in the lumbar cord, and soon afterwards in the leg area of the right motor

cortex. At this time no virus could be detected in the cervical cord, the arm area of the right motor cortex or the left motor cortex; but later it was found to be present in the cervical cord, the leg area of the left motor cortex and the arm area of the motor cortex of both sides. It is difficult to explain these findings by any theory except that of spread by axis-cylinders.

Of equal importance is the observation of Jungeblut and Spring (1930) that after transection of the spinal cord in the thoracic region and the subsequent intracerebral inoculation of poliomyelitis virus, neither lesions nor virus could be detected in the lumbar segments, despite the fact that the circulation of the cerebro-spinal fluid was not obstructed.

In attempts to elucidate the mode of transmission of the virus, particular attention has been paid to the sequence of changes following intranasal inoculation. As has been mentioned previously, Flexner and Clark (1912), after infection by the intranasal route, were able to detect virus in the olfactory bulbs and adjoining regions of the cerebrum, before it could be found in the brain-stem or spinal cord. They looked upon these results as confirmatory of spread by the perineural lymphatics. Hurst (1932) has pointed out, however, that if the virus spread by means of the perineural lymphatics into the cerebro-spinal fluid, there is no particular reason why it should localise initially in the olfactory bulbs; but if it spread by means of the axis cylinders, the results are easily explainable.

Extensive investigations with regard to the spread of the virus, following infection by the intranasal route, have been made by Faber

(1933, 1938) and Faber and Gebhardt (1933). In the earlier stages of the disease, Faber (1938) found that the pathological changes were limited to the olfactory bulbs. Later, well marked lesions occurred in the brain-stem and at the same time the secondary olfactory centres, including the amygdaloid nucleus and uncinate gyrus, were involved. Finally, the spinal cord was implicated. In this connection the recent work of Bodian and Howe (1939) may be mentioned. Following the intranasal inoculation of poliomyelitis virus into monkeys with one olfactory tract divided, a marked preponderance of lesions in the rhinencephalon and in the tegmentum of the mid-brain on the side of the intact tract, was noted.

A number of authors, including Brodie and Elvidge (1934), Schultz and Gebhardt (1933), Lennette and Hudson (1935) and Gordon and Lennette (1939) have shown that interruption of the olfactory pathway prevents infection from the intranasal instillation of poliomyelitis virus. They look upon these findings as indicative of axonal spread. However, as Hurst (1936) has pointed out, this assumption is open to the objection that the operative procedures necessary for the interruption of the olfactory pathway may have led also to the cicatrization of the accompanying perineural lymphatics.

Using the cathode ray oscillograph technique, O'Leary, Heinbecker and Bishop (1932) found that the conductivity of the nerve fibres of monkeys suffering from poliomyelitis remained unaltered until the stage when lesions in the related neurones were clearly detectable. They argued that if the virus spread along the axis cylinders, impairment of conductivity should occur earlier in the disease, and suggested, therefore,

that the virus might travel between the nerve fibres, rather than in them. In contesting their argument, Hurst (1936) wrote, "It is probably impossible finally to prove or disprove this hypothesis, which in this case rests upon the assumption that the passage of virus along the axon is necessarily detrimental to that structure. This by no means follows from the evidence available. In several diseases due to neurotropic viruses it is possible to detect nuclear inclusions, indicating deranged nuclear metabolism, while the cytoplasm is still normal..... It may be that the presence of some viruses in the cytoplasm is relatively innocuous, and that it is by perversion of nuclear metabolism that impaired function of the cell and cytoplasmic degeneration are ultimately brought about. Were this so the conductivity of the axon would remain unaltered during transmission of the virus."

Cowdry (1934) found difficulty in understanding the mechanism whereby viruses spread in the axis cylinders, and pointed out that the processes were " ... tubes of capillary size through which no chemical substance could travel without being arrested by practically complete absorption on the walls." He considered that if the axon were composed of aqueous material of low viscosity, the passive carriage of viruses might be possible. The micro-dissections of de Renyi (1932) showed, however, that the axis cylinder is composed of a gelatinous substance which retains its shape even when cut into fragments. For this reason Cowdry was of the opinion that if viruses did spread in the axon, they must do so by a process of active proliferation along it.

That such a mechanism is possible is suggested by the recent work

of Nicolau and Kopciowska (1938), who, after the intra-neural injection of herpes virus, were able to demonstrate minute bodies lying in the axis cylinders.

It is also suggested by the experiments of Demme (1930), who found that after the inoculation of poliomyelitis virus intraneurally, the incubation period varied with the length of the nerve. Thus after the injection of the virus into the facial nerve, the incubation period was three days, into the median nerve, 4 - 6 days, and into the sciatic nerve, 5 days.

In conclusion, the available evidence clearly establishes the importance of the axonic pathway in the propagation of the virus in the central nervous system. It is to be admitted, however, that at some stage of the process, the perineural lymphatics and the cerebro-spinal fluid may play minor roles in the transport of the virus.

Recently, German and Trask (1938) reported that various denervation procedures not only failed to prevent infection, following intradermal injection of poliomyelitis virus into the denervated area, but, in the majority of cases, resulted in an increased susceptibility. Whether the theory of axonic transmission must be modified to bring it into agreement with these results, remains to be determined.

C. THE HISTOPATHOLOGY OF THE CENTRAL NERVOUS SYSTEM IN POLIOMYELITIS.

The vast change in the interpretation of the histopathological findings in poliomyelitis during the last thirty years can be presented most conveniently, by contrasting the views of the earlier and of the more recent investigators.

Representative of the views of the earlier workers is the monograph of Peabody, Draper and Dochez (1912), whose findings were based on the examination of eleven human cases, all of which died in the acute stage. These authors considered that the earliest change was an acute interstitial meningitis, characterised by hyperaemia and the collection of small mononuclear cells, probably lymphocytes, in the perivascular spaces of the vessels of the leptomeninges.. Later, the spinal cord was involved by the spread of the inflammatory process inwards along the sheaths of the vessels. The perivascular infiltration led to a certain amount of obstruction of the lumina of the vessels, but, in addition, there was often some effect, either toxic or mechanical, on the intima which resulted in haemorrhages and oedema. Although the possibility of a direct toxic action of the virus on the nerve cells could not be eliminated, it was considered more probable that their degeneration was secondary to the direct pressure of the haemorrhages, oedema and exudate, aided by the anaemia which followed compression of the vessels. Recovery of the function of the nerve cells was possible in cases in which the pressure and anaemia had not been prolonged or excessive. Neuronophagia of the necrotic nerve cells was brought about by polymorphonuclear leucocytes. To a lesser degree the same sequence of changes was found in the brain and brain-stem and also in the posterior root ganglia.

The most generally accepted interpretation of the micropathology of the disease nowadays is that of Hurst (1929). His conclusions were based on the experimental disease in which it was possible to trace the precise development of lesions from the incubation period onwards. For that

reason greater weight must be attached to his opinion than to that of the former group of investigators, whose findings were based on the end result of the action of the virus on the nervous system, in which the features of the primary lesion and secondary reaction are, of necessity, so intimately intermingled as to make their disentanglement almost impossible.

Hurst's studies showed clearly that the nerve cells were affected primarily, and that the involvement of the interstitital tissues was a secondary phenomenon. The actual priority of the lesions of the ganglion cells has been known since Rissler's time, but Hurst was the first to stress its importance. Hurst found that the changes in the neurones varied from mild chromatolysis to complete and apparently sudden necrosis. The affected cells showed progressively, varying degrees of tigrolysis, swelling of the nucleus and cytoplasm, shrinkage of the former, extrusion of the nucleolus, vacuolation of the cytoplasm, disappearance of fibrils and, finally, granular degeneration. With more virulent strains the ganglion cells were killed almost at once, and then their outline remained unaltered, their nucleus faded, and neuronophagia was delayed. The neuronophagia subsequent to the death of ganglion cells was brought about mainly by the microglial cells, though polymorphonuclear leucocytes initiated the process.

Following the primary injury to the nerve cells, Hurst found that there was a secondary reaction of the interstitial tissues which was characterised by vascular congestion, perivascular, tissue and pial infiltration, oedema, and, as has been mentioned above, neuronophagia.

The perivascular infiltration affected both arteries and veins.

It was predominantly lymphocytic, though in addition there was a variable proportion of adventitial and endothelial cells, and in the early stages a small percentage of polymorphonuclear leucocytes.

Tissue infiltration manifested itself as a diffuse cellular increase in the gray matter. Although it was most severe in the gray matter of the anterior horns and in the gray commissure, often the posterior horns were involved also. In addition, numerous focal accumulations of cells occurred, which frequently corresponded in position with damaged nerve cells. In the earlier stages, especially with virulent strains, the tissue infiltrate contained a considerable number of polymorphonuclear leucocytes, but most of these had disappeared within 48 hours after the first nervous symptom. The bulk of the tissue infiltrate was composed of microglial cells which had undergone metamorphosis and proliferation. These cells were identical with the "polyblasts" which were considered by Wickman to be derived from the lymphocytes. In addition to the microglia there was a small proportion of lymphocytes. These tended to increase in number with less virulent strains and in the later stages of infection.

Hurst drew attention to the fact that there was no true meningitis, and pointed out that the pial infiltration resulted from the discharge of surplus cells from the distended perivascular spaces. It therefore made its first appearance at the points of entry or exit of the blood vessels, but did not necessarily correspond, however, with the areas of gray matter which were affected most severely. Cells of the lymphocytic series formed the bulk of the pial infiltrate, but, in addition there

were some endothelial cells and, in the earlier stages, a small number of polymorphonuclear leucocytes.

With regard to haemorrhages, Hurst concluded that they played no part in the pathological process, and were merely the result of post-mortem trauma.

D. INTRANUCLEAR INCLUSIONS IN NERVE CELLS IN POLIOMYELITIS.

The first report on the occurrence of intranuclear inclusion bodies in the nerve cells in poliomyelitis was made by Covell (1930). This author examined the central nervous system of 33 monkeys sacrificed at various stages of the disease, and noted the presence of intranuclear inclusions, not only in the ganglion cells of the spinal cord, but also in those of the brain-stem. The bodies were most numerous in animals killed shortly after the onset of the paralysis. When one to three weeks were allowed to elapse after paralysis had occurred, they were found less frequently. The inclusions were acidophilic, sharply defined bodies, measuring 0.25 u - 3 u in diameter, and surrounded by a distinct halo. Usually one or two were present in each cell. They were seen only in cells undergoing degenerative changes. They reacted negatively to the Feulgen test for thymonucleic acid, and were not doubly refractile to light. Covell thought that the inclusions were comparable with those found in other diseases caused by filterable viruses, but left the question of their specificity for poliomyelitis, an open one.

In the following year Hurst (1931) confirmed this finding in 25 out of 28 rhesus monkeys suffering from the experimental disease, and also

in one human case. Among the 24 normal and pathological controls examined with negative results were 2 monkeys with rabies, 5 with vaccinial encephalitis, 1 with sepsis, and 1 with tuberculosis of the central nervous system. In addition, there was one case of post-vaccinal encephalitis in man, and several examples of other human diseases. Hurst considered that these inclusions were of the same nature as those reported in herpetic encephalitis, equine encephalo-myelitis and other virus diseases, and believed that they might represent a nuclear reaction to the virus. He concluded that they were apparently characteristic of the disease.

Inclusion bodies were reported also by Stevenson (1932), who considered that some of them seemed to be nothing more than a clumping of the intranuclear network.

Wolf and Orton (1932), claimed that intranuclear inclusion bodies, similar to those described by Covell and by Hurst, were demonstrable in the nerve cells of individuals who had died from diseases other than poliomyelitis. They suggested that such bodies were merely non-specific products of degeneration of the nucleoplasm.

In reply to these views Hurst (1934) contended that the inclusions occurring in normal cells were usually smaller and less acidophilic, their contours were less distinct, and they often showed little projections joining them to the general, weakly acidophilic, nuclear network. Moreover, they never showed evidence of internal heterogeneity, which was of frequent occurrence in the bodies described as nuclear inclusions.

It may well be that a final decision on the question of the specificity of these bodies must await the development of new technical methods.

Appendix.

TRANSMISSION OF POLIOMYELITIS TO THE EASTERN COTTON RAT AND TO WHITE MICE.

In 1939, Armstrong (1939 a) reported the successful transmission of a recently isolated strain of poliomyelitis virus (Lansing strain) from the monkey to the Eastern cotton rat (Sigmodon hispidus hispidus). The disease thus produced was characterized clinically by the occurrence of flaccid paralysis of the extremities, while pathological examination of the central nervous system revealed lesions similar to those observed in poliomyelitis both in man and in rhesus monkeys (Lillie and Armstrong (1940)). Subsequently, Armstrong (1939 b) was successful in transmitting the disease from the cotton rat to white mice.

The work of Armstrong (1939 a) has been confirmed by Toomey and Takacs (1940). Attempts to produce the disease with strains of virus other than the Lansing strain were, however, unsuccessful.

Results similar to those of Armstrong have been reported recently by Jungeblut and Sanders (1940) using the SK New Haven strain of poliomyelitis virus. The symptoms and lesions produced in mice were comparable in all respects with those of the disease in man and in the monkey. While the murine virus was highly pathogenic for mice and cotton rats, it possessed only limited pathogenicity for rhesus monkeys and none for albino rats, guinea-pigs and rabbits. Monkeys inoculated with live murine virus proved in a certain degree resistant to subsequent infection with the homologous poliomyelitis monkey virus. If the

latter observation can be confirmed, and extended to the production of immunity to poliomyelitis in man, a discovery will have been made as far-reaching in its consequences as that of Pasteur in connection with rabies.

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C. THE PRESENT INVESTIGATION 1.

The following account is based on a paper which has been published already (Swan (1939)).

The cases were obtained during an epidemic of poliomyelitis occurring in South Australia in the last part of 1937 and the early part of 1938, i.e., in the summer and autumn seasons. In a total population of 591,000, from 1st November, 1937, to 30th June, 1938, 341 cases were notified with 21 deaths. The case mortality rate was, therefore, approximately 6 p.c.. The 341 cases were part of a severe epidemic which involved also Victoria and Tasmania. The early cases in Victoria were very acute and often bulbar in type (Robertson (1940), Burnet (1940)).

(1) CASE NOTES 2.

Case 1.

N.P., male, aged 7 years was admitted on 12/12/37. Four days previously he came home from school complaining of headache and pain in the neck. He had fever the next day and began vomiting. On the following day he still vomited frequently, and developed a nasal voice with some regurgitation of fluids. He could still swallow. Later he complained of "pins and needles" in his shoulders.

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1. This work was carried out under a grant from the Council of National Health and Medical Research, Australia.

2. For the provision of the case notes I am indebted to the Board of the Metropolitan Infectious Diseases Hospital and to the Medical Superintendent, Dr. Alan Finger.

Examination. The temperature was 101.4°F., the pulse rate 112 and the respiratory rate 26. The face was flushed and he held himself somewhat rigidly. Except when addressed, he was apathetic. His voice was nasal. There was paresis of the left side of the palate and of the face. The spine and neck were moderately stiff, and flexion of the neck caused "pins and needles" in the right flank. Kernig's sign was negative. There was no gross weakness of the limbs but marked tremor was present. The deep reflexes were present. Breathing, both intercostal and diaphragmatic was normal.

Course. On the next (5th) day his general condition was much worse. Both lower limbs were weak, speech unintelligible and he was unable to swallow. For some hours before death there was respiratory difficulty. He died at 3.10 a.m. on the 6th day of the illness. Before death his temperature was 102.6°F. and his pulse rate 160.

Autopsy (performed 12 hours after death). There was enlargement of the spleen and of the mesenteric glands. The brain was slightly congested. Section of the brain-stem showed congested small vessels.

Case 2.

S.B., female, aged 8 years, was admitted on 26/12/37 after having been ill for three days. After one day of listlessness, she complained of frontal headache, and later, pains in the back and abdomen. On the following day she was restless and the pains were still present. The next morning (3rd day) her right arm was weak, and there was marked tremor and head retraction.

Examination. The temperature was 99.4°F., the pulse rate 100 and respiratory rate 38. The patient was a well-nourished child, rather apprehensive and with obvious dyspnoea. There was marked tremor of the extended

bands, especially the right. Her head was retracted and the neck and spine were rigid. The cranial nerves were normal. Apart from absence of the knee jerks, there was no abnormality of the lower limbs. Of the upper limbs, the left was normal but the right was definitely weak. The diaphragm was over active and there were practically no intercostal movements.

Course. Though she was placed in a respirator, her general condition deteriorated rapidly and she died some hours later. Death was preceded by convulsive spasms and cyanosis.

Autopsy (performed within 8 hours of death.) There were no abnormal findings on macroscopic examination of the brain and spinal cord.

Case 3.

R.M., female, aged 14 years, was admitted on 7/1/38. At noon, two days previously headache commenced and she vomited once. In the evening she was better, but at 3 a.m. she awoke and found that one leg was weak. Next morning both legs were weak, and a little later both arms became weak. At 10 a.m. she could not speak. Respiratory difficulty began at mid-day and by the time she reached hospital (2 p.m.) she was unconscious, cyanosed and moribund. Within a few minutes of admission she died in convulsions. The temperature and pulse rate were not taken.

Autopsy (performed within 2 hours of death.) There was no enlargement of the mesenteric glands. The brain was congested, and there was a large amount of post-mortem subarachnoid bleeding, suggesting to those with no knowledge of the case history, spontaneous subarachnoid haemorrhage.

Case 4.

H.L., male, aged 20 years, was admitted on 23/1/38. Six days previously he had pain in the back and front of the chest. The next day he had a sore throat, and on the following day a very severe occipital headache. This lasted until the morning of admission.

Examination. The temperature was 100°F., the pulse rate 110 and respiratory rate 24. The patient was a well-developed aboriginal half-caste lying apprehensively in bed. The neck and spine were rigid. The cranial nerves were normal. He had weakness of the left triceps muscle. His left lower limb was flaccidly paretic. There was very little intercostal movement, but there was full movement of the diaphragm.

Course. On account of increased respiratory difficulty he was placed in a respirator shortly after admission. The next day his general condition was worse. There was inactivity of both the diaphragm and intercostal muscles. All four limbs were very weak and he had retention of urine. Later that day he was unable to swallow. Paroxysmal tachycardia developed which responded to pressure on the carotid bifurcation. He died that evening following a short period of convulsive spasms. His temperature reached 105.4°F., and his pulse rate 156.

Autopsy (performed 16 hours after death.) There were no abnormal findings on macroscopic examination of the central nervous system.

Case 5.

K.P., male, aged 5 years, was admitted on 31/1/38. Six days previously he was restless at night. He was better on the next two days. Then he complained of pains in the neck and was drowsy and feverish. Anorexia was

marked and he was very constipated. Loss of power in the right side of the face followed two days later.

Examination. The temperature was 101°F., the pulse rate 120, and respiratory rate 30. The neck and spine were very stiff. There was paresis of the right side of the face, and of the left triceps muscle. Movement of the intercostal muscles was very slight.

Course. The facial paresis was much more marked next day. On the following day the temperature rose to 102.4°F., and difficulty in swallowing developed. Next day the temperature rose to 104°F., and coma led to death.

Autopsy (performed 5 hours after death). Unfortunately only the olfactory bulbs and the trigeminal ganglia were available for examination.

Case 6.

H.R. male aged 46 years, was admitted on 3/2/38. Two days previously he woke in the morning with stiffness of his shoulders and neck. Headache developed and became more severe during the day. The next day he noticed stiffness and weakness of the right arm. Considerable retching occurred and his limbs appeared to twitch.

Examination. The temperature was 98.4°F., the pulse rate 78, and respiratory rate 24. The patient was a big middle-aged farmer lying comfortably in bed. The lungs were normal except for greater expansion of the left side of the chest. There was no movement of the diaphragm, yet there were no signs of respiratory distress. The cranial nerves were normal. The neck and spine were slightly stiff, and both arms were weak.

Course. Some hours later respiratory difficulty became apparent, and he was placed in a respirator. The next day dysphagia developed and

the patient became rapidly worse. Death followed a short period of unconsciousness with cyanosis and convulsions.

Autopsy (performed 5 hours after death). In the spinal cord there was slight meningeal congestion. The upper cervical cord was much softer than the lumbar cord, and showed congested small vessels on section. The brain-stem was similar. The mesenteric glands were enlarged, and there were pleural adhesions on the right side of the thorax.

Case 7.

M.B., male, aged 11 months, was admitted on 14/2/38, after having been ill for three days. The illness commenced with feverishness and vomiting. He appeared better the next day, but on the morning of admission he woke up "limp," and "did not move himself much." His neck seemed stiff.

Examination. The temperature was 100.2° F., the pulse rate 128 and respiratory rate 38. The patient was a sick apathetic baby. There was no tremor, and the back and spine were rigid. The cranial nerves were apparently normal. There was paresis of the intercostal muscles, with consequent over-action of the diaphragm, but no respiratory distress. The limbs, so far as could be determined, were normal. The reflexes were present.

Course. There were no marked changes until two days later. In the early morning of 16/2/38 respiratory distress developed, and the patient was transferred to a respirator. Death occurred at 7.35 p.m., and was preceded by irregularity of the pulse rate. There was no definite evidence of paralysis of the limbs throughout the course of the illness.

Autopsy (performed 14 hours after death). There were no pathological changes to be found on macroscopic examination of the brain and spinal cord.

Case 8.

R.N., male, aged 38 years, was admitted on 27/2/38. Two days previously he noticed aching pains in the back and thighs. He suffered from nausea but did not vomit. Next day he had pain and stiffness in the neck. On the morning of admission he noticed weakness of his right leg.

Examination. The temperature was 99.2°F., pulse rate 104 and respiratory rate 20. The patient was a big, well-built, sun-tanned man lying quietly in bed. There was a fine tremor of the hands, and the neck and back were very stiff. The cranial nerves were normal. All the muscles of the right lower limb were very weak. The upper limbs and respiratory muscles were normal.

Course. On the following day paresis of the left leg was apparent. There was retention of urine and constipation, associated with abdominal distension. These persisted until death. On 3/3/38 he had intermittent dysphagia, and the upper intercostal muscles and the triceps muscles became weak. He became very restless and was controlled by sedatives. Dysphagia and respiratory difficulty increased, and he died in convulsions, with rapidly mounting pyrexia on 5/3/38.

Autopsy. There were no abnormal findings on macroscopic examination of the central nervous system.

Sex and Age-incidence: Mortality: Type of Case.

Of 21 patients dying in the epidemic, 12 were male and 9 were female. The age distribution of the 341 cases (including the 21 fatal) is shown in Fig. 1, from which it will be seen that 159 cases, or 46.6 p.c., were 10 years of age or over, while 45 cases, or 13.2 p.c., were 20 years of age or over. These figures are suggestive of the tendency of poliomyelitis in recent years to affect more and more the higher age groups ("Poliomyelitis" International Committee (1932), Burnet (1940)).

The number of fatal cases, 21, is too small to allow of definite conclusions. However, 13 of these, or 62 p.c., were over 10 years of age, while 7, or 33 p.c., were over 20 years of age, suggesting greater mortality as well as greater incidence in the higher age groups.

Of the cases considered in this thesis, six were male and two female. Six of these eight cases had clinical evidence of bulbar involvement. Fischer and Stillerman (1937) found that all their fatal cases showed evidence of bulbar involvement during their illness, while Silverman (1931) found that there was a certain parallelism between the incidence of cases of bulbar and bulbo-spinal paralysis and the fatality rate.

Scott-Brown (1931) found in his series of 15 cases of poliomyelitis, of which five were bulbar in type, that tonsillitis or pharyngitis was present in every case. On the other hand, in this series it occured only in Cases 1, 4 and 8.

(2) MATERIAL AND METHODS.

With the exception of the olfactory bulbs and trigeminal ganglia, which were fixed with Zenker-acetic fluid, the brain, and the portions of the spinal cord which were not preserved in glycerine, were fixed with formol-saline. After removal of the brain-stem, the brain was divided by sagittal section into two halves. A series of approximately eight slices was made in the frontal plane through one half of the brain. These slices, together with appropriate sections across the brain-stem and such sections of the spinal cord as were available, were embedded in celloidin. Sections were cut at 15u and stained by routine histological methods.

The olfactory bulbs and trigeminal ganglia were embedded in paraffin. Both were cut in serial section at 5u, and stained with iron-alum haematoxylin and eosin.

(3) PATHOLOGICAL CHANGES.

A. CEREBRUM.

This was available for examination in 6 of the 8 cases of this series.

(1) MENINGES.

Infiltration of the pia and the arachnoid was mild and patchy, and for the most part related to vessels. It was more apparent in the depths of the sulci, than on the surfaces of the gyri. The cells of the infiltrate were lymphocytes with occasional polymorphonuclear leucocytes.

In most of the cases this mild scattered meningeal infiltration was seen in the central sulcus, and on the pre- and postcentral gyri. It was also present in the hippocampal fissure and over the anterior perforated

substance. Occasionally it occurred in the lateral fissure. In Case 4, it was present also in the calcarine fissure, and in Case 1, on the outer aspect of the tuber cinereum and the infundibulum. Cases 6 and 7 differed from the others in that meningeal infiltration was for the most part absent, and nowhere amounted to more than a few scattered cells.

In some regions, such as the precentral gyrus, and the anterior perforated substance, the meningeal infiltration was related to underlying pathological changes in the parenchyma. Hurst (1929) maintains that in such cases the pial infiltration is brought about by the discharge of surplus cells from the overcharged perivascular spaces of the grey and white matter into the pial meshes.

Meningitis in the calcarine fissure, however, cannot be explained thus. Környey (1933 a) considers that meningeal infiltration without underlying parenchymal damage may be due either to a reaction to the virus present in the meninges, or to a general reaction of immunity of the mesoderm of the central nervous system. However, as Horanyi-Hechst (1935) points out, this does not explain the insular form of the infiltration. He suggests that the conditions governing the circulation of the cerebro-spinal fluid may play a part in this. The fact that the meningeal infiltration is most apparent in the depths of the sulci agrees to a certain extent with this statement. Possibly there is a slower velocity of flow of the cerebro-spinal fluid within the depths of the sulci, and the inflammatory cells tend to lodge in these "back-waters."

(2) CORTEX.

With one exception the only area affected was the precentral gyrus, almost exclusively in the area giganto-pyramidalis of Brodmann. In all

of the cases the vessels of this area showed moderate perivascular infiltration in all six cortical laminae. The infiltrate consisted of lymphocytes and other mononuclear cells, and when traced to the surface became continuous with that in the meninges. In addition perivascular infiltration could be seen in the white matter immediately subjacent to the affected cortex. Tissue infiltration was of moderate intensity and was focal in type. It was present in all laminae and consisted of swollen microglial cells and polymorphonuclear leucocytes. The presence of the latter is of considerable importance, for it is characteristic of a recent lesion. It is probable that lesions of the area giganto-pyramidalis are secondary to those of the motor nuclei of the spinal cord and brain-stem, and that the infection passes upwards along the pyramidal tracts. Pette (1930) and Stiefler and Schenk (1933) also have observed the presence of comparatively large numbers of polymorphonuclear leucocytes in poliomyelitic lesions of the area giganto-pyramidalis.

Of the nerve cells, the giant pyramidal cells of Betz were chiefly involved.(3) Only occasional cells were affected, many being apparently normal. Most of the affected cells showed moderate to marked chromatolysis, sometimes associated with swelling of the cell and eccentricity of its nucleus. Occasionally necrosis and neuronophagia occurred. A few smaller pyramidal cells lying in the third lamina showed moderate chromatolysis and rarely were undergoing neuronophagia.

Krayenbühl (1928), Hurst (1929), André-Thomas and Lhermitte (1929), Pette (1930) and Spielmeyer (1932) were the first to draw attention to

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(3) = Photograph 3.

isolated lesions of the motor cortex in both human and monkey poliomyelitis. Pette (1930) and Környey (1933 b) have shown that not only is the area giganto-pyramidalis the area of election for poliomyelitic lesions of the cortex, but that there is laminal electivity in this area, the third and fifth layers being mainly affected.

Hurst (1930) noted that the lesions of the motor cortex in monkey poliomyelitis were most marked in the dorsal third (leg centre). More recently Horanyi-Hechst has found a similar state of affairs in the majority of his cases of human poliomyelitis, and in a proportion has correlated the distribution of lesions in the precentral gyrus with those in the brain-stem and spinal cord. Thus in the majority of cases in which the leg centre was most severely affected, the lumbar segments of the spinal cord showed the most marked changes.

Of the six cases of this series, Cases 1 and 4 showed the most severe changes in the dorsal third (leg area). In Case 6 the upper and lower thirds were equally involved, while in Case 7 the lower half was more severely affected. In the remaining two cases there was no difference in the intensity of the lesions of the various parts of the motor cortex. It was not possible to correlate the lesions of the bulbo-spinal motor centres with those of the area giganto-pyramidalis. For the most part, however, the clinical findings were the only indication of localisation of lesions in the spinal cord, since appropriate sections were available only in Cases 4 and 6.

In several of the cases an occasional vessel which showed mild perivascular infiltration was seen in the frontal cortex, while in

Case 4 similar perivascular cuffing was seen in the cortex of the cingulate sulcus; cell loss and tissue infiltration, however, were absent.

Horanyi-Hechst agrees with Környey that poliomyelitic foci and changes in the nerve cells of the cerebral cortex in human poliomyelitis are found only in the precentral region, particularly in the area giganto-pyramidalis. The only dissentient view he could find in the literature was that of Spielmeyer, who found lesions in the cornu Ammonis of one case. In Case 7 of this series a small focus of glial cells and one polymorphonuclear leucocyte was seen in the posterior part of the cornu Ammonis. It lay in the stratum lucidum (Cajal), just external to the double layer of pyramidal cells. In its neighbourhood there were a few vessels with mild perivascular infiltration. Therefore, while agreeing with Környey and Horanyi-Hechst so far as the neopallium is concerned, I cannot accept their opinion in regard to the archipallium.

(3) BASAL GANGLIA.

(a) Corpus Striatum.

1. Caudate nucleus: Lesions were absent in every case.

2. Lentiform nucleus: In two cases there were a few vessels in the putamen with mild perivascular infiltration. In addition there was a small area of mild diffuse infiltration in one of these cases. In both the nerve cells were normal.

The globus pallidus was involved in four cases, the lesions in each consisting of mild perivascular infiltration of a few vessels. In none

were the nerve cells affected. In Case 6 there was calcification of the walls of the vessels. In those cases in which lesions occurred in the globus palladus, the putamen was unaffected.

3. Internal capsule: An occasional vessel with mild perivascular infiltration was seen in three cases.

(b) Claustrum.

This was never involved. Medial to it, however, a few vessels with mild perivascular infiltration were seen in the external capsule of one case.

The changes found in the corpus striatum and claustrum are similar to those reported by Stiefler and Schenk, and by Környey (1933 b). Horanyi-Hechst found in a few of his cases, besides perivascular infiltration, poliomyelitic foci both in the globus pallidus and the putamen, Occasionally there was mild perivascular infiltration in the claustrum.

(c) Thalamus.

Lesions were found in the thalamus in all cases. Usually they consisted of mild perivascular cuffing. The nerve cells were never affected.

Special attention was paid to the site of the lesions within the thalamus. The anterior nucleus was never affected. The lower end of the medial nucleus and the lateral nucleus were each involved in two cases. The ventral nucleus was more often involved, lesions occurring in five of the six cases. In the ventral nucleus the medial part was always most involved. Apart from the mild perivascular cuffing,

diffuse infiltration of glial cells, together with an appreciable number of polymorphonuclear leucocytes were seen in the ventral nucleus of two cases.

The ventral nucleus of the thalamus serves as a relay station for the medial lemniscus and spinothalamic tracts. In addition it receives fibres from the brachium conjunctivum and red nucleus. It may well be, therefore, that the lesions of the ventral nucleus are secondary to lesions of the spinal cord, brain-stem and cerebellum.

Horanyi-Hechst admits that the changes in the diencephalon are generally later in origin than those of the brain-stem and the spinal cord. He could not find in the changes in the thalamus, however, any topographical relationship either to the internal capsule (spread of the virus along the pyramidal tracts), nor to the termination of the lemnisci. Both Stiefler and Schenk, and Környey (1933 b) found that the main changes occurred in the ventral nucleus.

(d) Hypothalamus.

The hypothalamic region was involved in all six cases, though different nuclei were affected in every case. Lesions consisted of moderate perivascular infiltration, associated sometimes with mild to moderate tissue infiltration, which was usually diffuse but occasionally focal in type. Death of nerve cells was never seen. In some sections the infiltrate, apart from glial cells and lymphocytes, contained appreciable numbers of polymorphonuclear leucocytes. The supraoptic nucleus was involved in three cases, the tuber cinereum in two cases, and the tegmental field H of Forel in two cases.

The dorso- and ventro-medial hypothalamic nuclei were each affected in one case. The subthalamic nucleus (Luys) showed mild perivascular infiltration in only one case.

Stiefler and Schenk found that the changes were most marked in the neighbourhood of the third ventricle and decreased in intensity laterally; in this respect the present cases are all similar. Horanyi-Hechst found changes most frequently in the nucleus paraventricularis, but even here cell necrosis occurred but rarely.

(e) Metathalamus.

The lateral geniculate body was never affected. The medial geniculate body was affected in one case, the lesions consisting of mild perivascular cuffing, and moderate focal infiltration with glial cells and polymorpho-nuclear leucocytes.

(f) Epithalamus.

The pineal body showed marked vascular congestion in one case. The nucleus of the posterior commissure (Darkschewitsch) was seen in one case, and showed perivascular cuffing and focal infiltration. The Nissl granules were not clear cut, and a few cells were undergoing neuronophagia.

(4) RHINENCEPHALON.

In view of the widely accepted belief that the olfactory nerves are the most probable portal of entry for the virus of poliomyelitis, special attention was paid to the rhinencephalon. Apart from those in the region of the anterior perforated substance, the lesions were

suprisingly insignificant.

(a) Olfactory bulbs.

These were available in four cases. Though they were cut completely in serial sections, no lesions were detected.

(b) Anterior perforated substance.

The changes consisted in moderate perivascular cuffing and tissue infiltration usually focal in type. They occurred in four cases. The medial and lateral striate vessels pass through the anterior perforated substance to supply the basal ganglia. The perivascular infiltration, therefore, can be explained as due to an overflow of inflammatory cells from the lesions in the basal ganglia into the spaces of Virchow-Robin. It is improbable, therefore, that lesions in the anterior perforated substance indicate the portal of entry of the virus.

(c) The amygdaloid nucleus was always free from lesions.

(d) Lesions occurred in the cornu Ammonis in only one case. They have been described already in the section on the cerebral cortex.

(5) CRANIAL NERVES.

In two cases there was mild diffuse mononuclear infiltration at the periphery of the trigeminal ganglia. The ganglion cells were normal. The optic nerves and tracts were always unaffected.

(6) CHOROID PLEXUS.

Apart from calcification in the vessels in Case 6, no lesions were seen.

B. BRAIN-STEM AND CEREBELLUM.

(1) MENINGES.

Meningeal infiltration was similar to that seen in the cerebrum. It occurred in the interpeduncular fossa and over the ventral aspect of the pons and the medulla, and often in the cerebellopontine angle and between the cerebellar folia. Its occurrence on the ventral aspect of the brain-stem cannot be related to the underlying pyramidal tracts, to the pontine nuclei or to the inferior olivary nucleus, because these rarely showed lesions. However, the ventral aspect is the region of entrance and exit of most of the blood vessels, and it is possible that the occurrence of meningitis here is due to the overflow of inflammatory cells from the diseased tegmentum along the perivascular sheaths. The presence of meningitis in the cerebellopontine angle could be explained on similar lines, but in both cases the conditions governing the flow of cerebro-spinal fluid may play a part. Thus Hurst (1932), after the injection of Indian ink or trypan blue into the cisterna magna of monkeys, stated that, "the maximum colouration was seen on the inferior surface of the cerebellum, along the basilar artery and anterior cerebral arteries, and around the interpeduncular fossa, spreading thence by the Sylvian fissure and adjacent convolutions to the vertex and medial surface." The evidence suggests, therefore, that whatever the origin of the cells infiltrating the meninges, their distribution is influenced by the flow of the cerebro-spinal fluid.

(2) MIDBRAIN.

Lesions in the midbrain were almost entirely confined to the tectum and the tegmentum; the cerebral peduncles were usually spared, but a few vessels showed perivascular cuffing. The inflammatory changes consisted of vascular congestion, oedema, moderate to severe perivascular cuffing, occasional extra-adventitial infiltration, and tissue infiltration, both diffuse and focal. The infiltrate consisted of swollen microglial cells and lymphocytes with a few polymorphonuclear leucocytes.

In the superior colliculi there was marked perivascular infiltration, but tissue infiltration was milder and more diffuse. In three cases the motor cells of the superior colliculi showed mild chromatolysis.

As a rule, perivascular and tissue infiltration were well marked in the reticular formation and in the gray matter surrounding the aqueduct of Sylvius. Occasional cells of the oculomotor nucleus showed moderate chromatolysis, but there was no death of cells.

Only the parvo-cellular portion of the red nucleus was examined. Apart from moderate perivascular infiltration especially towards the periphery of the nucleus and occasional areas of mild diffuse and focal infiltration, the lesions were not marked. The nerve cells were normal.

The results so far are similar to those of Horanyi-Hechst.

In the substantia nigra of four cases there was moderate to severe perivascular infiltration. In one of these, tissue infiltration was absent, in the others it was mild and diffuse. The cells were normal.

The substantia nigra in the remaining two cases was markedly affected, extremely severe tissue and perivascular infiltration involving almost the whole structure with the exception of a small portion superolaterally. The pigmented nerve cells were embedded in masses of inflammatory cells, and most of them were moderately to severely chromatolytic. Necrosis with neuronophagia occurred in some. A few apparently normal cells were seen among the masses of inflammatory cells.

The first four cases are similar to those described by Stiefler and Schenk, Horanyi-Hechst, and Környey (1933 b), who consider that necrosis of cells in the substantia nigra is rare. The other two resemble those of Guizetti (1933), who maintains like Marinesco, Manicatide and State-Draganesco (1929) and André-Thomas and Lhermitte (1929) that necrosis of these cells is common.

(3) PONS AND MEDULLA.

In the pons and the medulla, as in the midbrain, the seat of the pathological processes was the tegmentum. Except for occasional vessels with mild perivascular cuffing lying in the pontine nuclei, arcuate nuclei, olivary nuclei, and the pyramidal tracts, the ventral region was unaffected; very rarely small foci of glial cells were seen here. The nerve cells were never involved.

The pontine region was severely affected in five cases, and moderately in one. The severity of the inflammatory process usually diminished slightly as it was traced from pons to medulla. The most intense changes were seen in the tissues forming the floor of the fourth ventricle, especially in the mid-line, but they were also well marked

in the lateral walls. They consisted of oedema, vascular congestion, occasional small haemorrhages, perivascular infiltration, and tissue infiltration both diffuse and focal. Associated with these was the death of nerve cells.

Hurst (1929), on account of the lack of reaction to the effused blood, interprets the minute haemorrhages as the result of post-mortem trauma. Landon and Smith (1934), on the other hand, maintain that they are due to the action of a toxin, presumably virus itself, on the endothelium of the blood vessels, which are damaged to such a degree that they permit diapedesis of red blood corpuscles or even actual haemorrhage. The latter theory is improbable because the virus of poliomyelitis has never been shown to affect any but nerve cells. Sabin and Olitsky (1936) have found that its specificity is so complete that, unlike most other viruses, it has never been grown in tissue-culture except of human embryonic brain. Moreover viruses do not produce toxins, so that if a toxin is supposed to damage the vascular endothelium, it must be produced in degenerating nervous tissue. Lennette and Reames (1937, 1938) have shown that in experimental poliomyelitis there is a slight increase in the permeability of the blood-central nervous system barrier to sodium nitrate, but not to protein and globulin; it seems unlikely that red blood corpuscles could pass through damaged walls impermeable to protein.

Perivascular cuffing of vessels was extremely severe, and in addition there was often extra-adventitial infiltration. Infiltration with swollen microglial cells and a few lymphocytes, both diffuse and focal, was seen throughout the tegmentum. More rarely a few polymorphonuclear leucocytes were seen. The intensity of the inflammatory reaction in the pons was often equal to that seen in severely affected segments of the spinal cord.

Chromatolytic changes and necrosis occurred more severely and more frequently in the nerve cells of the motor nuclei, than in those of the sensory nuclei of the brain-stem.

In the motor nucleus of the 5th nerve, there were a few necrotic cells in one case, but usually the changes went no further than those of moderate chromatolysis. The abducent nucleus showed moderate cell loss in one case. The facial nucleus showed mild cell loss in one case, moderate in three cases, and severe in one case. There was a tendency, noticed in the other motor nuclei, for the nucleus on the one side to be much more affected than its fellow on the opposite side. In the lateral vestibular nucleus (Deiter's), in all of the cases at least half of the cells were undergoing neuronophagia, while there was moderate to severe chromatolysis of most of the remainder. A few apparently normal cells were seen among the closely packed cells of the infiltrate. The nucleus ambiguus and the hypoglossal nucleus were equally affected In three cases there was moderate cell loss, while in two there was almost total loss of the cells comprising the nucleus. The nucleus supraspinatus showed moderate cell loss in three cases. Moderate to severe chromatolysis was often seen in the large motor cells of the reticular formation. Occasional cells were necrotic and underwent neuronophagia. In two cases mild cell loss occurred in the dorsal motor nucleus of the vagus.

The pigmented pontine nuclei of the locus coeruleus were affected in five cases. In two there was moderate chromatolysis, while in the others occasional cells were necrotic and were undergoing neuronophagia. An expremely interesting feature of the neuronophagia was the presence of melanin granules within the cytoplasm of the phagocytes. This has

been reported previously both in the substantia nigra and locus coeruleus by Marinesco, Manicatide and State-Draganesco (1929). Creutzfeldt (1925) reports a similar finding in the substantia nigra in von Economo's disease. All these observations give definite evidence for the occurrence of neuronophagia.

Horanyi-Hechst says that he has never seen actual phagocytosis, that is, the presence of fragments of cell bodies or tigroid or fibril fragments within the phagocytes, and prefers to call the process neurocytolysis. It is certain that inflammation severe enough to cause the death of nerve cells would be sufficient to cause lysis of the tigroid bodies and neurofibrils, making it difficult to recognize by these criteria that neuronophagia was occurring. In this connection the work of Levaditi and Pignot (1914) who observed definite evidence of neuronophagia in the posterior root ganglia, may be mentioned.

In the mesencephalic nucleus of the 5th nerve mild cell loss was found in two cases. This is not unexpected for the nucleus has an embryological origin similar to that of the posterior root ganglia, in which lesions are found in both human and experimental poliomyelitis (Forssner and Sjövall (1907)). The principal sensory nucleus of the 5th nerve was mildly affected in five cases, a few cells being necrotic. The superior olivary and ventral cochlear nuclei were affected similarly in two cases. Though perivascular infiltration often occurred in the hilum of the inferior olivary nucleus, its cells were always normal.

The arcuate nucleus was also unaffected. Focal infiltration was present in the medial and lateral tegmental processes of the pontine nuclei and in the nucleus of the spinal tract of the 5th nerve, but the nerve

cells were unaffected. There was occasional necrosis of nerve cells in the cuneate and gracile nuclei, but it was not proportional to the tissue infiltration.

The localization of the pathological process in the brain-stem to its tegmental portion has been a point of much interest since Harbitz and Scheel (1907 a) first drew attention to it. Seifried and Spatz (1930) believe that the dorsal parts of the medulla and pons are especially involved by the disease because the virus spreads by way of the cerebro-spinal fluid. Horanyi-Hechst says that he would like to believe this, but that the assumption is not in accordance with all the histological facts. Nor can he explain the localization of the process by the spread of the virus along the axons.

The intensity of the pathological process in the floor of the fourth ventricle, especially towards the midline, prompts consideration of a tract to which no attention has hitherto been paid in poliomyelitis, that is, the medial longitudinal bundle, which extends through the whole length of the brain-stem, and lies close to the median plane and to its fellow of the opposite side.

The most important element in the medial longitudinal bundle consists of fibres from the vestibular to the oculomotor group of nuclei of both sides. It also contains fibres which connect with the formatio reticularis of the medulla and pons, the sensory nucleus of the 5th nerve and probably all other motor nuclei of the pons and medulla. It extends as high as the nucleus of the posterior longitudinal bundle (situated in the grey matter ventro-lateral to the third ventricle), and below is continuous with the anterior intersegmental tract of the spinal cord. Through the latter it is

in intimate relationship with the anterior horn cells, a factor of the utmost importance.

It is possible, therefore, that virus could pass from the diseased anterior horn cells into the anterior intersegmental tract, from there to the medial longitudinal bundle, and thence to the nuclei of the tegmentum of the medulla and pons, and to the third ventricle. On the other hand if the initial lesions occurred in the pons and medulla, the anterior horn cells could be secondarily involved through the same paths.

Passage of the virus along the anterior intersegmental tract might explain the step-like character of the ascending type of poliomyelitis, the infection passing from segment to segment of the spinal cord, and eventually reaching the brain-stem by means of the median longitudinal bundle. The reverse would hold for the descending type.

(4) CEREBELLUM.

In the molecular layer occasional vessels were seen with mild perivascular cuffing. The nerve cells were variably affected. There were no lesions of the Purkinje cells, but the cells of the ventro-medial portion of the dentate nucleus were mildly chromatolytic. In their neighbourhood there was moderate perivascular and focal infiltration. André-Thomas and Lhermitte (1929), on the other hand, found marked changes in the neurones of the dentate nucleus, but associated microglial reaction was absent.

The roof nuclei were much more severely affected in the two cases in which they were examined.(4) They were literally packed with inflammatory cells. Nearly all of the nerve cells were undergoing neuronophagia.

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(4). = Photograph 4.

Severe perivascular infiltration and occasional small haemorrhages occurred in the middle cerebellar peduncles, and were present in a milder form in the superior and inferior cerebellar peduncles. In one case there was mild perivascular and diffuse infiltration in the anterior medullary velum.

The severity of the pathological process in the nucleus fastigius runs pari passu with that in Deiter's nucleus. Possibly the virus, after manifesting its properties in the latter and thus becoming increased in quantity, passes to the nucleus fastigius along the fastigio-bulbar tract which connects them.

C. SPINAL CORD.

Most of the spinal cords were placed in glycerine, in order to obtain virus for experimental study. Sections through cervical, thoracic and lumbar segments were available in Cases 4 and 6, through the thoracic and lumbar in Case 2, through cervical in Cases 3, 7 and 8, and through thoracic in Case 1.

Meningitis was more marked than in the cerebrum or the brain-stem, but the same types of cells occurred. As a general rule the infiltration of the pia was more apparent in the lower than in the upper segments, an observation previously made by Wickman (1911). It was more intense in the antero-median fissure and over the ventral aspect of the cord.

Occasionally there was disparity between the intensity of the meningitis and the underlying inflammation of the parenchyma. Thus in a section from the lumbar cord of Case 6, meningitis was severe, yet within the substance of the cord, tissue infiltration was mild and

localized to the region of the central canal, while the anterior horn cells, apart from occasional vacuolation, were normal.(1) In the cervical region of the same case, there were severe infiltrative changes and death of nerve cells, yet the meningeal infiltration amounted to no more than a few scattered cells.

Lesions in the parenchyma of the spinal cord were severe in lumbar and cervical regions but only moderate in the thoracic region in Case 4. In Case 6 there were severe lesions in the cervical and thoracic regions, but the lumbar region was only mildly involved.

In most of the eight cases the sections showed severe vascular congestion and oedema. Perivascular infiltration was marked and there was often extra-adventitial infiltration. The former occurred equally in the grey and white matter. At times it was possible to trace the inflammatory cells along the perivascular sheaths to the pia where they became continuous with the infiltration there.

Tissue infiltration was confined to the grey matter and was of two types, diffuse and focal. It was most intense in the anterior horns and in the grey matter surrounding the central canal. Occasionally the posterior horns were almost as heavily involved, but usually less so; rarely not at all. Tissue infiltration was always more intense immediately adjacent to the vessels.

The cells comprising the infiltrate varied at different levels of the spinal cord. In segments at a later stage of the pathological process the vast majority of cells were swollen microglia, together with a small number of lymphocytes and rare polymorphonuclear leucocytes. In segments more recently attacked by the virus, there were a few microglial cells

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(1) = Photograph 1.

at the rod-cell stage and occasional lymphocytes, but polymorphonuclear leucocytes predominated in the infiltrate. It was thus possible to trace the spread of the disease in the spinal cord and to correlate it with the clinical symptoms. For instance, in Case 6 where the initial palsy occurred in the upper limb, the cervical cord showed a later stage of the pathological process, the thoracic cord an intermediate stage and the lumbar cord an early stage. In the cervical region there was a total loss of anterior horn cells, while those in the lumbar region showed only occasional vacuolation.

In general the pathological picture was that of a primary degeneration of the neurons, secondarily attacked by polymorphonuclear leucocytes. Later these leucocytes were replaced by swollen microglial cells and lymphocytes. The phagocytosis of the necrotic nerve cells was brought about by the microglia.(2) The minute hæmorrhages which occasionally occurred in the grey matter were not always related to the severity of the inflammatory reaction.

Loss of nerve cells was almost entirely confined to the anterior horns, but sometimes cells belonging to Clarke's column or more rarely the intercalated neurons of the posterior horn were affected. The lesions of the anterior horn cells varied from mild chromatolysis to severe changes, eventually passing on to necrosis and later to neuronophagia. When ganglion cells of the anterior horns were spared, they were always those of small or medium size; the large ones were apparently more susceptible to the virus. When Clarke's column was affected it was often only on one side of the spinal cord. Sometimes vacuolation of the cells occurred. Tissue infiltration was often well marked in the lateral

(2) = Photograph 2.

horns which contain the connector cells of the sympathetic system. In rare cases some of these cells were severely chromatolytic, but actual loss of cells was never detected.

The central canal occasionally contained some hyaline exudate and a few mononuclear cells. In one case it was obliterated by proliferation of the ependymal cells. Landon and Smith (1934) have noticed obliteration of the central canal in over one-third of their cases. It must be pointed out that this often occurs in man with increasing age, and is not necessarily the result of disease.

(4) DISCUSSION.

A. THE SELECTIVE AFFINITY OF THE VIRUS.

The predilection of the virus for the motor cells of the anterior horns of the spinal cord has long been known, but the reason for it remains obscure. Recent workers have maintained that loss of nerve cells in the spinal cord is a manifestation of selective affinity of the virus for the cells of the voluntary motor system, and is independent of vascular factors. The fact that in the present cases the lateral horn cells were usually unaffected when anterior horn cells were severely involved, although both have the same blood supply, leads to the same conclusion. This was previously pointed out by Horanyi-Hechst.

One of the main objects of this research was to determine whether nerve cells similar in structural and physiological properties to the anterior horn cells, but lying elsewhere in the central nervous system

than the spinal cord, showed the same susceptibility to the virus. Malone (1913) has described already the distribution within the central nervous system of such cells, that is, cells of the motor type. He recognizes them by their large size, by their sharply polygonal form, and by their relatively coarse, well-defined Nissl granules. Directly or indirectly they innervate striated muscle. They include; the Betz cells; the cells of the globus pallidus; the nucleus intercalatus corporis mammillaris, and the substantia reticularis hypothalami of the hypothalamus; the magno-cellular portion of the red nucleus; the nucleus of the posterior commissure (Darkeschewitsch); the pars reticularis of the substantia nigra, but not the pars compacta; the motor cells of the superior colliculus; the motor cells of the reticular formation of the brain-stem; the motor nuclei of the cranial nerves; Dieter's nucleus; and the roof and dentate nuclei of the cerebellum.

In the cerebral cortex specificity for this type of cell is almost absolute, for apart from the lesions of the Betz cells only a few smaller pyramidal cells of the third lamina were affected. The granule cells were never involved.

In the cerebellum a similar specificity was shown since the brunt of the pathological process was borne by the nucleus fastigius; the Purkinje cells, on the other hand, were never affected. It is curious, however, that the dentate nucleus was only mildly affected, for it contains motor cells similar to those of the nucleus fastigius.

From histological knowledge one would have predicted marked lesions in the globus pallidus. The results show that here the cells, contrary to the findings in the experimental disease, were always free from lesions.

According to Malone the pigmented portion of the substantia nigra (that is, the pars compacta) does not contain cells of the motor type. Yet death of nerve cells was common here, and uncommon in the non-pigmented pars reticularis containing motor cells. This was also the experience of Horanyi-Hechst, who suggests that the involvement of the pars compacta is due to the presence of melanin. He points out that the pigmented cells of the locus coeruleus which likewise contain melanin, were also involved.

So far then as the globus pallidus and the substantia nigra in human poliomyelitis are concerned, the motor cells are apparently not very susceptible to the virus. Similar findings were noted in the hypothalamus of these cases.

The nucleus of Darkeschewitsch was involved in the one case in which it was examined. Horanyi-Hechst found that this nucleus was injured in half of his cases, so that evidently it is susceptible to the virus.

The magno-cellular portion of the red nucleus did not appear in the present sections. From the findings of others it appears that this nucleus may sometimes be involved, but not often (Stiefler and Schenk, Horanyi-Hechst, Környey (1933 b)).

In the cases reported here the motor cells of the superior colliculi were mildly involved in three of the six cases, the motor cells of the reticular formation and of the nuclei of the cranial nerves showed occasional and variable involvement, while Deiter's nucleus was regularly and severely involved.

As a general rule the motor nuclei of the brain-stem were more regularly and more severely affected than the sensory nuclei, except for the pigmented cells of the locus coeruleus.

Kino (1928), from a study of two cases, maintains that there is selective affinity of the virus for the motor nuclei of the brain-stem. Horanyi-Hechst found this in only three of his 28 cases. Környey, from an extensive investigation of both human and monkey poliomyelitis, can find no evidence of selective affinity in the brain-stem. In experimental poliomyelitis Warburg (1931) and Luhan (1937) reported similar findings. Possibly the difference in these results is dependent on the strain of the virus.

André-Thomas and Lhermitte (1929) maintain from an examination of one case that the virus has an elective affinity for motor cells throughout the central nervous system. It is clear, however, that there is no absolute specificity of the virus for cells of the motor type in the cranial part of the nervous system, except perhaps in the cerebral cortex and the cerebellum. But neither is it absolute in the spinal cord, for though the brunt of the pathological process is borne by the anterior horn cells, occasional cells of Clarke's column and of the posterior root ganglia are also affected. At the same time it may be said that cells of the motor type are more often and more severely affected than any others.

B. THE PORTAL OF ENTRY OF THE VIRUS.

(1) THE OLFACTORY ROUTE.

Since the hypothesis was propounded originally by Flexner and Lewis (1910), it has been widely accepted that the virus of poliomyelitis reaches the central nervous system from the nasopharyngeal mucosa by passage along the filaments of the olfactory nerves to the olfactory lobes of the brain, and thence to the medulla and the spinal cord.

In the present cases, apart from a small poliomyelitic focus in the cornu Ammonis of one, and the involvement of the anterior perforated substance in four, lesions in the rhinencephalon were absent. As was pointed out, the infiltration of the anterior perforated substance can be explained on other grounds.

In the four cases in which they were examined, the olfactory bulbs were free from pathological change. This was the experience of Horanyi-Hechst in 15 cases, of Harmon (1937) in nine cases, of Sabin (1939) in 12 cases, and of Harbitz and Scheel (1907 b) and Stillerman and Fischer (1938) each in one case. Clinically, the cases reported by Stillerman and Fischer and by Sabin were bulbar in type. Robertson (1940), in his study of the olfactory bulbs obtained from eleven cases in the recent Victorian epidemic, found inflammatory changes in only one of them; even here there was no indication that the reaction was certainly due to poliomyelitis. Landon and Smith (1934) found characteristic inflammatory changes in less than a quarter of 56 olfactory bulbs, obtained from 40 cases of the disease. They suggested three possible explanations; first, that the inflammatory changes, unlike those eleswhere in the nervous system, might be extremely short-lived; second, that the virus might pass through the olfactory bulb without leaving traces of its passage; third, that the olfactory pathway in human beings is not necessarily as frequent a route of entry of the virus as is commonly held. Of the three possible explanations they were in favour of the third, and suggested that nerve roots elsewhere are equally important portals of entry.

It is well known that the virus of poliomyelitis can pass along peripheral nerves without giving rise to inflammatory or degenerative changes. We might accept this explanation also for the olfactory bulbs if it were not that it is in direct contradiction to the results obtained in the experimental animal. Sabin and Olitsky (1937) examined the olfactory bulbs of 18 monkeys inoculated with the virus intranasally. In all of them they found characteristic infiltrative changes of the outer layers of the olfactory bulbs, associated with necrosis and neuronophagia of the mitral cells. In 13 further monkeys infected by the intracerebral, subcutaneous, intraocular and intrasciatic routes respectively, with one doubtful exception, no lesions were detected in the olfactory bulbs. It will be seen, therefore, that in the monkey the olfactory bulbs act as reliable indicators of the portal of entry of the virus.

When one considers the close resemblance of the lesions in man and the monkey elsewhere in the central nervous system, it seems unlikely that the virus could pass by way of the olfactory nerves in man without producing changes in the olfactory bulbs and the rest of the rhinencephalon similar to those found in the monkey. Yet apart from the small number of positive cases described by Landon and Smith, the results are uniformly negative. One is driven to the conclusion either that infection by the olfactory route in man is much less common than was formerly thought, or that olfactory infection is not commonly fatal. Burnet's work (1936) on louping-ill in the rat suggested to him that the latter alternative accounted for immunisation of the average child infected with poliomyelitis.

Using vesicular stomatitis virus, Sabin and Olitsky (1938) came to a similar conclusion.

The insignificance of the lesions of the rhinencephalon in human cases of poliomyelitis prompts consideration of the evidence for and against the olfactory route, and discussion of alternative portals of entry.

The orthodox opinion, recently reaffirmed by Flexner (1936), is based ultimately on the fact that in rhesus monkeys intranasal inoculation is the only regularly successful method of attaining infection by "normal" routes. (Burnet, Jackson and Robertson (1939)). As has been mentioned already, a number of observers have noted that surgical interruption of the olfactory pathway prior to intranasal inoculation of the virus prevents the development of the disease. A similar protective action follows the application of various protein coagulants to the nasal mucosa (Armstrong and Harrison (1935), Schultz and Gebhardt (1937), etc.).

The experimental findings with regard to the portal of entry in the rhesus monkey are not necessarily applicable, however, to the disease in man. King (1939) believes that the relative importance of the nasal route in monkey poliomyelitis may be purely an artificial condition, brought about by some degree of change in the virus. He points out that with fresh strains subcutaneous and intradermal inoculations readily result in infection, but on repeated passage the property of cutaneous infectivity is soon lost. Furthermore, certain other viruses (louping ill, equine encephalomyelitis, yellow fever and Japanese encephalitis B) may under altered conditions show a predilection for the olfactory route,

although under natural conditions they are highly infectious by subcutaneous inoculation.

(2) THE TONSILLO-PHARYNGEAL ROUTE.

The possibility of the tonsillo-pharyngeal region as a portal of entry of the virus was originally suggested by Bülow-Hansen and Harbitz (1899). Until recent years, however, there has been little evidence to support their view.

Since 1928, over 60 cases of poliomyelitis following tonsillectomy and / or adenoidectomy have been reported. Of these, at least 47 were bulbar in type. The cases reported include those of Ayer (1928), Aycock and Luther (1929), Silverman (1931), Fischer and Stillerman (1937), Stillerman and Fischer (1938), Eley and Flake (1938), Anderson and Dixon (1938), Culley (1938), Scholes (1938), Koskoff, Amschel and Lebeau (1939) and Stebbins, Gillick and Ingraham (1939).

The comments of Stillerman and Fischer (1938) in their recent paper are very much to the point. "The extirpation of the tonsils and adenoids removes a protective barrier, and may provide sufficient trauma to the regional nerves to permit the passage into the central nervous system of the virus, which might not otherwise have entered." These authors go on to point out that the nerves from the tonsillar and pharyngeal walls have their nuclei in the bulb. From this the occurrence of the bulbar type of poliomyelitis, when infection takes place by the tonsillo-pharyngeal route, is readily explained.

From the experimental aspect Sabin (1938) has found that when the virus of poliomyelitis is injected into the tonsillo-pharyngeal region of Macaca mulatta, the disease is, almost without exception, bulbar or bulbo-spinal in type. From these results, however, we have no evidence that the virus is capable of penetrating the intact mucous membrane of the tonsillo-pharyngeal region. Sabin, like Brodie and Elvidge (1934), points out that the disease fails to develop following nasal instillation of the virus in Macaca Mulatta whose olfactory pathways have been interrupted, despite the fact that most of the virus runs down into the pharynx.

On the other hand, Burnet, Jackson and Robertson (1939), using Macacus cynomolgus (Macaca irus), have succeeded in producing infection by swabbing of the tonsillo-pharyngeal region with virus. In a later paper, Burnet (1940) states that so far eight monkeys have been specifically infected by this route. In five of these the initial paralysis was bulbar in type. The clinical signs in two of these cases were confirmed histologically. The other three monkeys had initial paralysis of the hind limbs, and it was concluded that these animals were probably infected through the intestine after swallowing the virus. Except for two cases, all these animals had received preliminary spraying with zinc sulphate.

The clinical evidence of Scott-Brown (1931) and from the recent South Australian and Victorian epidemics suggests that the virus in man behaves as it does in Macaca irus, i.e., that it is capable of penetrating the intact mucous membrane of the tonsillo-pharyngeal region.

(3) THE GASTRO-INTESTINAL ROUTE.

The initial symptoms of gastro-enteritis and the post-mortem findings were considered by Bülow-Hansen and Harbitz (1899) to be indicative of the entrance of the virus of poliomyelitis through the gastro-intestinal tract. Opponents of this theory have pointed out, however, that gastro-intestinal symptoms are common features of any pyrexial disease of childhood. Others believe that the intestinal lesions are not necessarily indicative of infection by this route, and that they might equally well be the result of swallowed virus (Flexner (1912), Marinesco, Manicatide and State-Draganesco (1929)), or be part of a generalised reaction of the lymphatic tissues to the infection (Abramson (1918)).

Wickman (1911) contended that primary lower limb palsy was the result of infection via the gastro-intestinal tract. Römer (1911) and Fairbrother and Hurst (1930) pointed out, however, that a similar initial localisation of the paralysis occurred in a high percentage of monkeys inoculated with virus intracerebrally. This finding is contrary to the experience of Burnet, Jackson and Robertson (1939); these authors therefore support Wickman's contention. In this connection it may be mentioned that infection by the gastro-intestinal route does not necessarily lead to primary lower limb palsy. Kling, Levaditi and Hornus (1934) and Burnet, Jackson and Robertson (1939) each found in one case that after the injection of poliomyelitis virus into a loop of intestine that the initial palsy occurred in the upper limbs.

The results of attempts to produce infection by the gastro-intestinal route in monkeys have been extremely variable. While Leiner and von Wiesner (1910), Kling, Levaditi and Lépine (1929, 1931, 1933), Demme (1930), Pette, Demme and Környey (1932), Saddington (1932), Toomey (1933, 1934 a, 1934 b), Kling, Levaditi and Hornus (1934) and Burnet, Jackson and Robertson (1939) have obtained positive results, others, including Landsteiner and Levaditi (1909, 1910, 1922), Leake (1918), Amoss (1928), Schultz (1929), Thompson (1930), Clark, Schindler and Roberts (1930), Clark, Roberts and Preston (1932), Plotz (1934) and Flexner (1936) have met with no success in attempts to produce infection by this route.

A number of the positive results must be considered with reserve. Both Clark, Roberts and Preston (1932), and Flexner (1936) have drawn attention to the fact that attempts to produce poliomyelitis by feeding might result in infection by the nasal or tonsillo-pharyngeal routes, especially if regurgitation of virus occurs. The same applies to the use of the stomach tube, since the same regions may be contaminated during its withdrawal. It must be pointed out, however, that successful results have been obtained by the injection of the virus into a loop of intestine, by which the chances of such contamination have been largely eliminated.

It is noteworthy that most of the successful results have followed the use of Macaca irus (cynomolgus). Even with the use of this species of monkey, however, the results have been inconstant. Burnet, Jackson and Robertson (1939) believe that this may be dependent on the strain of virus. It may well be, also, that the property of gastro-intestinal

infectivity, like that of cutaneous infectivity, is diminished or lost on repeated passage of the virus.

Care should be taken not to dismiss the possibility of infection by the gastro-intestinal route in man too lightly, for poliomyelitis virus is certainly more highly infectious for susceptible human beings than it is for Macaca irus. While there is as yet no fully conclusive evidence of infection by this route in man, it is of interest to note that a number of epidemics have been described in which the transmission of the disease has been attributed to milk.

It appears probable that infection with the virus of poliomyelitis is possible by all three routes in monkeys. In man infection through the olfactory nerves may occur, but is either less common than was formerly thought or the virus is localized here and immunisation results. Cases with initial bulbar palsy are probably due to infection by the tonsillo-pharyngeal route. It would be possible for cases with initial palsy in the lower limbs to be due to infection through the gastro-intestinal route, but the evidence is conflicting and is based mainly on experimental work which has no counterpart in human infection. Stillerman and Fischer (1938) have referred to the work of Leake (1935), who has found that, in paralysis developing after the inoculation of poliomyelitis virus, the level of the spinal cord first affected corresponds to the extremity inoculated, so that if gastro-intestinal infection occurred the legs would be affected first. At the moment, however, there is no fully conclusive evidence of infection by this route.

The three portals of entry mentioned already, are probably not the only ones. In as yet unpublished results I have succeeded in infecting a Macaca Mulatta monkey through the intact conjunctiva or the cornea, after preliminary cauterization of the lacrimal punctae, and, to make doubly sure, spraying of the nose with saturated picric acid. A second experiment failed to confirm this result. The positive result, however, is interesting in view of the reports of conjunctivitis in some epidemics.

Lumsden (1938) believes that the disease may be derived from an animal reservoir with some biting insect as vector. This theory must be kept in mind, in view of recent reports which show that freshly isolated strains of poliomyelitis virus are highly infective by intradermal inoculation (Trask and Paul (1936), Stimpert and Kessel (1940)).

The portals of entry of the virus in the cases described in this thesis cannot be determined definitely. Cases 1 and 5 with initial bulbar palsy resulted probably from infection by the virus by the tonsillo-pharyngeal route. One would conclude that Cases 4, 5, 6 and 7 were infected by other than the olfactory route, because of the absence of lesions in the olfactory bulbs. It would be unwise to offer any suggestions in regard to the remainder of the cases in view of the conflicting experimental evidence at our disposal.

There are many aspects of the pathology of poliomyelitis which for the time being must remain uncertain, despite the vast amount of research work which has been performed during the last hundred years.

A stage is being attained, however, when the words of Jaeger concerning cerebro-spinal meningitis, will be applied appropriately to poliomyelitis. He wrote, "The diffusion of this scourge appears to us now like a mountain range free from mist; only peaks without foundation are visible; yet we are perceiving now more and more the great bases upon which the peaks arise."

(5) SUMMARY AND CONCLUSIONS.

An account has been given of the history of the pathology of poliomyelitis and of the recent advances.

Examination of the central nervous system of eight cases of poliomyelitis by routine neuro-histological methods led to the following conclusions;

The distribution of the meningeal infiltration is modified by the flow of the cerebro-spinal fluid.

Necrosis of nerve cells in the cerebral cortex is confined to the area giganto-pyramidalis, and almost entirely to the Betz cells of that area.

The rhinencephalon (including the olfactory bulbs) was surprisingly little involved, lesions occurring only in the anterior perforated substance and in one instance in the cornu Ammonis.

Evidence for the process of neuronophagocytosis was seen in the case of the pigmented cells of the locus coeruleus.

Selective affinity of the virus for cells of the motor type is not absolute except perhaps in the cerebral cortex and the cerebellum.

Nevertheless motor cells are more often and more severely involved than sensory cells.

It is suggested that the medial longitudinal bundle is an important pathway for the spread of the pathological process.

Infection by the virus of poliomyelitis by the olfactory route is either less common than was formerly thought, or is not commonly fatal, i.e., the virus is localized in the olfactory bulbs, and immunisation takes place.

Cases with initial bulbar palsy probably result from infection by the tonsillo-pharyngeal route.

There is no fully conclusive evidence for infection by the virus by the gastro-intestinal route in man.

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PHOTOGRAPHS.

(1) Spinal Cord: Vacuolation of anterior horn cells.

(2) Spinal Cord: Advanced stage of neuronophagocytosis. The microglial cells are arranged in the shape of the previously present anterior horn cells.

(3) Cerebral Cortex:- Focal infiltration of Betz cells lying in the 5th. layer of the area giganto-pyramidalis of Brodman.

(4) Cerebellum: Severe lesions involving the roof nuclei, with marked destruction of nerve cells.

Essential Facts of the Case Histories.

Case	1	2	3	4	5	6	7	8
Sex	Male	Female	Female	Male	Male	Male	Male	Male
Age	7 years	8 years	14 years	20 years	5 years	46 years	11 months	38 years
Fever	+	+	?	+	+	+	+	+
Vomiting or nausea	+		+			+	+	+
Headache	+	+	+	+		+	—	—
Stiffness of neck or spine	+	+	?	+	+	+	+	+
Tremor	+	+	?			+	—	+
Dysphagia	+		+	+	+	+	—	+
Retention of urine			—	+			—	+
Initial palsy	Lt. Palate Lt. Face	Rt. Arm	Leg	Lt. Triceps Lt. Leg	Rt. Face	Rt. Arm	Intercostal muscles	Rt. Leg
Type of case	Bulbo-spinal	Upper spinal	Ascending type	Spino-bulbar	Bulbo-spinal	Spino-bulbar	Spinal	Spino-bulbar
Duration of illness from initial symptoms	6 days	3 days	2 days	7 days	9 days	2 days	5 days	7 days
Duration of illness from initial palsy	4 days	1 day	1 day	2 days	3 days	1 day	3 days	5 days

Table 1.

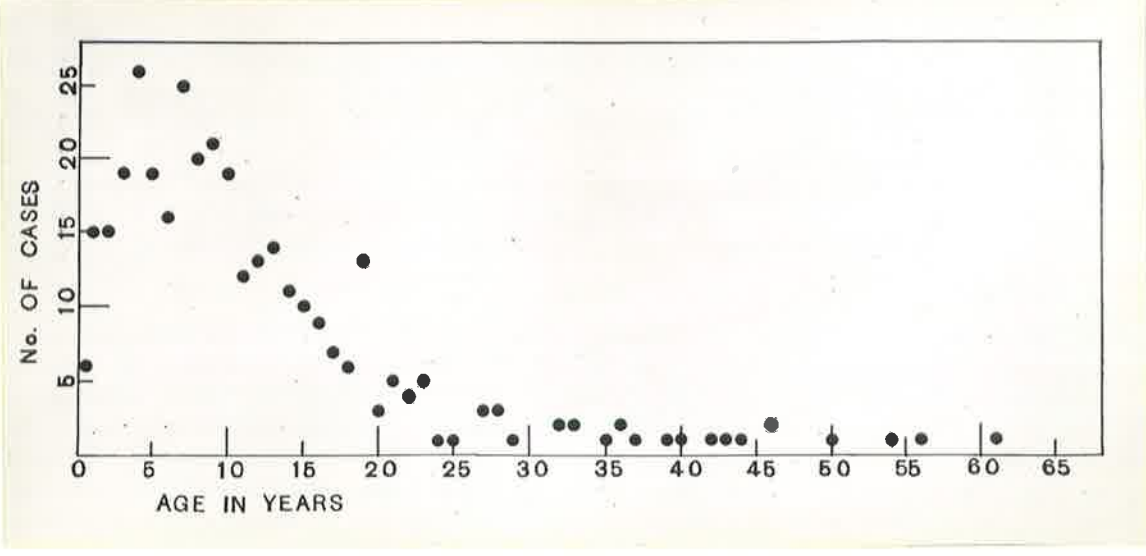
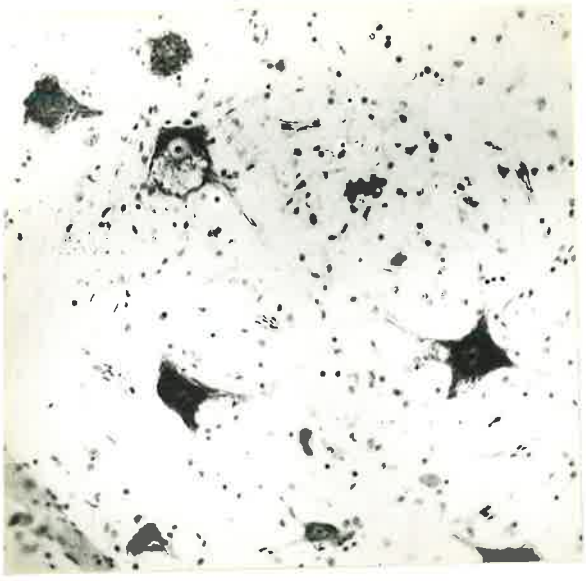
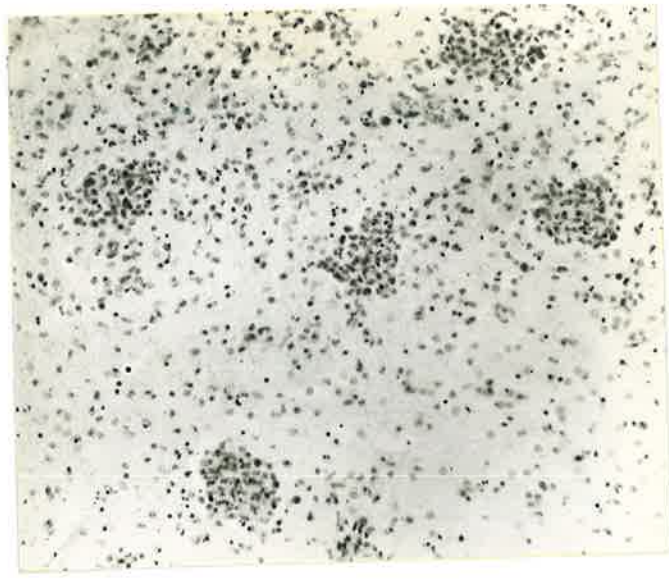


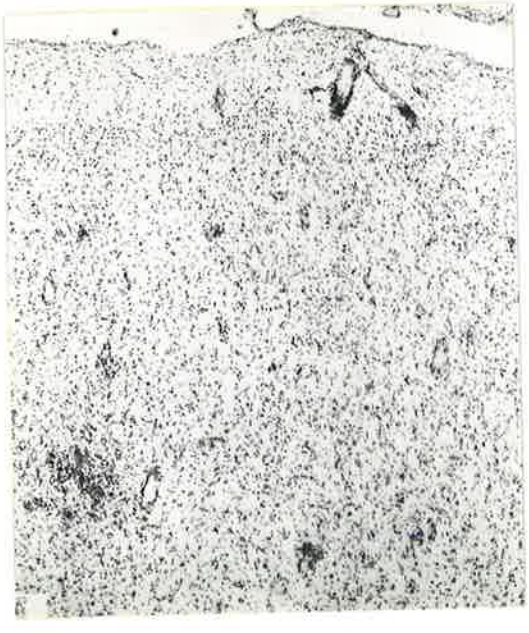
Figure 1.



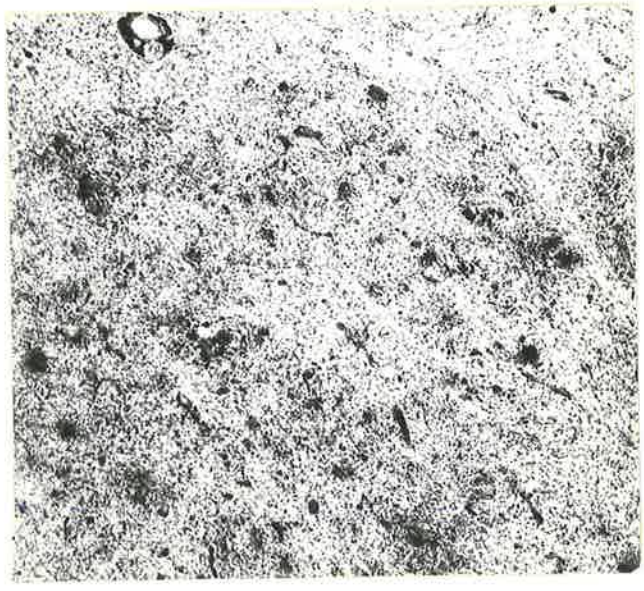
Photograph 1.



Photograph 2.



Photograph 3.



Photograph 4.

STUDIES IN EXPERIMENTAL POLIOMYELITIS.

(1) THE EFFECT OF THE INOCULATION OF POLIOMYELITIS VIRUS INTO DENERVATED AREAS OF SKIN, AND THE MODE OF PROPAGATION OF THE VIRUS FROM SUCH AREAS TO THE CENTRAL NERVOUS SYSTEM.

By Charles Swan (1)

(From the Institute of Medical and Veterinary Science, Adelaide)

In his suggested basis for classification of viruses affecting the nervous system, Hurst (1936) stated that the stricter neurotropes, including the virus of poliomyelitis, when inoculated into a denervated area did not lead to infection.

Beginning their investigations with this assumption, German and Trask (1938) found, contrary to expectation, that the intradermal injection of poliomyelitis virus into denervated areas not only failed to prevent infection, but, in the majority of cases, resulted in an increased susceptibility to the virus.

The present paper deals with a series of experiments designed to confirm the work of German and Trask, and to throw light on the method of transmission of the virus from denervated areas to the central nervous system.

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(1) Working with the aid of a grant from the National Health and Medical Research Council, Australia.

MATERIALS and METHODS

(1) THE VIRUS

The virus was derived from two local strains isolated during the major epidamic in Australia of 1937. It was prepared by combining approximately equal parts of spinal cord obtained from the third, fourth fifth and sixth passages of a Victorian strain and the first passage of a Tasmanian strain. The mixture thus prepared was submitted to two passages through Macaca irus (M. cynomolgus). In the first experiment the spinal cord of the second passage served as a source of virus. In the remainder of the experiments, except where otherwise stated, a representative mixture of later passages of the virus, obtained in the course of this and other investigations was used.

The spinal cords were preserved in 50% glycerine in normal saline solution buffered by phosphates to a pH of 7.0. They were kept in the refrigerator at a temperature of -10°C.

On the day of use they were prepared freshly as 10% suspensions by grinding with sterile washed sand and normal saline solution. In Experiment 3., the normal saline solution was replaced by equal parts normal monkey serum and normal saline solution, in order to prevent intravascular clotting. In Experiment 1., the suspension of spinal cord was centrifuged at 1500 R.P.M., for five minutes. Later, however, a standard of 3000 R.P.M., for 15 minutes was adopted.

The usual dose administered intradermally was 2 cc., in 10 piqures of 0.2 cc.

(2) MONKEYS.

A total of 23 apparently healthy monkeys, of which 21 were Macaca mulatta (M. rhesus) weighing from 3.5 to 4.5 kgm., and 2 were Macaca irus

weighing approximately 1 kgm., was used.

Daily rectal temperatures were recorded for up to four weeks following inoculation.

At post-mortem, sections of the cervical and lumbar regions of the spinal cord, together with the olfactory bulbs, were saved for histological examination. In certain instances the brain and brain-stem were retained also. The remainder of the spinal cord was preserved as a source of virus.

(3) DENERVATION PROCEDURES.

In all cases the site chosen for operation was on the left side. Ether anaesthesia, after induction with A.C.E. mixture, was used as a routine.

(a) SKIN FLAPS.

In the formation of isolated skin grafts, the two-stage method described and illustrated by German and Trask was adopted. It aimed at producing circular denervated areas of skin approximately 10 cm., in diameter.

An incision was made in the form of a semicircle, and a flap composed of skin and superficial fascia was raised from the underlying muscle for a distance of about 6 cm. After placing a black silk thread marker at the apex of the angle between the flap and its original bed, the flap was replaced and the incision sutured with silk.

About 10 days later a second incision was made. This was similar to the first but extended in the opposite direction. It connected with the ends of the original incision and thus completed a circle. A flap

was elevated as before until the site of attachment of the pedicle of the first flap was undercut completely. This was facilitated by the presence of the black silk thread. The second flap was then replaced in its original bed and the incision was sutured as in the first stage.

The following denervation procedures were based on the work of Kramer and Todd (1914), Potts (1914), Woollard and Phillips (1932), and Malméjac and Haimovici (1936). According to these authors high section of the main nerve trunks would lead to complete degeneration of both somatic and autonomic fibres of the distal portions of the fore and hind limbs. It should be pointed out, however, that while subscribing to this view so far as the somatic nerves are concerned, Brauecker (1929) and McDowall (1938), believe that a small number of sympathetic fibres may still persist.

(b) DENERVATION of the DORSUM of the HAND.

This was performed through an incision centred over the clavicle and passing along the medial border of the biceps muscle and the posterior border of the sterno-mastoid muscle. By dissection it was possible to display the brachial plexus throughout its course. The plexus, from the commencement of its trunks to the division of the cords into the nerves of supply to the upper limb, was removed. In addition the supraclavicular and intercosto-brachial nerves were divided, a portion of each nerve being removed in order to ensure adequate separation of the proximal and distal ends.

(c) DENERVATION of the DORSUM of the FOOT.

Through an anterior vertical incision in the mid-inguinal plane, the femoral nerve was exposed and divided at its point of emergence from behind the inguinal ligament. No attempt was made to divide the obturator nerve since it plays no part in the innervation of the foot. Posteriorly a vertical incision was made at the mid-point between the ischial tuberosity and the greater trochanter. After division of the gluteus maximus muscle the sciatic nerve was displayed and a portion of it was removed.

EXPERIMENT 1.

The initial experiment was devised not only to compare the intradermal infectivity of the virus in denervated and normal areas, but also to determine whether the time interval elapsing between the completion of the operative procedures and the inoculation was of significance.

In three monkeys the virus was inoculated into denervated skin flaps, which at the time of inoculation had been completed for 63, 29 and 14 days, respectively. Four normal animals served as controls. One of these was injected intracerebrally, and the remainder in areas of skin similar in size and situation to the flaps.

The plan and results of the experiment are presented in Table 1.

TABLE 1.

radermal inoculation of poliomyelitis virus into denervated and normal skin.

Period (in days) between completion of flap and inoculation	Site and type of inoculation.	Dosage of 10% virus	Day of onset of paralysis	Distribution of paralysis (in order of appearance) and severity.
63	Denervated skin flap on outer aspect of left thigh, intradermal.	2 cc.	6.	Right arm +++ right leg +.
29.	"	2 cc.	-	None
14.	"	2 cc.	11.	Both arms and extensor muscles of neck +++.
Control	Area of normal skin similar in situation and size to flap, intradermal.	2 cc.	-	None
"	"	2 cc.	-	None
"	"	1.3 cc.	-	None
"	Left hemisphere, intracerebral.	1 cc.	5.	Right arm and leg ++.

+++ = Severe paralysis.
++ = Moderate ".
+ = Mild "

The above results show that following intradermal inoculation of virus into areas of skin deprived of nerve supply, severe poliomyelitis occurred in monkeys S 10 and S 16, in which the skin flaps had been completed for 63 and 16 days respectively, but not in S 11 (29 days); whereas the animals acting as controls, with the exception of S 26 which had been inoculated intracerebrally, failed to develop the disease. It should be noted, however, that one of the controls, S 20, received 1.3 cc. of virus instead of the usual dose of 2 cc.

In S 10, despite the fact that an interval of 63 days had elapsed since the operative procedures, the enhanced susceptibility still persisted.

The initial paralysis in monkeys S 10 and S 16 occurred in the right arm and the arms and extensor muscles of the neck, respectively. Such a localisation of the paralysis is contrary to that which might have been expected if the virus had travelled from the site of inoculation to the spinal cord along axonic pathways.

EXPERIMENT 2.

In the second experiment 13 monkeys were used. Two of these (S 31 and S 35) had been submitted to operative procedures devised to produce denervation of the dorsum of the foot and hand, respectively. Following operation a period of approximately 100 days had been allowed to elapse, by which time it was considered that complete degeneration of the somatic nerve fibres and of the vast majority, if not all, of the autonomic fibres would have occurred.

Two animals (S 30 and S 32) on which 93 days previously, denervated skin flaps had been prepared on the thigh and arm respectively, were

included for the purposes of comparison and also to determine whether enhanced susceptibility which had been shown to be present at 63 days still persisted. With all of the denervated monkeys a further animal was used as a control.

Finally, in an attempt to reduce the likelihood of transport of virus along nerve fibres which might have regenerated during the interval between operation and inoculation, it was decided to test the effect of nerve section just prior to inoculation in an animal (S 37) on which a denervated skin flap had been completed 66 days previously. In this animal and in a control monkey (S 46) the femoral and sciatic nerves were divided by operative procedures similar to those described for denervation of the dorsum of the foot. In addition a long horse-shoe shaped incision was made which passed along the line of the inguinal ligament, then along the iliac crest, and finally downwards and outwards along the lower border of the gluteus maximus muscle. The skin and the subcutaneous tissues lying below the level of the incision were then elevated from the underlying structures for a distance of approximately one inch. While these operative procedures no doubt led to the interruption of all somatic neural connections to the flap which may have regenerated, no such claim can be made for nerve fibres belonging to the autonomic system. Nevertheless, it would seem that their number would be greatly reduced.

On account of the possibility of haematogenous transport of the virus it was decided to test the blood of monkeys submitted to denervation procedures, i.e. S.30, S 31, S 32, S 35, and S 37, for virus at intervals of ½, 2 and 24 hours after inoculation. By means of cardiac puncture 9 cc., of blood were withdrawn into a glass syringe containing 1 cc. of

2% sodium citrate in normal saline. After thorough mixing the blood specimens obtained at the times specified were pooled. The three specimens of pooled blood were each tested for the presence of virus by means of combined intracerebral and intramuscular inoculations. Specimen 2 was incomplete as, despite several attempts, no blood could be obtained from S 37 two hours after inoculation.

The plan and results of Experiment 2., are embodied in Tables 2., and 3.,

TABLE 2.

Intradermal inoculation of poliomyelitis virus into denervated and normal skin.

Operation, and number of days elapsing between its completion and inoculation.	Site of intradermal inoculation of 2 cc., of 10% virus.	Day of onset of paralysis	Distribution of paralysis (in order of appearance) and severity.
Skin flap, outer aspect of left thigh, 93.	Skin flap.	-	None
Control	Area of skin similar in size and situation to flap on S 30.	8	Right leg +++, left leg +++, arms +.
Skin flap, deltoid region of left arm, 93	Skin flap	-	None, though temperature rose to 104°F. on 10th day.
Control	Area of skin similar in size and situation to flap on S 32	-	None, though temperature rose to 104.3°F. on 8th day.
Denervation of dorsum of left foot, 105.	Dorsum of foot	-	None, though temperature rose to 104.8°F. on 7th day
Control	Dorsum of left foot	12	Right leg +. Paralysis quickly disappeared.

TABLE 2. (Continued)

Denervation of dorsum of left hand, 102.	Dorsum of hand	7	Arms and extensor muscles of neck +++, left leg +++, right leg ++.
Control.	Dorsum of left hand	12.	Right leg ++, left leg +.
Skin flap, outer aspect of left thigh, 66. Femoral and sciatic nerves etc., divided just prior to inoculation. (See text).	Skin flap.	11.	Legs +++.
Control. Femoral and sciatic nerves etc. divided just prior to inoculation. (See text)	Area of skin similar in size and situation to flap on S 37.	-	Found dead on 7th day. Animal showed neither rise in temperature nor paralysis.

TABLE 3.

ence of poliomyelitis virus in the blood of monkeys inoculated intradermally.

Interval between inoculation of S 30, S 31, S32, S 35 and S 37, and collection of blood samples	Dosage of pooled blood and sites of inoculation.	Day of onset of paralysis	Distribution of paralysis (in order of appearance) and severity.
½ hour	0.5 cc., intracerebrally left hemisphere; 10 cc., intramuscularly, thighs.	-	None
2 hours	0.5 cc., intracerebrally left hemisphere; 10 cc., intramuscularly, thighs.	9	Left side of face +++, limbs +
24 hours	1 cc., intracerebrally left hemisphere; 10 cc., intramuscularly, thighs	-	None, though temperature rose to 106F., on 6th day.

It will be seen from Table 2., that only two of the five monkeys (S 35 and S 37) which had been submitted to denervation procedures became infected, while in contrast to the first experiment three of the five control animals (S 40, S 47 and S 52) contracted the disease. On comparing the intensity of the disease, however, it will be noted that while the denervated monkeys developed severe paralysis, only one of the control animals (S 47) manifested paralysis of the same degree.

In contrast to the first experiment the paralysis in the animals subjected to denervation procedures occurred initially in the limb which had been inoculated. This did not occur, however, in the control monkeys with intact neural connections. S 40 and S 47 which were injected in the left lower limb developed their initial paralysis in the opposite hind limb, while S 52 which was inoculated in the dorsum of the left hand became paralysed first in the right lower limb.

The virus was present in the pooled specimens of blood obtained 2 hours after inoculation. Though the result is inconclusive, it is possible that virus was present also 24 hours after inoculation, as the monkey in which the blood was tested developed a temperature of 106°F., on the 6th day, and was subsequently resistant to intracerebral inoculation.

One month after the first inoculation the survivors of the second experiment were reinoculated. Virus of the same passage as was used in Experiment 1., was administered in the hope that it might prove more effective by the intradermal route than the later passages. None of the animals, however, developed either a rise in temperature or paralysis. Finally, they were tested for immunity by intranasal

administration of the virus on three successive days. One animal (S 32) became paralysed on the 7th day, while the others remained healthy. It is of interest to note that in S 32 the paralysis was extremely severe, and was slightly more marked in the arm on which the denervated skin flap was situated.

EXPERIMENT 3.

The third experiment was planned in order to determine whether the presence of a denervated skin flap would still result in an enhanced susceptibility and whether the initial paralysis bore any relationship to the area of denervation if the virus were administered by another route. Two monkeys, S 43 and S 42, were inoculated intravenously with 5 cc., of 10% virus on three successive days. The former animal had had a skin flap prepared on the outer aspect of the left thigh 36 days previously. The latter monkey acted as a control. Both animals became paralysed on the fifth day. In S 43 the initial paralysis occurred in the left arm; later the left leg and the right leg were involved. In the control animal, S 42, the legs were affected first; later the arms were moderately involved.

The intensity of the paralysis was slightly more marked in S 43, but the difference was not sufficient to support a claim of increased susceptibility.

In S 43 there was no relationship between the site of the denervated skin flap and the localisation of the initial paralysis.

HISTOLOGICAL FINDINGS.

In the majority of cases the clinical findings were confirmed by microscopical examination of the spinal cord. In addition in monkeys, S 16, S 35, S 37, S 42, S 43, and S 47, the olfactory bulbs were examined with negative results.

Histological examination of the spinal cord and brain-stem of monkey S 66, in which the pooled specimens of blood obtained two hours after inoculation were tested, revealed lesions typical of experimental poliomyelitis.

DISCUSSION.

The results as a whole show that the intradermal administration of poliomyelitis virus to eight monkeys subjected to denervation procedures led in four to paralysis, which in all instances was severe in type. Three of a similar number of control animals developed the disease when inoculated in the same site, but in only one of them was the disease of similar intensity.

It is difficult to explain the difference in the results of the first and second experiments. In the former, two of the denervated animals became paralysed, while the controls remained healthy. In the latter, while a similar number of animals subjected to denervation procedures developed the disease, three of the control animals were affected also. The difference may be due to some change in the virus brought about by further passages by various routes. One would have expected, however, that further passage would have diminished rather than increased the cutaneous infectivity of the virus.

German and Trask, in 10 out of 11 instances of intradermal inoculation into denervated areas, found that the limb first affected was on the side of the inoculation. In the present experiments, while in the denervated monkeys there was a definite relationship between the site of inoculation and the localisation of the initial paralysis in two out of four instances, it was surprising to note that in the control animals with intact neural connections no such relationship was apparent. Monkey S 52 is a case in point. This animal though inoculated in the dorsum of the left hand manifested its initial paralysis in the right lower limb.

The possible routes by which virus may reach the central nervous system from a denervated area are:

(1) by sympathetic nerve fibres left unsectioned.

(2) by regenerated nerve fibres.

(3) by the blood stream:

(a) by absorption by the central ends of the sectioned nerves.

(b) by excretion on to the nasal mucosa and thence by the olfactory pathway.

(c) directly through the blood vessels of the spinal cord or of the posterior root ganglia.

These alternatives will be considered in conjunction with the results of the investigation.

If the virus had spread to the central nervous system by means of automomic fibres left unsectioned by operative procedures, there should be a relationship between the site of inoculation and the localisation of the initial paralysis. S 10 and S 16 which were inoculated in the left thigh manifested their first paralysis, however, in the upper limbs.

Moreover, one of the control animals, S 52, in which the sympathetic and somatic nerve fibres were intact, after inoculation in the dorsum of the left hand became paralysed first in the right leg.

It is probable that transmission of the virus by regenerated nerve fibres can be eliminated in S 16 because of the relatively short interval (14 days) between the completion of the skin flap and inoculation. It can be eliminated definitely in S 37 because the nerve fibres were divided just prior to inoculation. The argument used in reference to transmission by the sympathetic fibres can also be applied here.

It is conceivable that the blood stream might play an intermediate role in carrying the virus to the peripheral nerves. If this theory were correct it might be expected that the virus would travel mainly, if not entirely, along the central ends of the sectioned peripheral nerves, for Hurst (1930) has shown that the virus does not travel readily following intraneural inoculation unless the nerve fibres are subjected to trauma. Against carriage of the virus by this means the argument used in reference to propagation via the autonomic fibres can again be applied. Furthermore, correlation of the initial palsy with the site of inoculation was absent in monkey S 43, despite the fact that the virus was introduced directly into the blood stream and thus had ample opportunity for coming into contact with the central ends of the sectioned nerves.

Lennette and Hudson (1935) believe that, following intravenous inoculation, the virus reaches the central nervous system by excretion on to the nasal mucosa and subsequent entry along the olfactory pathway. Sabin and Olitsky (1937) have shown that the olfactory bulbs are reliable indicators of infection by the nasal route. In the present

investigation examination of the olfactory bulbs showed that they were free from lesions, so that this explanation can be rejected also.

By a process of elimination it would seem probable that the virus reaches the central nervous system either through the blood vessels of the spinal cord or of the posterior root ganglia. In favour of the latter alternative is the observation of Flexner and Amoss (1914) that poliomyelitis virus administered intravenously is detectable in the posterior root ganglia before it is demonstrable in the central nervous system. Moreover, in the human disease sensory disturbances and hyperaesthesia are common initial symptoms. It would be possible for the operative procedures to result in a breakdown of the blood-central nervous system barrier. It is to be expected, however, that such a breakdown would be localised rather than generalised. So far as a localised breakdown is concerned, it can be eliminated because of the lack of relationship between the site of inoculation and the localisation of the initial palsy. Further investigation on the pathogenesis of infection by the intravascular route is necessary, however, before it will be possible to decide this question.

The results of the present experiments do not necessarily throw doubt on the theory of axonic propagation of the virus, for this hypothesis is based largely on experimental work in which strains of virus which had undergone repeated animal passage were used. King (1939) points out that recently isolated strains may behave quite differently and be highly infective by the subcutaneous and intestinal routes (Trask and Paul (1936, 1938)). In infection by the nasal route the virus almost certainly travels by axonic pathways. King considers

that the relative importance of this route in monkey poliomyelitis is purely an artificial condition and not necessarily related to the natural disease. He draws attention to the fact that other viruses such as louping-ill, yellow fever and Japanese encephalitis B., which under natural conditions are highly infective on subcutaneous inoculation may undergo modification on passage and show a predilection for the nasal route. It may well be that the transmission of poliomyelitis virus from the periphery to the neural axis can occur both by the blood stream and by nerve fibres. The results of the present investigation suggest that recently isolated strains prefer the former pathway. Once the central nervous system is reached, however, there is no reason to suppose that the virus travels by other than the exonic route.

SUMMARY.

Intradermal inoculation of poliomyelitis virus into denervated areas not only failed to prevent infection, but resulted in an increased susceptibility to the virus. This increase in susceptibility persisted for at least one hundred days after the operative procedures.

In animals subjected to denervation the virus was detectable in the blood two hours after intradermal inoculation.

In half of the denervated animals and all of the control monkeys there was lack of relationship between the site of inoculation and the localisation of the initial paralysis.

The possible routes by which virus may reach the neural axis from a denervated area are discussed.

It is concluded that the transmission of poliomyelitis virus from the periphery to the central nervous system is possible both by the blood stream and by axis-cylinders, but that recently isolated strains prefer the former pathway.

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STUDIES in EXPERIMENTAL POLIOMYELITIS.

(2) THE ALLEGED DEVELOPMENT of IMMUNITY FOLLOWING INTRANASAL INOCULATION of POLIOMYELITIS VIRUS INTO MONKEYS SUBJECTED TO PRELIMINARY BILATERAL SECTION OF THE OLFACTORY TRACTS.

By Charles Swan (1)

(From the Institute of Medical and Veterinary Science, Adelaide)

One of the most interesting problems in connection with the study of poliomyelitis is the mechanism by which the majority of the human population develop immunity to the disease. Burnet (1936) and Sabin and Olitsky (1938) from their investigations on the pathogenesis of the viruses of louping-ill and vesicular stomatitis, respectively, suggested, by analogy, that the virus of poliomyelitis after its entry by the filaments of the olfactory nerves might be halted at the olfactory bulbs and lead to a localised infection resulting in immunity.

Recently, Howe and Ecke (1937-38) reported that after intranasal administration of poliomyelitis virus a febrile reaction occurred at the same time as in control animals, in monkeys subjected to preliminary bilateral section of the olfactory tracts, provided that the olfactory bulbs were left in situ. In the control animals, but not in the monkeys which had undergone operation, the rise in temperature was followed by paralysis. Histological examination of the olfactory bulbs

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(1) Working with the aid of a grant from the National Health and Medical Research Council, Australia.

of the latter animals showed traces of perivascular infiltration and destruction of mitral cells. Howe and Ecke concluded that the olfactory bulbs were the seat of an inflammatory reaction which duplicated the initial stages of clinical poliomyelitis. They suggested that the localised infection might result in immunity.

The present investigation was undertaken with the object of confirming the work of Howe and Ecke and of determining whether the experimental procedures led to immunity.

MATERIALS and METHODS.

THE VIRUS.

A mixture of two local strains of virus isolated during the major epidemic in Australia of 1937-38 served as a source of virus.

Spinal cords were preserved in 50% glycerine in normal saline solution, buffered by phosphates to neutrality, and were kept in the refrigerator at a temperature of -10 C.

For intranasal inoculation a 10% suspension of spinal cord substance was prepared by grinding with sterile washed sand and normal saline solution. After the gross particles had been allowed to settle, the supernatant fluid was administered in a dose of 1.5 cc. per nostril on three successive days.

In the preparation of virus for intracerebral administration and for serum-virus mixtures, 1% and 2% suspensions, respectively were centrifuged at 3,000 R.P.M., for 15 minutes.

For the serum neutralization tests, 0.5 cc. of a 2% suspension of spinal cord substance was added to 0.5 cc. of the appropriate serum. The resultant mixture was kept at a temperature of 0° C. for three hours prior to its inoculation into the left cerebral hemisphere.

MONKEYS.

A total of eleven apparently healthy monkeys, all of which were Macaca mulatta (M. rhesus) weighing approximately 3.5 kgm., was used.

Daily rectal temperatures were recorded for up to four weeks following each inoculation.

At autopsy, sections of the cervical and lumbar regions of the spinal cord, together with the olfactory bulbs, were saved for histological examination. The remainder of the spinal cord was preserved as a source of virus.

OPERATIVE PROCEDURES.

Bilateral section of the olfactory tracts was performed under ether anaesthesia after induction with A.C.E. mixture. The dura-mater overlying the frontal lobes of the cerebrum was exposed by the elevation of a scalp flap and the removal of the underlying bone with the aid of nibbling forceps. The dura mater was incised and the superior longitudinal sinus was divided between ligatures. Division of the falx cerebri and elevation of the frontal poles disclosed the olfactory bulbs and tracts lying on the floor of the anterior cranial fossa. The olfactory tracts were sectioned, care

being taken not to dislodge the bulbs from the cribriform plates, thus preserving their vascular, lymphatic and remaining neural connections. The scalp flap was then replaced and sutured with silk.

EXPERIMENTAL PROCEDURES.

Seven monkeys were inoculated intranasally with poliomyelitis virus on three successive days. Four of these animals (S 23, S 22, S 25 and S 27) had been subjected to bilateral section of the olfactory tracts approximately 47 days previously; they developed neither pyrexia nor paralysis. The remaining three monkeys (S 28, S 33 and S 34) which acted as controls, became paralysed.

One month after the commencement of the first experiment, S 23, S 22, S 25 and S 27 together with a control animal, S 39, were tested for immunity by the intracerebral administration of 1 cc. of 1% poliomyelitis virus into the left hemisphere. One of the animals, S 23, remained healthy; the remainder developed paralysis.

Sera obtained one month after the start of the initial experiment were tested for the presence of antibodies. For this purpose equal parts of the sera of S 22, S 25 and S 27 were combined, while the serum of S 23 was tested separately. Serum obtained from a normal monkey was used as a control. As can be seen from Table 1., in which the results of the investigation are embodied, antibodies were not present in any of the sera in detectable amounts.

TABLE 1.

Effect of intranasal inoculation of poliomyelitis virus in monkeys, subjected to preliminary bilateral section of the olfactory tracts.

Monkeys	Result of intranasal inoculation.	Result of subsequent intracerebral inoculation	Result of serum neutralization test.
S 23	Neither pyrexia nor paralysis	Neither pyrexia nor paralysis	Negative.
S 22.	"	Paralysis on 17th day	Negative
S 25	"	Paralysis on 7th day	
S 27	"	Paralysis on 7th day	
Controls	S 28, S 33, and S 34 paralysed on 8th, 10th, and 8th days, respectively.	S 39 paralysed on 10th day.	

HISTOLOGICAL EXAMINATION.

In the monkeys subjected to bilateral section of the olfactory tracts histological examination of the olfactory bulbs proved negative. In contrast, the bulbs of the control animals which had been inoculated intranasally showed characteristic inflammatory changes.

DISCUSSION.

The investigation failed to confirm the work of Howe and Ecke, in that the intranasal inoculation of four monkeys after preliminary bilateral section of the olfactory tracts did not result in pyrexia, nor in infiltrative changes in the olfactory bulbs. The reason for this disparity in results is by no means clear, but it may possibly be dependent on the strain of the virus.

Only one of the four animals proved resistant to subsequent intracerebral inoculation, though in another the incubation period was prolonged. As is well-known, monkeys occasionally prove refractory to the intracerebral inoculation of poliomyelitis virus, so that the above findings are not necessarily indicative of the development of immunity.

The negative result of the serum neutralization tests is no criterion of lack of immunity of the nervous system to the virus (Brodie, Fischer and Stillerman (1937), Burnet and Jackson (1939). Considered in conjunction with the results of the intracerebral test, however, it would appear that no appreciable immunity resulted from the experimental procedures.

The results are thus similar to those of Gordon and Lennette (1939). In their experiments, however, section of the tracts was followed by cauterization of the bulbs, thus eliminating the possibility of the occurrence of a localised infection of the latter following subsequent intranasal inoculation.

SUMMARY.

Intranasal inoculation of monkeys with poliomyelitis virus after preliminary bilateral section of the olfactory tracts resulted neither in pyrexia, infiltrative changes in the olfactory bulbs, nor in a detectable immunological response.

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STUDIES IN EXPERIMENTAL POLIOMYELITIS.

(3) THE EFFECT of FARADIC STIMULATION on the LOCALISATION of PARALYSIS INDUCED by the INTRANASAL INOCULATION of POLIOMYELITIS VIRUS.

By Charles Swan (1)

(From the Institute of Medical and Veterinary Science, Adelaide)

From time to time, trauma has been suggested as an aetiological factor in the development of diseases of the nervous system. Pritchard (1934) reported a case in which during the incubation period of poliomyelitis a particular muscle of the upper limb was fatigued excessively. The subsequent paralysis manifested itself most severely in the fatigued muscle which later became completely atrophic; the remainder of the affected muscles showed complete or almost complete recovery. In an epidemic of poliomyelitis at a boarding school, de Rudder and Petersen (1938) found in a proportion of cases that strenuous physical exertion was a factor in increasing susceptibility to the disease.

Although the above reports suggested the present investigation, it must be stressed at the outset that the experimental procedures adopted in no way lead to the simulation of normal muscular fatigue. For the induction of such fatigue it would be necessary to stimulate

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(1) Working with the aid of a grant from the National Health and Medical Research Council, Australia.

the anterior horn cells synaptically, that is, by means of a reflex, To those with experience in the handling of monkeys, the difficulties of such a procedure, especially when the use of an anaesthetic is precluded, are obvious.

In the present paper an account is given of an attempt to determine whether faradic stimulation of the muscles of a particular limb during the incubation period of poliomyelitis might be a factor in increasing the susceptibility of the anterior horn cells to the virus and in the localisation of the initial paralysis.

MATERIALS and METHODS.

VIRUS.

The virus was a mixture of two local strains, both of which had been isolated during the major epidemic of Australia of 1937-38.

The spinal cords which served as a source of virus were preserved in 50% glycerine in normal saline solution, buffered by phosphates to neutrality, and were kept in the refrigerator at a temperature of -10° C.

For intranasal inoculation a 10% suspension of spinal cord substance was prepared by grinding with sterile washed sand and normal saline solution. After the gross particles had been allowed to settle, the supernatant fluid was administered in a dose of 2 cc. per nostril on three successive days.

MONKEYS.

Eight monkeys, all of which were *Macaca mulatta* (*M. rhesus*), weighing about 3.5 kgm., were used.

Rectal temperatures were taken daily until paralysis ensued.

APPARATUS USED for FARADIC STIMULATION.

The apparatus comprised an induction coil and a three volt dry-cell battery connected together in series. In addition a buzzer was connected in parallel with the induction coil.

To the secondary circuit of the induction coil two electrodes were connected. One of these, the indifferent electrode, consisted of a plate of sheet zinc measuring 1⅛ inches by 2⅛ inches, and covered with eight thicknesses of cotton gauze. The other, the stimulating electrode, was composed of a glass rod, 5 inches long, around the lower half of which copper wire had been wound spirally. Superimposed over the copper wire and the portion of the glass rod around which it had been wound were eight layers of cotton gauze.

The strength of the stimulus used was determined in the first place empirically. When tested on our forearms the stimulus caused marked tetanization of the muscles and a moderate degree of pain.

Actual measurement showed that the frequency of the stimulus was approximately 60 per second. The voltage output on open circuit was approximately 50 volts R.M.S., and 1100 volts peak.

EXPERIMENTAL PROCEDURES.

In all, five monkeys were stimulated while the remaining three acted as controls.

Faradic stimulation was begun on either the first or second day after the initial intranasal inoculation, and was applied for a period

of half an hour per day until the onset of paralysis, when it was discontinued.

In all cases the left upper limb was selected as the site for stimulation.

Anaesthesia was induced with A.C.E. mixture and maintained with ether. As soon as an adequate depth of anaesthesia had been reached, the animal was placed on its back on a large rubber mat, and tied in position by means of ropes attached to both ankles and the right wrist. The indifferent electrode, previously moistened in saline, was then inserted between the posterior aspect of the left arm and the rubber mat, the weight of the limb ensuring adequate contact. The induction coil was switched on, and the stimulating electrode, which had been soaking in saline, was applied in turn to the motor points of the muscles belonging to the various groups of the arm, forearm and hand until fatigue occurred. By the time the more distal groups of muscles had been fatigued, the proximal groups, which had been tetanized earlier, showed some degree of recovery and responded to further stimulation. In the half hour period it was possible to repeat this cycle about four times.

The results of the experiment are shown in the following table.

Effect of Faradic Stimulation on Localisation of Paralysis.

No. of monkey.	No. of days stimulated.	Paralysis, distribution (in order of appearance) and severity.
S 50	8	Left arm +++, right arm + and legs +.
S 51	8	Legs +++, left arm +++, right arm ++.
S 69	17	Right Leg +, left arm +.
S 70	9	Left arm +++ and right arm ++, legs ++.
S 71	9	Legs +++, left arm ++, right arm +.
S 48 (Control)	0	Right leg +++ and left leg ++, left arm +.
S 49 (Control)	0	Legs +++ and right arm +++, left arm +.
S 73 (Control)	0	Legs ++, arms +++.

+++ = Severe paralysis
++ = Moderate "
+ = Mild "

It will be seen from the above table, that of the five monkeys subjected to stimulation, only two, S 50 and S 70, manifested their initial paralysis in the limb which had been stimulated. The initial palsy in the latter animal was associated with involvement of the opposite arm. In the remainder of the monkeys, S 51, S 69, and S 70, the paralysis began first in the lower limbs. Later, however, when

the upper limbs became affected, the paralysis was always more severe in the arm which had been stimulated.

In the control animals, S 48, S 49 and S 73, the lower limbs were always the first to become paralysed, but in S 49 the initial palsy occurred in the right arm as well. A comparison of the intensity of the paralysis of the upper limbs, shows that in one case the arms were involved equally, while in the remaining two, the left and the right arms were each involved more severely on one occasion.

In general the paralysis was not more severe in the animals subjected to stimulation. Indeed, S 69, which had been stimulated on seventeen successive days, was the least affected.

DISCUSSION.

The stimulated limb was the site of the initial palsy in only two out of five monkeys. As a similar localisation of the initial paralysis occasionally occurred in other experiments in which monkeys were infected by the intranasal route, it cannot be concluded that the site of the initial palsy is influenced by electrical stimulation.

Nevertheless the paralysis in the upper limbs was always more marked in the arm subjected to stimulation, suggesting that faradic stimulation increases the susceptibility of anterior horn cells to the virus. This increase in susceptibility may be the result of impulses passing antidromically along the axons to the motor cells.

SUMMARY.

Faradic stimulation of a particular limb during the incubation period of poliomyelitis did not influence the localisation of the initial paralysis. The stimulated limb, however, was always paralysed more severely than its fellow on the opposite side.

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THE METHOD OF TRANSMISSION OF THE VIRUS OF INFECTIOUS MYXOMATOSIS OF RABBITS TO THE CENTRAL NERVOUS SYSTEM.

by Charles Swan. (1)

In studies on the concentration of the virus of infectious myxomatosis of rabbits in various organs at different periods after intratesticular inoculation, Hurst (1937 c) noted that, either the virus appeared first in the lumbar region of the spinal cord, or was present at this level in higher concentration than elsewhere in the neural axis.

These results, which suggest transmission of the virus by neural pathways, were somewhat unexpected, as Hurst (1937 b) had shown earlier that this virus possesses only slight neurotropic affinities. Accordingly, the present investigation was undertaken.

MATERIALS AND METHODS.

(1) VIRUS.

Infected testes and spleens obtained from rabbits killed five days after intratesticular inoculation served as a source of virus. These organs were preserved in 50% glycerine in normal saline solution, buffered by phosphates to neutrality, and were kept in the refrigerator at a temperature of -10°C.

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(1). This work was carried out with the aid of a grant from the National Health and Medical Research Council, Australia.

For intradermal inoculation a 10% suspension of infected tissue was prepared by grinding with sterile washed sand and normal saline solution. After gross particles had been allowed to settle, the supernatant fluid was inoculated in a dose of 0.2 cc.

For intravenous administration a 10% suspension of infected tissue was prepared similarly, but the saline solution was replaced by normal rabbit serum in order to prevent intravascular clotting. The suspension was centrifuged for five minutes at 3,000 R.P.M. The supernatant fluid was injected in a dose of 1 cc.

(2) RABBITS.

Apparently normal rabbits, weighing about 1,500 grams, were used.

(3) OPERATIVE PRODECURES.

All operative procedures were performed under ether anaesthesia. Denervation of the dorsum of the foot was effected by division of the femoral and sciatic nerves at the level of the inguinal ligament and the greater sciatic foramen, respectively.

(4) POST-MORTEMS.

Under ether anaesthesia the anterior wall of the thorax was elevated and a specimen of blood was withdrawn from the right ventricle. After defibrination the specimen was set aside for examination later. The vascular system was then washed out with three litres of normal saline, according to the technique of Hurst (1936).

Representative sections of the nervous system were then removed. They included:

(1) the olfactory bulbs and adjacent portions of the frontal lobes;

(2) the middle cerebrum (frontal section passing just behind the optic chiasma);

(3) the posterior cerebrum (occipital lobes);

(4) the brain-stem (level of fourth ventricle);

(5) the spinal cord; (a) cervical region,
(b) dorsal region,
(c) lumbar region; and

(6) the posterior root ganglia (representative ganglia from the cervical, dorsal and lumbar levels).

(In subsequent tables the above sections are represented by the letters, O.B., M.C., P.C., B.S., C.C., D.C., L.C., and P.R.G., respectively).

From each of the above specimens of nervous tissue a 10% (10^{-1}) suspension was prepared by grinding with sterile washed sand and normal saline solution. These suspensions were centrifuged at 3,000 R.P.M., for five minutes. The concentration of virus was then determined by intradermal inoculation of serial 10 fold dilutions in the backs of fresh rabbits.

The specimen of defibrinated blood was also tested for the presence of virus, but no attempt was made to determine its titre.

EXPERIMENTAL PROCEDURES AND RESULTS.

(1) INTRADERMAL INOCULATION OF THE VIRUS.

A series of eleven rabbits was inoculated with the virus in the dorsum of the left foot, and the animals were killed at intervals ranging from three to five days. The results are presented in the following table.

TABLE 1.

Distribution of the virus of infectious myxomatosis of rabbits in nervous system following intradermal inoculation into left foot.

No. of days after inoculation.	O.B.	M.C.	P.C.	B.S.	C.C.	D.C.	L.C.	P.R.G.	Blood.
3.	0.	0.	0.	0.	0.	0.	0.	N.T.	+
3.	0.	0.	0.	0.	0.	0.	0.	N.T.	+
3.	10^{-1}	0.	0.	0.	10^{-1}	0.	0.	0.	+
4.	10^{-3}	10^{-1}	10^{-2}	0.	10^{-2}	10^{-1}	10^{-2}	N.T.	+
4.	10^{-2}	0.	0.	10^{-1}	10^{-1}	10^{-2}	0.	N.T.	+
4.	10^{-3}	0.	10^{-1}	0.	0.	0.	0.	N.T.	+
4.	10^{-3}	10^{-1}	10^{-2}	0.	10^{-2}	0.	0.	10^{-3}	+
4.	10^{-1}	10^{-1}	10^{-1}	0	0.	10^{-2}	10^{-2}	10^{-2}	+
5.	10^{-5}	10^{-5}	10^{-5}	10^{-4}	10^{-5}	10^{-4}	10^{-4}	N.T.	+
5.	10^{-3}	10^{-2}	10^{-1}	10^{-1}	10^{-1}	0.	0.	N.T.	+
5.	10^{-3}	10^{-4}	10^{-2}	10^{-4}	10^{-2}	10^{-2}	10^{-3}	10^{-3}	+

+ = virus present, but concentration was not determined.
0 = no virus detected.
N.T.= no test made.

The results show that the virus was present in the blood from at least the third day onwards. In the nervous system, on the other hand, virus was detectable in only one of three cases at the end of 72 hours, and then only in minimal amounts and in widely separated areas.

From the fourth day onwards virus was always present in the nervous system. For the most part the virus appeared to be distributed at random, but the olfactory bulbs were involved more constantly than other regions.

The level of the spinal cord corresponding to the site of inoculation was not affected prior to other levels of the neural axis. Indeed, in half of the cases the lumbar cord remained free from virus. There was therefore no evidence of spread of the virus via neural pathways. To confirm this observation the second experiment was undertaken.

(2) INTRADERMAL INOCULATION OF THE VIRUS INTO A DENERVATED AREA.

Nineteen rabbits were injected with virus in the dorsum of the left foot. In nine of these animals denervation of the foot had been effected three weeks before. In the remainder three months had elapsed. The results are embodied in Tables 2. and 3.

It will be seen that the injection of virus into denervated skin produced no significant alteration either in the time elapsing between inoculation and the involvement of the nervous system, or in the distribution of the virus. Spread of the virus from the periphery to the neural axis by means of axis-cylinders would appear to have been eliminated.

TABLE 2.

Distribution of the virus of infectious myxomatosis of rabbits in the nervous system following intradermal inoculation into left foot three weeks after denervation had been performed.

No. of days after inoculation.	O.B.	M.C.	P.C.	B'S.	C.C.	D.C.	L.C.	P.R.G.	Blood
3.	0.	0.	0.	0.	0.	0.	0.	0.	+
3	10^{-2}	0.	10^{-2}	0.	0.	0.	10^{-2}	10^{-3}	+
3	0.	0.	0.	0.	0.	0.	0.	0.	+
4.	10^{-2}	0.	0.	0.	0.	0.	0.	N.T.	+
4	10^{-2}	10^{-2}	10^{-2}	0.	0.	10^{-2}	10^{-4}	10^{-3}	+
4	10^{-1}	10^{-1}	10^{-1}	10^{-1}	10^{-1}	10^{-3}	10^{-3}	10^{-4}	+
5.	10^{-4}	10^{-4}	10^{-3}	10^{-3}	10^{-3}	10^{-4}	10^{-4}	N.T.	+
5.	0.	0.	0.	10^{-2}	0.	0.	10^{-2}	10^{-3}	+
5.	10^{-2}	10^{-3}	10^{-2}	10^{-2}	N.T.	10^{-3}	10^{-3}	N.T.	+

+ = virus was present, but concentration was not determined.

0 = no virus detected.

N.T. = no test made.

TABLE 3.

Distribution of the virus of infectious myxomatosis of rabbits in the nervous system following intradermal inoculation into left foot three months after denervation had been performed.

No. of days after inoculation.	O.B.	M.C.	P.C.	B.S.	C.C.	D.C.	L.C.	P.R.G.	Blood.
3.	0.	0.	0.	0.	0.	0.	0.	0.	+
3.	0.	0.	0.	0.	0.	0.	0.	0.	+
3.	0.	0.	0.	0.	0.	0.	0.	0.	+
4.	10^{-2}	10^{-2}	10^{-1}	0.	0.	0.	0.	N.T.	+
4.	10^{-1}	0.	0.	0.	0.	0.	0.	N.T.	+
4.	10^{-3}	10^{-1}	10^{-3}	0.	10^{-1}	0.	0.	N.T.	+
4.	10^{-4}	10^{-3}	10^{-3}	10^{-4}	10^{-4}	10^{-3}	10^{-4}	N.T.	+
5.	10^{-2}	10^{-2}	0.	10^{-1}	0.	0.	10^{-1}	N.T.	+
5.	10^{-3}	10^{-3}	10^{-3}	10^{-3}	10^{-2}	10^{-4}	10^{-3}	N.T.	+
5.	10^{-4}	10^{-5}	10^{-5}	10^{-5}	10^{-4}	10^{-5}	10^{-4}	N.T.	+

+ = virus was present, but its concentration was not determined.

0 = no virus detected.

N.T. = no test made.

(3) INTRAVENOUS INOCULATION OF THE VIRUS.

The object of the third experiment was to determine the distribution of the virus in the nervous system following intravenous administration.

A total of nine rabbits was inoculated in the marginal vein of the left ear, and the animals were sacrificed on either the second, third or fourth day after injection.

The results are summarized in Table 4.

TABLE 4.

Distribution of the virus of infectious myxomatosis of rabbits in the nervous system following intravenous inoculation.

No. of days after inoculation.	O.B.	M.C.	P.C.	B.S.	C.C.	D.C.	L.C.	P.R.G.	Blood
2.	10^{-1}	10^{-2}	10^{-1}	10^{-2}	10^{-2}	10^{-1}	0.	N.T.	+
2.	0.	0.	0.	0.	0.	0.	0.	0.	+
2.	10^{-2}	10^{-2}	10^{-3}	10^{-1}	0.	10^{-2}	10^{-2}	10^{-3}	+
3.	10^{-1}	0.	10^{-1}	10^{-1}	0.	10^{-1}	10^{-2}	10^{-3}	+
3.	10^{-2}	10^{-3}	10^{-2}	10^{-3}	10^{-3}	10^{-3}	10^{-2}	10^{-5}	+
3.	10^{-3}	10^{-3}	10^{-3}	10^{-3}	10^{-3}	10^{-4}	10^{-3}	10^{-5}	+
4.	10^{-3}	10^{-3}	10^{-3}	10^{-1}	0.	10^{-2}	10^{-1}	10^{-1}	+
4.	10^{-3}	10^{-3}	10^{-3}	10^{-3}	10^{-1}	10^{-3}	10^{-3}	10^{-3}	+
4.	10^{-3}	10^{-4}	10^{-2}	10^{-2}	10^{-2}	10^{-2}	10^{-2}	10^{-4}	+

\+ = virus was present, but its concentration was not determined.

0. = no virus detected.

N.T. = no test made.

Following intravenous inoculation virus was detectable in two out of three animals at almost all levels of the nervous system at the end of 48 hours. The random distribution of the virus which occurred after intradermal administration still persisted, but, unlike the former experiments, more levels of the neural axis were involved at one and the same time, and the olfactory bulbs were not affected more often or more severely than elsewhere in the nervous system.

A feature of interest was the high concentration of the virus in the posterior root ganglia. With one exception, the titre of the virus here was equal to, and sometimes greater than, the titre in the rest of the neural axis.

DISCUSSION.

In the present investigation, all of the evidence (the appearance of the virus in the blood stream before it was detectable in the nervous system, the random distribution of the virus in the latter, the fact that the level of the spinal cord corresponding to the area of inoculation was not the initial site of invasion of the virus, and the observation that the virus travelled readily from a denervated area) leads to the conclusion that the virus, when inoculated in a peripheral site, travels to the nervous system via the blood stream and not by neural pathways.

The mechanism whereby the virus reaches the central nervous system from the blood stream must now be considered. In studies on the pathogenesis of the virus of equine encephalomyelitis, Hurst (1936) suggested that the virus is deposited by the blood on to the nasal

mucosa, whence it travels via the perineural lymphatics of the olfactory nerves to the subdural space. This hypothesis would serve to explain only a proportion of the present cases.

Alternatively, Hurst (1936) suggested that the process might be a slow "growth through" the haemato-encephalic barrier, a theory which has found strong support in the recent work of King (1938, 1939). King has shown that when the virus of equine encephalomyelitis is inoculated peripherally, it enters the blood stream and produces its initial effect on the walls of the blood vessels. The nervous system is involved secondarily.

In view of the fact that the virus of infectious myxomatosis of rabbits produces remarkable proliferative and degenerative changes in the walls of the vascular system (Hurst (1937 a)) it is reasonable to assume, taking into account the evidence of the present study, that this virus behaves similarly to that of equine encephalomyelitis. In other words, the virus of infectious myxomatosis of rabbits, when injected peripherally, enters the blood stream and produces lesions in its walls which allow the virus to be sown indiscriminately throughout the nervous system.

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