

AN EXPERIMENTAL INVESTIGATION OF AN  
AUSTRALIAN EPIDEMIC OF ACUTE  
ENCEPHALO-MYELITIS<sup>1</sup>.

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<sup>1</sup> A full epidemiological, clinical and histological account of the disease may be read in the Annual Report of the Microbiological Laboratory of the Department of Public Health, Sydney, N.S.W., for the year 1917.

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### I. INTRODUCTION.

THIS investigation concerns an epidemic disease, which for convenience we have called "X disease," prevalent in certain parts of New South Wales during the late summer of the years 1917 and 1918.

The disease was at first thought to be "acute poliomyelitis"—the Heine-Medin disease. Increased observation, however, revealed important discrepancies; thus (1) it was confined to "outback" towns or districts remote from one another and from the metropolis (Sydney), and did not synchronise with any metropolitan epidemic of "acute poliomyelitis"; (2) it was extremely fatal (we have notes of 134 cases, of which no less than 94 died); (3) it attacked a number of adults (34 cases); (4) signs of intense cerebro-spinal irritation (convulsions, rigidity, increased reflex activity, mental confusion and pyrexia) dominated the clinical picture, paralysis, intercurrent or residual, being infrequent; and (5) the histological picture differed from that of "acute poliomyelitis," inasmuch as the changes were distributed throughout the central nervous system and did not fall with special intensity on the spinal cord.

Impressed by the peculiar features of the disease, we set in train the series of experiments herein recorded. The list comprises 62 experiments on monkeys, 52 on sheep, four on calves, one on a horse, and sundry experiments on dogs, kittens, rabbits, guinea-pigs and a fowl. Hence the research has been extensive. We may also say now that, while various side issues have been followed up with interesting results, the finding of chief importance is that the disease is communicable not only to the monkey, but to the sheep, the horse and the calf, that is, to animals which, so far as we are aware, have hitherto proved resistant to "acute poliomyelitis"—which is an additional discrepancy.

### II. INOCULATION, MATERIALS AND METHODS.

The usual procedure in obtaining and maintaining the virus for inoculation purposes was the following:

At the autopsy of the human being or animal dead from the disease, thin

slices of tissue were taken from the frontal, parietal, occipital and temporo-sphenoidal regions of the cerebrum, from the cerebellum, from the pons and medulla, and from the cervical, dorsal and lumbar areas of the spinal cord. These were then straightway put into 33 per cent. glycerine diluted either with water, or in the earlier cases with normal saline solution, in which they were kept in an ice chest until wanted; or the material was at once emulsified with sterile powdered quartz in a mortar with the diluent, and stored similarly after a proper milky emulsion had been made. To preserve material from one or two of the earlier human cases the strength of the glycerine solution was 50 per cent. In some cases the emulsion was made with normal saline solution only when it was proposed immediately to inoculate a test animal. When small blocks of tissue had been preserved in glycerine, they were emulsified in a mortar just before inoculation. Pasteur-Chamberland F. and Berkefeld filtrates of this primary emulsion of the brain or spinal cord were sometimes used, whilst in other cases, after light centrifuging, the supernatant fluid was treated with various sera.

Other materials used for inoculation comprised swabbings from the nasopharynx emulsified in 33 per cent. glycerine solution, a Pasteur-Chamberland F. filtrate of faeces from a human case, and an emulsion of fowl ticks.

Nearly all the inoculations were made intracerebrally. The usual procedure was to tie the animal out on a frame and to anaesthetise it, first with chloroform and then with ether; in some cases chloroform alone was used. After cleaning the scalp and removing the hair, a small lineal incision was made in the parietal area down to the bone. Then by means of a small trephine, a puncture was made through the skull by means of the pin in the centre of the trephine, the teeth of the trephine keeping the pin in position. It was found better to use a trephine than an awl or other instrument. As a rule the time when the pin of the trephine had pierced the cranial vault could be easily estimated by the teeth of the trephine beginning to give. Occasionally, when a little undue force was used, the disc of bone loosened by the trephine became somewhat depressed. In one or two of the earlier cases the trephine tablet was removed. This was apt to lead, when the disease developed, to a hernia cerebri. The puncture having been made and by means of a surgical needle proved to be through the bone, a moderate sized needle of a large 5 c.c. syringe was inserted deeply into the cerebral substance, and about 1 c.c. of the emulsion injected. At one time the injection was made with some force, and the needle end was moved in various directions, with the deliberate object of destroying a certain amount of brain tissue in the neighbourhood of the injection and so facilitating the "taking" of the virus. It frequently happened that on withdrawing the syringe fluid escaped along the needle track but in several cases wherein this occurred the animals nevertheless contracted the disease.

The animals appeared to recover perfectly from the operation, usually within a few hours, and next day, with few exceptions, showed no results of

the operative treatment, in spite of the fact that definite injury must have been done to the brain.

In some of the earlier cases not only was the emulsion injected into the brain substance, but a certain amount was injected subdurally as well.

Intraperitoneal and intrasciatic inoculations were made in the usual way. In two cases lumbar puncture was performed, once in a monkey and once in a sheep, and a few c.c. of normal serum were injected into the spinal canal. Then an incision was made in the thigh or neck of the animal and about 0·5 c.c. of the emulsion was injected intravenously. No results followed from any of these inoculations. In the case of the intravenous introductions, the failure was possibly due to the small amount of virus employed.

### III. DETAILS AS TO THE VIRUSES EMPLOYED.

1. *Estimated day of illness when the virus was obtained.* This varied from one to eight days; in most cases it was obtained on the third to the fifth day. In the three human cases, the patient had died on the third, second and fifth days of the disease respectively.

2. *Hours after death when the virus was removed from the body.* With the exception of the cases in which the virus was obtained from human beings, the post-mortem examinations were made immediately after death or within a few hours. The three human cases had been dead 5, 12 and 21 hours respectively.

3. *Period during which the virus was preserved in vitro.* In a number of the animal experiments this was only a few hours. The longest periods during which it was preserved in glycerine and retained its activity were seven days in one human case and fifteen days in a monkey case.

4. *Menstruum in which the virus was preserved.* When the virus was immediately injected into a further test animal, it was usually suspended in normal saline solution. In most of the other cases it was preserved in 33 per cent. glycerine solution, the dilution of the glycerine being sometimes with normal saline solution, sometimes with sterile water. The percentage of glycerine was usually 33, but in one instance it was 50.

### IV. THE DISEASE AS MANIFESTED IN MONKEYS (*MACACUS RHESUS*).

Twenty monkeys altogether were successfully inoculated with the virus as proved by full histological examination after death. Details of the individual animals will be found in the Appendices.

1. *The incubation period.* This varied from 5 to 23 days, the average of the 20 cases being 10·25 days, or eliminating the very long incubation period of 23 days, 9·5 days. In only one instance was the incubation period as long as 23 days; in one it was 17 days; in one, 15 days; in two, 12 days; in three, 11 days; in three, 10 days; in two, 9 days; in one, 8 days; in three, 7 days; in one, 6 days; and in two, 5 days.

2. *Length of illness.* This varied from  $2\frac{1}{2}$  to 11 days. In most instances the animals were killed *in extremis*. In several instances it is possible, more especially in the case of A. 110, Monkey 3925, that the animal might have survived a considerably longer period, or have recovered. In the majority of cases, however, the animal had reached an almost moribund state in from 3 to 6 days.

3. *The general course of the disease.* In half, at least, of the affected monkeys, the first sign of the onset of the disease was a peculiar anxious facial expression. This sign might precede by a day other manifestations, or might be accompanied by evidences of incoördination and by exaggerated or irregular muscular movements and increased reflex action. In most instances there was a gradual progression of this incoördination and exaggerated muscular movement, sometimes accompanied by convulsive muscular contortions, and occasionally by true convulsions, until eventually the animal lay prostrate on the bottom of the cage. In some instances paresis of a limb, accompanied by incoördination, was a recognisable and early manifestation, and in one case prostration was the first indication of a "take." In some cases consciousness was apparently not lost until just before the animal was killed or died. The dominant features of the disease were exaggerated muscular movements and intense incoördination, though a few individual monkeys were quieter, and showed less incoördination, and more paresis.

4. *The symptoms and signs manifested on the first day of illness.* In ten of the twenty monkeys an anxious or frightened expression was easily recognisable on the first day of illness, and as already indicated was frequently the first intimation that the virus had "taken." In eight cases the monkeys were described as being nervous or jumpy, or as walking gingerly; in eight also there was definite incoördination of muscular movement. These two classes comprised fourteen of the twenty monkeys. Some spasticity of the limbs was noticed in one monkey, and convulsions were recorded in one. In six monkeys there was definite paresis of a limb, and in one the animal was found prostrate.

Regrouping the above signs, it will be found that on the first day of illness fifteen of the twenty monkeys showed exaggerated movements, incoördination, spasticity, or convulsions, whilst only seven showed paresis and none paralysis.

5. *Later manifestations.* The most note-worthy feature of these cases, seen in nineteen of the twenty monkeys, was an incoördination of movement of a form apparently due more to exaggeration of muscular efforts in attempts to balance than to paresis. In twelve of the animals, including eleven of the above, there were greatly exaggerated movements of limbs, and in three, including two of these twelve and one of the nineteen, definite convulsions. Thus, nineteen monkeys showed incoördination and thirteen exaggerated movements or convulsions, the whole twenty animals being included in these two categories.

The incoördination presented itself as inability to maintain the balance easily, and difficulty in quickly adjusting the limbs and hands to the various movements attempted. The exaggerated muscular movements were especially

noticeable when the animal attempted to jump, and became much more pronounced when it was disturbed and excited. Thus, in jumping on to a perch in the cage, the animal might miss the perch and pass below, or pass above and hit its head against the roof of the cage, or lose its hold and tumble off. In other cases, especially when disturbed, the animal would career wildly round the cage, dash its head against the sides, and apparently be unable to control the propulsive movements which it had initiated. Frequent bruising and injury, especially of the head, occurred under these circumstances. Often these movements were almost convulsive in character.

Definite convulsions occurred in three monkeys, and were merely a supreme exaggeration of the muscular movements already described. They were unaccompanied by a definite loss of consciousness. The animal during the attack would lie partly prostrate, or seized by violent contortions would rotate on the bottom of the cage, or possibly attempt to steady itself by efforts at grasping the bars. Such attacks sometimes lasted several minutes, and left the animal exhausted.

Irregular muscular contractions and twitches, apart from more purposive movements of limbs, were noticed in two animals. A general tremor, or a tremor of limbs or of the head, occurred in two cases, the movements in one resembling those of paralysis agitans. Some spasticity or rigidity of the limbs occurred in three and head retraction in five cases. Five of the animals uttered staccato or barking cries.

Paresis or paralysis was noted in fourteen of the twenty monkeys. In three there was slight paresis of a limb, in five there was marked paresis, and in six there appeared to be paralysis of a limb or other part. Two animals showed squint. In three there was ptosis, marked in two cases. The eyesight may possibly have been affected in one monkey. Three monkeys were drowsy, somnolent, or intensely sleepy.

Terminal prostration was manifested in nearly all the animals, varying from a slight degree, wherein there was inability to rise, with some remaining power of movement in a limb or limbs, to a condition so complete that perhaps an intelligent look in the eyes and slight movements of the tail tip or of a hand or foot were the only recognisable indications of life.

The temperature was frequently subnormal towards the end, sometimes markedly so, and in one case fell to 29° C.

#### V. THE DISEASE AS MANIFESTED IN SHEEP.

1. *Incubation period and general course.* Thirteen successful inoculations confirmed by full histological examination were obtained in sheep. In ten of these the virus came from monkeys, and in the remaining three from previously inoculated sheep. As a converse to the conveyance of the disease from the monkey to sheep, the virus was conveyed from a sheep (A. 65, Sh. 3855) to a monkey (A. 66, Monkey 3860), and from this monkey to a series of these animals. All the inoculations were intracerebral under an anaesthetic, and the virus

had been suspended either in 33 per cent. glycerine or in normal saline solution. Also the inoculations were all made within two days of the death of the previously infected host. After an incubation period of three to twelve days the first symptoms of illness were noticed. The duration of the illness until the animal died, or was *in extremis* and killed, ranged from one to five (or seven) days, except in one case which died thirty days after symptoms were first noticed.

The early symptoms were somewhat vague, as might be expected in an animal showing such low mental development. Hanging of the head and disinclination to feed were taken as suspicious; usually there were quiverings of the lips and ears, and sometimes champing of the jaws, suggestive of cud-chewing; occasionally there was dribbling from the mouth, or a mucous discharge from the nose. Later some retraction of the head usually appeared, whilst the animal, still able to stand and walk, tended to circle to one side.

The final stage was sometimes rapid—in one case lasting an hour—and was frequently characterised by convulsions and great respiratory irregularities. Occasionally convulsive seizures occurred earlier in the disease, and the animal temporarily recovered; indeed, some sheep recovered permanently.

The following notes of a case, where death took place soon after the convulsions first occurred, exemplify the early symptoms:

In the morning it was moving about and eating a little. In the afternoon it was seen in the paddock turning slowly in a circle, with its head down as if trying to reach the grass. After making several revolutions it fell on its side and began nibbling, not grass but a small native plant (*Pimelea*) of unattractive appearance. Shortly afterwards its head became retracted and there were slight convulsive movements, whilst the lips and nostrils were trembling and moving irregularly. It was breathing quickly and there were occasional to and fro movements of the forelimbs, less so of the hind limbs. Later the head became markedly retracted, and the limbs rather rigid and partly convulsed. The segments of the hoof of one of the front feet were sometimes widely separated. The animal seemed to be unconscious. More definite convulsive movements occurred occasionally. At the end of one of these, respirations became highly irregular and then ceased, and the animal died an hour after it had been noticed circling round.

2. *Analysis of the symptoms and signs met with in the sheep.* The following summary deals only with the thirteen sheep in which histological examination proved the presence of "X disease." Those that showed slight symptoms, apparently of "X disease," and recovered, are not included.

Convulsive movements or fits, sometimes intense, occurred in eleven of the thirteen sheep. During these attacks the head was thrown back, the limbs were moved convulsively and sometimes pawed the air, and fine twitchings occurred in the lips, nostrils, and ears. In five cases, before the development of more untoward symptoms, the sheep tended to walk in a circle. Quivering or fine tremors of the lips, ears, nostrils, etc., sometimes extending to the whole



body, occurred in nine cases, and were frequently seen, apart from attendant convulsions. Under similar circumstances, champing movements of the jaws were noticed in two cases, and grinding of the teeth in another. Stiff neck or retraction of the head was noticed during the course of the illness in six cases. In two, the legs were rigid or there was a stiff, jerky gait. Protrusion of the tongue occurred in one animal, "staggers" in one, and restlessness in one. One animal was noted as being drowsy. In most cases terminal unconsciousness or coma accompanied the convulsions. Some weakness of the hind legs was seen in one case. In nine instances rapid or irregular breathing occurred during the course of the illness. Sometimes a series of very rapid respirations were followed by a pause, and then the respirations began again. Other interesting signs were running of the nose or a mucous discharge in two sheep, and dribbling from the mouth in two others.

3. *Sheep surviving intracerebral inoculation, but showing slight symptoms, probably of encephalo-myelitis.* A. 82, Sh. 3894. On June 17th this animal was inoculated intracerebrally with the same material as successfully conveyed the disease on the same date to two other sheep, A. 80 and A. 81. Twelve days later the animal seemed ill and was lying down, and was "off its food," but showed no paresis. Beyond these slight symptoms on this date it remained well until July 6th, when blood was taken from it under an anaesthetic. Four days later it died, apparently from the after-effects of the anaesthetic. No histological lesions of the disease were detected.

It is possible that the slight symptoms shown on one day were due to a very mild attack of encephalitis from which the animal recovered, and that the lesions had disappeared by the time the animal died, eleven days later. Under any circumstances the series of experiments to which this case belongs shows that A. 82 possessed a decided relative, if not an absolute, immunity to the disease.

A. 90, Sh. 3903. On June 27th this animal was inoculated intracerebrally with material that gave a positive result in the case of two other sheep, A. 89 and A. 91. On July 2nd it was breathing fast, held its head down, was drowsy and did not eat. On July 3rd it held its head down when resting, was not eating, and did not seem to run about so much or so quickly as usual, while the hind legs seemed to go down easily when pressure was exerted on the back. On July 4th it was still sluggish and not eating, but next day it was well and remained so afterwards.

This animal had been previously inoculated, under the designation A. 68, with material from a positive sheep, which successfully infected the monkey, A. 66, done on the same date. It is possible that the first inoculation, intended to be intracerebral, was made into the frontal sinus, at any rate it was resultless. The sheep had also received a second inoculation, under the designation of A. 86, from Sh. A. 75, which upon histological examination was found not to show the lesions of encephalo-myelitis.

It seems highly probable that the symptoms from which this sheep suffered after the third injection were due to a mild attack of encephalomyelitis from which it recovered. And it can hardly be denied, especially when its previous inoculations are considered, that the animal possessed a relative, if not an absolute, immunity to the virus.

A. 102, Sh. 3914. On July 7th this animal was inoculated intracerebrally with material which successfully conveyed the disease to two sheep A. 103 and A. 105, and to a monkey, A. 100. It became ill on July 12th and had a temperature of 106° F. On July 13th the temperature was lower, 105°, and from then to the 16th it remained well excepting for occasional twitchings and rapid breathing. It seemed, however, very weak and was "gone in the legs," and the nose was running. On July 19th it seemed well.

Blood was taken under an anaesthetic on July 31st. The serum from this blood was used, on August 2nd, for mixing with a virus before its injection into A. 117, M. 3937. This monkey developed the disease twenty-three days later, which is an unusually long incubation period.

The symptoms presented by this sheep suggest that it may have had a mild attack of encephalitis, from which it recovered. This is perhaps supported by the unduly prolonged incubation period resulting in monkey A. 117, which may be possibly attributed to some immunising power possessed by the sheep serum. The course of the disease in the monkey, however, when it developed, was not altered.

A. 125, Sh. 3948. On August 14th this animal was inoculated intracerebrally with an emulsion from a positive sheep, A. 121. Next day it was a little sick, as the result of the operation. On August 18th it seemed cramped and could not use its hindquarters for a few minutes, and then seemed to be convulsed. On August 19th it showed convulsive movements, lasting for about two hours. Thereafter it remained well until August 25th when one hind leg seemed to be slightly contracted and spastic. It was well next day and remained so afterwards.

This sheep had received a previous inoculation intracerebrally on July 7th with material from a monkey, which produced the disease on the same date in two other sheep, A. 103 and A. 105, and in a monkey, A. 100. It had been unaffected by this first inoculation.

It is possible that the symptoms shown by this sheep, A. 125, were due to a mild attack of encephalitis. The result of the first inoculation, however, shows that it must have possessed a marked relative, if not an absolute, immunity to the virus. If the symptoms manifested after the second inoculation were not due to a mild attack of encephalitis, the result would confirm the view that it possessed absolute immunity. Otherwise a relative or varying immunity would be suggested.

A. 130, Sh. 3968. On August 28th this animal was inoculated intracerebrally with material from a monkey which on the previous day had successfully infected A. 129, M. 3967. On September 5th it seemed sick and was not feeding.

It was walking about, the head was drooping and the respirations were rapid. Next day it seemed worse, was "off its food," and was lying down continually, whilst in the afternoon it was circling round with the head somewhat retracted. On September 7th the lips were twitching at times, and it was shivering all over and had a slight limp in one foreleg. On the afternoon of September 8th it seemed worse, the lips were twitching, it kept poking out its tongue, and was circling round and not eating. On September 9th it had two fits, each of about five minutes' duration, during which it lay down, kicked with its fore and hind legs, and seemed unconscious. On September 10th it was still sick but was able to stand. On being made to move it could walk, but occasionally stumbled and tended to fall on one side. From this date it remained well.

This sheep, under the designation of A. 119, on August 2nd had received a previous inoculation, intracerebrally, of a Berkefeld filtrate of monkey material. It had been unaffected by this first inoculation.

The symptoms manifested by this sheep are strongly suggestive of a mild form of encephalitis, from which it recovered. It would appear that the Berkefeld filtrate contained no virus or a sub-infective dose. It is possible that the injection of this filtrate produced some immunity against the second inoculation, leading to the recovery of the sheep, but this is very doubtful.

A. 131, Sh. 3969. On August 28th this sheep was inoculated in the same way and with the same material as A. 130. It had also received a previous inoculation on August 2nd with the same Berkefeld filtrate. On September 5th the animal showed some paresis of the left foreleg, but could run about and was still feeding. The respirations were rapid. Next day it seemed worse. On September 7th it seemed better and could run about and feed. On September 10th it seemed well except for a little stiffness in one hind leg. At no time did it show any twitchings or head retraction.

It is possible, though doubtful, that the slight symptoms shown by this sheep were due to a mild attack of encephalo-myelitis. The remark under A. 130 again applies.

*Comment.* The histories of A. 125 and A. 130 are distinctly suggestive of mild attacks of encephalo-myelitis. Those of A. 90, A. 102 and A. 131 are also suggestive but less so. In the other sheep, A. 82, the result is very uncertain.

If some of these sheep were "mild takes," then there is evidence of bridging between the fatal disease conveyed to some individual sheep and the complete immunity possessed by others.

4. *Inoculation of sheep in series.—Insusceptibility of some animals.* In four instances, series of sheep, five or six in number, were all inoculated at the same time, in the same way, and under the same conditions, with materials consisting of various parts of the brain and spinal cord of successfully inoculated monkeys. The material used varied according to circumstances, but in most cases was a mixed emulsion of various portions of the brain and spinal cord, and in other cases tissue from the frontal and occipital regions only, or from

the medulla alone. In one series the emulsion was treated with sheep sera. In two animals of another series a Berkefeld filtrate of the emulsion was used. The general results obtained from these four series show conclusively that the material might in one individual sheep produce the typical disease, whilst the same material introduced in exactly the same way, at the same time and under the same conditions in another sheep might fail to produce any illness whatsoever. In several instances reinoculations of these apparently immune sheep with material presumably virulent again failed, suggesting, as is indicated in other experiments, that the immunity thus possessed is a real one, and that the occurrence of non-infection in the series was not due to any over-looked inhibitive factor. Therefore it must be considered as established that some individual sheep are susceptible to the virus and develop the disease when the virus is actually introduced into the middle of the cerebrum, while other individual sheep fail to react in any noticeable way after the virus has been so introduced. These results are very important inasmuch as the inference to be drawn from them may, with some reason, be applied to human beings, and may explain why some persons are attacked with the disease while others, apparently equally exposed to the virus, remain unaffected. Hitherto the general opinion as regards "acute poliomyelitis" may be said to have been that many individuals in the community harboured the virus in their naso-pharynx or in some other situation, and that the virus only occasionally gained access to the central nervous system. In other words, it appeared possible that any carrier might contract this disease, provided immunity had not been established, if the organism should gain entrance to the central nervous system. The results of these sheep inoculations show, however, that even after the virus has been introduced into the central nervous system infection may not necessarily result, but that individuals of the species may be absolutely insusceptible to the virus in spite of not having been already immunised to it.

The following is an account of the results of the four series of sheep inoculations mentioned:

(1) A. 80 to A. 85, Sheep 3892 to 3897. The first four of these animals received an inoculation of material from the usual selected areas of the brain and spinal cord; the fifth, material from the frontal and occipital areas of the brain only; and the sixth from the medulla alone. The last-mentioned animal died within three days of the operation, leaving five animals to be accounted for. The sheep inoculated with the frontal and occipital cortex only remained unaffected and later, under the designation A. 109, Sh. 3921, was successfully inoculated with the disease. The inference to be drawn is that in this particular case the material from the frontal and occipital regions of A. 72, Monkey 3873, either did not contain the virus or contained it in a sub-infective amount. Of the four sheep inoculated with the general emulsion, two developed the typical disease, one was unaffected, and one died 23 days after the operation without having shown definite signs of the disease, and without showing the specific histological lesions.

*Summary.* Of four sheep inoculated with the same material, at the same time and under the same conditions, two developed the disease and two did not.

(2) A. 89 to A. 94, Sheep 3902 to 3907. All these animals were inoculated with material from A. 78, Monkey 3890. The first three received the usual mixed emulsion of various parts of the brain and spinal cord; the fourth and fifth, material from the medulla alone; and the sixth material from the frontal and occipital regions only. Of these animals, two of those receiving the general emulsion and one of those receiving the medulla alone developed the disease, while the other three sheep were unaffected.

*Summary.* Neglecting the sheep which received material from the frontal and occipital regions and for which there was no control to show that this material contained the virus, we find that of five sheep in which the virus was introduced into the brain, three developed the disease and two failed to do so.

(3) A. 101 to A. 106, Sheep 3913 to 3918. These animals received an emulsion of the usual parts of the brain and spinal cord of A. 87, Monkey 3900. This emulsion was mixed with normal saline solution for the injections into two sheep; with the serum of a previously inoculated but unaffected sheep for two others; and with the serum of a normal healthy sheep for the remaining two. One of the last-mentioned sheep died in five days before any symptoms were likely to have manifested themselves. The two receiving the emulsion and saline were unaffected, but one of each of the other pairs developed the disease.

*Summary.* Of five sheep in which the virus was introduced into the brain, two developed the disease and three were unaffected.

(4) A. 119 to A. 123, Sheep 3939 to 3943. These animals were treated with material from A. 110, Monkey 3925. The first two received a Berkefeld filtrate and were unaffected; the other three received an emulsion of the usual parts of the brain and spinal cord. Of these, one developed the disease and two were unaffected.

*Summary.* Of three sheep in which the unfiltered virus was introduced into the brain, one developed the disease and two were unaffected.

5. *Sheep showing natural immunity to intracerebral inoculation of the virus.* Eight sheep, viz. A. 63, (76, 93, 115), A. 69 (94), A. 83 (114), A. 101 (125), A. 104 (126), A. 111, A. 122 and A. 68 (86, 90), are shown, in Appendices XX, XXI and XXV, to have been naturally immune to the disease even when the virus was introduced actually into the brain.

## VI. THE DISEASE AS MANIFESTED IN THE CALF.

The disease was conveyed successfully to one calf, as proved by histological examination after death. In two other cases slight symptoms arose, and in one were almost certainly those of encephalo-myelitis. Both of these animals recovered.

The chief symptoms in the calf, in which the presence of the disease was proved, were restlessness and a tendency to "go" in the front legs, followed by a clear discharge from the nostrils, and on the third day by weakness of the legs and a gait which tended to be circular. The illness terminated in death after general convulsive seizures, with rigidity of limbs, muscular tremors, retraction of the head and arching of the back.

## VII. THE DISEASE AS MANIFESTED IN THE HORSE.

Only one inoculation was made into this species of animal and it was successful, as proved by histological examination. The incubation period was nine days. On the first day of illness the animal tended to move towards one side. The next day there were in addition twitchings of the facial muscles, staring eyes and perhaps partial blindness. On the third day the animal was lying down with the head drawn to one side, and irregular movements of the limbs were observed. This was followed by intense and rapid convulsions, during which the animal was unconscious, alternating with short quiescent periods.

## VIII. OTHER EXPERIMENTS AND VARIOUS INFERENCES DRAWN.

1. *The treatment of the virus with various sera before intracerebral inoculation into monkeys.* Four groups of monkeys pass under this category, viz., A. 9 and 10, A. 20 and 21, A. 27 and 28, and A. 116 and 117.

A. 9 and 10. The serum used in the case of A. 9 was pooled from three children who had had infantile paralysis some time previously. In one case infantile paralysis had occurred  $2\frac{1}{2}$  years before, whilst in the other two cases the disease was probably as remote or more so. The children were under treatment, at the Royal Alexandra Children's Hospital, for the residual paralysis. The serum used in A. 10 was obtained from a healthy medical man who had never suffered from infantile paralysis. The usual emulsion of the human virus was mixed with equal amounts of the respective sera, and kept in an incubator for an hour, and then in the ice-chest over-night. Twenty-four hours after the mixing the injections were made.

A. 8 acts as a control to these two monkeys, inasmuch as the untreated emulsion was inoculated intracerebrally the day previously. A. 8 developed the disease nine days after inoculation, and had a length of illness of six days; A. 9 developed the disease twelve days after inoculation, and had a length of illness of eleven days; and A. 10 developed the disease fifteen days after

inoculation, and had a length of illness of three days. The three monkeys were killed *in extremis*.

From these results it would appear that, by the method employed, the sera of cases which had had infantile paralysis two or three years previously were not capable of neutralising the virus of encephalo-myelitis ("X disease"). It is interesting to note, however, that the two monkeys inoculated with the treated virus had a much longer incubation period than the one inoculated with the untreated virus; and as remarkable that A. 10, in which normal serum was used, had a longer incubation period than A. 9, in which pooled sera, wherein protected bodies might have been expected, were used. It may be noted, however, that whilst the disease in A. 10 ran a rapid course after a long incubation period, in A. 9 it ran a very slow course of eleven days, after a somewhat shorter incubation period. It may be further noted that A. 117, another monkey in which the emulsion was treated with a serum, had a phenomenally long incubation period of twenty-three days; on the other hand A. 124, inoculated with an untreated emulsion, had an incubation period of seventeen days.

*Conclusions.* The treatment of the emulsion of the virus with human serum may possibly lengthen the incubation period. Sera obtained from old cases of infantile paralysis were not proved capable of annulling the virus.

A. 20 and 21. These inoculations need not be further discussed, inasmuch as the emulsion employed was obtained from a monkey which histologically showed no evidence of encephalo-myelitis.

A. 27 and 28. The serum employed in the case of A. 27 was derived from a patient who was believed to have had "X disease" in the previous year. The blood had been forwarded by train and the serum kept in an ice-chest for a few days before use. In the case of A. 28, the serum was a normal human one. The method employed was that already described in connection with A. 9 and 10. Both monkeys failed to "take." As there were no control successful "takes" with material from this human case, there is unfortunately no evidence that the virus was present in the emulsion. Therefore nothing can be learned from the experiments.

A. 116 and 117. These monkeys received injections of a virus from A. 110, Monkey 3925. The virus consisted of an emulsion in normal saline solution of tissues from the usual regions in the brain and spinal cord. After being emulsified, the material was centrifuged at a low speed for two minutes, which yielded a supernatant milky fluid and a deposit of coarser fragments. The fluid was divided into two portions, and to each an equal amount of one of the sera to be mentioned was added. The mixtures were shaken, then incubated for two hours, then kept at room temperature for an hour, and finally injected intracerebrally into the respective monkeys. The object of this procedure was to see whether, during the time given and with the amount of serum employed, the infectivity of the virus would be annulled.

A. 116, Monkey 3936, received the supposed virus mixed with serum from

A. 89, Sheep 3902. This sheep was inoculated on June 27th with material from a previous monkey. It showed slight symptoms on July 2nd, 3rd and 4th, which might be considered as indicating a mild form of encephalo-myelitis. On July 5th it seemed well again. On July 18th 20 c.c. of blood were removed under an anaesthetic. It was used for immunity experiments on sheep. The animal was bled again on July 31st under an anaesthetic. Two days later it was sick, apparently from lung trouble, was worse next day and died on August 4th. Histological examination showed lesions of encephalo-myelitis, apparently in a stage of early resolution, suggesting that the symptoms manifested from July 2nd to 4th were due to a mild form of this disease from which the animal was in process of recovery. A. 116, Monkey 3936, remained unaffected by the inoculation of the virus combined with the serum of this sheep.

A. 117, Monkey 3937, received the virus intermixed with the serum of A. 102, Sheep 3914. This sheep had been inoculated on July 7th with material from a previous sheep, A. 101, Sh. 3913. It became ill five days later with a raised temperature, and thereafter had occasional twitchings, breathed rapidly, seemed very weak, was "gone" in the legs, and had a running nose. It appeared perfectly well on July 19th. Blood was withdrawn under an anaesthetic on July 31st. The symptoms manifested by this sheep suggest that it may have had a mild form of encephalo-myelitis from which recovery had resulted. A. 117, Monkey 3937, was found prostrate on the bottom of the cage twenty-three days after the inoculation. It exhibited intense incoördinated movements on being disturbed, and histological examination after death showed typical lesions of encephalo-myelitis.

*Discussion of the results.* First it is necessary to point out that the monkey supplying the virus employed in these two experiments, namely A. 110, Monkey 3925, at the time it was killed appeared as though it might have recovered from the disease. The possibility, therefore, is that the strain of virus at this stage possessed less virulence than it had originally, so that even without any other treatment it might have failed to convey the disease to a monkey, or have only produced the disease after a long incubation period, or in mild form, when it did appear. The results show that A. 116 did not contract the disease and that A. 117 contracted it after an unduly prolonged incubation period of twenty-three days. It is to be noted, however, that when the disease did arise in A. 117, it appeared in an intense form. After making due allowance, therefore, for the possibility that the activity of the virus was waning, the negative result in A. 116 is in support of the view that the serum of A. 89, Sheep 3902, actually did neutralise the virus under the circumstances of the experiment. It might have been expected, moreover, that the serum from this sheep, which was actually shown later to have suffered from encephalo-myelitis, would have possessed immune bodies—if such are developed in this disease—at the time when it was withdrawn. As regards A. 117, the inferences are less clear. The sheep whose serum was employed may or may not have had encephalo-myelitis. The unduly prolonged incubation period may or may not have



been the expression of some immune properties held by this serum. The serum may in fact have delayed the appearance of the disease without modifying its course when it did appear.

2. *The treatment of the virus with various sera before intracerebral inoculation into sheep.* Four sheep, A. 103, 104, 105 and 106, were inoculated with material from A. 87, Monkey 3900. The usual emulsion in normal saline solution was centrifuged at a low speed for about seven minutes. The supernatant fluid was then divided into two portions and each mixed with an equal amount of serum—with serum from a normal sheep, in the case of the last two, and with serum from A. 82, Sheep 3984, in the case of the first two animals. After incubation for two hours, the mixtures were kept at room temperature for a further two hours, and then injected intracerebrally into the respective sheep.

The serum-yielding sheep, A. 82, had been inoculated with material from A. 72, Monkey 3873, on June 17th. On June 29th it seemed ill but showed no paresis. It was bled under an anaesthetic on July 6th for the purpose of the present experiments and died on July 10th, apparently from the after-effects of the anaesthetic. Histological examination showed no lesions of encephalo-myelitis. It should be noted that two companion sheep to A. 82, viz., A. 80 and A. 81, both developed the disease after identical intracerebral inoculations.

A. 103, Sheep 3915, developed the disease, whilst A. 104, Sheep 3916, showed no symptoms, being probably an immune animal.

A. 105, in which the emulsion was treated with serum from a normal sheep, developed the disease, but A. 106, similarly treated, died from lung trouble in five to six days, perhaps before signs of the disease could have manifested themselves.

*Discussion of results.* The serum of A. 82, Sh. 3894, failed to neutralise the virus in the method employed when injected into A. 103, Sh. 3915. As explaining this result, there is no evidence that A. 82, Sh. 3894, actually had encephalo-myelitis, so as to be in the position of possessing anti-bodies in its serum.

The positive results in A. 105, together with the positive results in A. 103, both show that by the method employed sheep serum alone does not neutralise the virus.

A. 111, 112 and 113. As regards these inoculations, the procedure was that adopted in the preceding series, save that the mixtures were incubated for only 1¼ hours and were inoculated an hour later. Again, as showing that the virus was present in the material used, A. 110, Monkey 3925, acts as a control since this received an injection differing only in the replacing of the serum by an equal amount of normal saline solution, and the animal developed the disease. The serum employed for the three sheep was that from A. 89, Sheep 3902, taken on July 18th. A short summary of the history of A. 89 has been already given in discussing the experiments on the two monkeys, A. 116 and

117, in the preceding section. It will be remembered that the serum employed in the case of these monkeys, and which apparently protected A. 116 against infection, was obtained from A. 89, Sheep 3902, on July 31st, whereas the serum employed in the case of these sheep was that obtained on July 18th. A. 111 showed no evidence of encephalo-myelitis after its inoculation; the other two animals died two and four days respectively after the inoculation before any symptoms could possibly have arisen.

*Discussion.* It is possible that A. 111, Sheep 3923, was protected against infection by the blood serum of A. 89, Sheep 3902. As, however, many sheep are naturally immune to the intracerebral injection of the virus, the question of the protective value of this serum at this date is by no means proved.

3. *Experiments suggesting that the virus is no longer present on the 8th or 10th day of illness in monkeys.* In the discussion on the "Failure in Monkeys of Certain Intracerebral Inoculations of Brain and Spinal Cord from Monkeys" (vide Appendix VI) it is suggested that the failure to infect A. 18, Monkey 3823, and perhaps A. 19, Monkey 3824, was due to the disappearance of the virus during the eight or ten days of illness that had elapsed before the death of A. 9, Monkey 3785.

4. *Experiments showing presumed dying out of the virus during prolonged storage in glycerine in the cold.* A. 17, Monkey 3836, was inoculated with material from a monkey on April 11th and was unaffected thereby. The same material on February 13th had successfully conveyed the disease to A. 14, Monkey 3805. That A. 17 was not immune was shown by its successful inoculation later as A. 55, Monkey 3848. The inference is that the virus had died out during the storage for two months in glycerine emulsion in the cold.

A. 96, Sheep 3935 was inoculated on July 29th with material from a monkey, A. 78, Monkey 3890, and remained unaffected. The same material on June 27th and 28th had successfully conveyed the disease to a monkey, a sheep and a horse. This sheep, A. 96, was not further tested to see whether it possessed natural immunity. The failure of the experiment may therefore be attributed either to the length of time the material was preserved in glycerine in the ice-chest, namely a month, or to natural immunity.

5. *Experiments possibly showing a waning in virulence of the virus.* A. 116, Monkey 3936, was inoculated with monkey material which had been treated with the serum of a "positive" sheep, A. 89. The monkey was unaffected by the inoculation, whilst another monkey, A. 117, M. 3937, which was inoculated on the same day with the same material, save the substitution of serum from another sheep, A. 102, developed the disease after an unduly prolonged incubation period of 23 days, the disease thereafter running its usual course. It is probable that the failure of A. 116 to "take" was attributable to the neutralising power of the sheep serum, and it is possible that the prolonged incubation period in A. 117 was due to the presence of similar neutralising bodies, but

to a less degree, in the second sheep's serum. On the other hand it is possible that the negative result in the first monkey, and the prolonged incubation period in the second, were due to a waning in activity of the virus leading to complete failure to "take" in some monkeys and a prolonged incubation period in others. It may be noted that A. 132, which is the same monkey as A. 116, also escaped disease when inoculated later with material from another positive monkey. This second failure may be attributed either to the animal having been rendered artificially immune by the first inoculation, or to the supposed waning of virulence leading to a "take" in some monkeys and a failure in others.

6. *Experiment suggesting the production of artificial immunity.* A. 132, Monkey 3977, which has just been discussed, may be an instance of artificial immunity resulting from the administration of an active virus which had been exposed to the serum of a sheep, which sheep had had encephalo-myelitis and still showed lesions of this disease.

#### IX. SUMMARY AND CONCLUSIONS.

1. The disease is an acute encephalo-myelitis produced by a virus akin to, but not identical with, that of the Heine-Medin disease.

2. The disease was readily communicated, with fatal results, to monkeys (*Macacus rhesus*) by intracerebral inoculation of a suitably-prepared emulsion of nervous substance (brain, cerebellum, pons, medulla and spinal cord) from the human subject dead from "X disease." Moreover, the virus was found to breed true in a succession of thirteen monkey (*Macacus rhesus*) generations.

3. The disease was not communicated to *Macacus cynomolgus* (several trials).

4. The disease was communicated by the above-mentioned method from monkey to sheep (10 times), from sheep back to monkey and on again from monkey to monkey.

5. A certain number of sheep, perhaps 50 per cent., were found wholly insusceptible to the disease; others suffered lightly and recovered.

6. The disease was communicated, with fatal results, by the same method, from monkey to horse (1 case) and to calf (1 case). Two calves suffered lightly after intracerebral inoculation of the usual virus-containing material taken from monkey and horse respectively.

7. The virus appears to be held back completely, or to a great degree, by the pores of a Berkefeld filter.

8. Storage of the virus-containing material in diluted glycerine, under cool conditions, for longer than a few days, reduced or annulled its nocive properties.

9. Drying of the virus-containing material in Petri dishes, in an incubator, probably destroys its activity.

10. In the case of the sheep, there was failure to induce the disease by swabbing the nostrils with virus-containing emulsion.

11. There is some evidence that in the case of the sheep and the calf a previous inoculation with the virus confers immunity.

12. One experiment suggested that artificial immunity might be induced in the monkey by inoculation of virus treated with serum from an "X disease" sheep.

13. Intracerebral inoculation of three dogs, one kitten, two rabbits and one hen failed to produce any signs of the disease; and similar inoculations of two guinea-pigs gave doubtful results.

14. Treatment of the virus-containing emulsion with (a) normal human serum, (b) serum from recovered human cases of "acute poliomyelitis" and (c) serum from "X disease" sheep prolonged the incubation period of the disease in the monkey but did not destroy the virus.

15. Normal sheep serum and serum from "X disease" sheep did not neutralise the virus in its operation on other sheep.

16. Two experiments suggested that the virus was no longer present in the monkey on the eighth or tenth day of illness.

17. Two experiments towards the end of the investigation suggested a waning in strength of the virus.

18. Intraperitoneal and intrasciatic inoculations of virus-containing material, also intracerebral inoculations of cerebro-spinal fluid, of a filtrate of faeces, of a "Noguchi culture," of an emulsion of fowl ticks, of naso-pharyngeal swabs from human cases and contacts, and inoculations into veins, all failed.

## X. APPENDICES<sup>1</sup>.

### APPENDIX I. *Tabulation of the kinds of Inoculations and Results in the respective Species of Animals.*

Each number refers to an individual experiment on an animal. Since in many instances it was necessary, when no result followed, to use the animal again for a further experiment, it is obvious that the number of animals actually used is considerably less than might be inferred from the number of experiments made.

MONKEYS: *Macacus rhesus* and *Macacus cynomolgus* = 62.

Positive results from the intracerebral inoculation of material from three human cases: A. 8, 9, 10, 33, 48 = 5

Positive results from the intracerebral inoculation of material from monkeys: A. 14, 49, 50, 55, 62, 64, 66, 72, 78, 87, 100, 110, 117, 124, 129 = 15

Animals dying shortly after the operation as a direct result of this or from early sepsis. (These cases will not be further considered): A. 7, 13, 22, 25, 47, 51, 128 = 7

<sup>1</sup> Throughout the Appendices the abbreviations M. and Sh. stand for Monkey and Sheep respectively.

*Acute Encephalo-myelitis*

Death in 12 days from pathogenic infection, without co-existent evidence of encephalo-myelitis: A. 11	= 1
Failure of intracerebral inoculations of the brain (and spinal cord) from eight human cases: A. 27, 28, 29, 37, 38, 39, 40, 41, 44, 45	= 10
Failure of certain intracerebral inoculations of the brain and spinal cord of monkeys: A. 17, 18, 19, 20, 21, 116, 132	= 7
Failure of intraperitoneal inoculations of human spinal cord: A. 6, 24	= 2
Failure of the intrasciatic inoculation of the brain and spinal cord from a human case: A. 5	= 1
Failure of the intracerebral inoculation of Pasteur-Chamberland F. filtrates: A. 12, 26, 54	= 3
Failure of the intracerebral inoculation of cerebro-spinal fluid: A. 1, 32	= 2
Failure of the intracerebral inoculation of a Pasteur-Chamberland F. filtrate of faeces: A. 31	= 1
Failure of the intracerebral inoculation of a "Noguchi culture"	= 1
Failure of the intracerebral inoculations of naso-pharyngeal swabs from contacts and a case: A. 3, 30	= 2
Failure of the intracerebral inoculation of the brain and spinal cord of a horse: A. 42	= 1
Failure of the intracerebral inoculation of an emulsion of fowl ticks: A. 43	= 1
Failure of the intraperitoneal inoculation of swabs from contacts: A. 2	= 1
Failure of the intrasciatic inoculation of swabs from contacts: A. 4	= 1
Failure of the introduction of the virus into a vein after lumbar puncture: A. 71	= 1

**SHEEP: = 52.**

Positive results from intracerebral inoculation of material from the brain and spinal cord of monkeys: A. 52, 65, 80, 81, 89, 91, 92, 103, 105, 121	= 10
Positive results from intracerebral inoculation of material from the brain and spinal cord of positive sheep: A. 98, 108, 109	= 3
Dying within four and a half days of the operation as a direct result of this, or from post-anaesthetic lung trouble or sepsis: A. 85, 99, 112, 113, 123	= 5
Surviving intracerebral inoculation but showing slight symptoms, possibly of encephalo-myelitis: A. 82, 90, 102, 125, 130, 131	= 6
Showing no symptoms after intracerebral inoculation of brain and spinal cord from human cases: A. 35, 46	= 2
Showing no symptoms after intracerebral inoculation of brain and spinal cord from infected monkeys: A. 53, 63, 69, 75, 83, 84, 93, 94, 96, 101, 104, 106, 111, 122	= 14
Showing no symptoms after intracerebral inoculation of brain and spinal cord from infected sheep: A. 68, 114, 115, 126	= 4
Showing no symptoms after intracerebral inoculation from a monkey histologically negative. (This case will not be further discussed): A. 23	= 1
Showing no symptoms after intracerebral inoculation from a sheep histologically negative. (This case will not be further discussed): A. 86	= 1
Showing no symptoms after swabbing the nose with virus-containing material: A. 70	= 1

- Showing no symptoms after the introduction of horse serum into the spinal canal and of a small quantity of virus into a vein: A. 76 = 1
- Showing no symptoms after intraperitoneal inoculation of the virus: A. 67 = 1
- Showing no symptoms after the intracerebral injection of dried tissue from the brain and spinal cord: A. 77 = 1
- Showing no symptoms after intracerebral inoculation of a Berkefeld filtrate of virus-containing material: A. 119, 120 = 2

**CALVES:** = 4.

- Positive result after intracerebral inoculation with material from the brain and spinal cord of a monkey: A. 57 = 1
- Showing symptoms of illness, possibly of encephalo-myelitis, after intracerebral inoculation of material from the brain and spinal cord of a positive monkey or a positive horse: A. 88, 107 = 2
- Showing no symptoms after intracerebral inoculation (a second inoculation) with brain and spinal cord from a positive sheep: A. 127 = 1

**HORSE:** = 1.

- Positive result from intracerebral inoculation of material from the brain and spinal cord of a monkey: A. 95 = 1

**DOGS:** = 5.

- Showing no symptoms after an intracerebral inoculation of material from the brain and spinal cord of a human case, a monkey case, or a sheep case: A. 16, 34, 56, 97 = 4
- Dying as a result of the operation. (This case will not be further discussed): A. 15 = 1

**KITTENS:** = 2.

- Showing no symptoms after intracerebral inoculation of brain and spinal cord from a positive monkey: A. 79 = 1
- Dying as the result of the operation. (This case will not be further discussed): A. 61 = 1

**RABBITS:** = 3.

- Showing no symptoms after intracerebral inoculations: A. 58, 73 = 2
- Dying as the result of the operation. (This case will not be further discussed): A. 59 = 1

**GUINEA-PIGS:** = 2.

- Showing indefinite symptoms and doubtful histological results after intracerebral inoculation of brain and spinal cord from infected monkeys: A. 60, 74 = 2

**HEN:** = 1.

- Showing no symptoms after intracerebral inoculation of brain and spinal cord from an infected monkey: A. 118 = 1

*Acute Encephalo-myelitis*

APPENDIX II. *Table of Monkey Inoculations. Positive Results.*

No. of Monkey	Date	Source of material	Day of illness when virus was obtained	Hours after death when p.m. examination made	Period virus was <i>in vitro</i>	Medium in which virus was preserved	Incubation period in days	Length of illness in days
A. 8, M. 3783	29/1/18	Case 27, Narrabri	3	} 21	1 day	} 33% glyc. in saline	9	6
A. 9, M. 3785	30/1/18	" 27 "	3		2 days		12	11
A. 10, M. 3786	30/1/18	" 27 "	3		2 days		15	3
A. 14, M. 3805	13/2/18	A. 8, M. 3783	6	Immediate	1 day	50% glyc. in saline	10	3
A. 33, M. 3803	13/2/18	Case 38, Wee Waa	2	12	1 day	33% "	12	6
A. 48, M. 3829	22/3/18	Case 32, Narrabri	5	5 hours	7 days	33% glyc. sol. in saline	11	3
A. 49, M. 3835	4/4/18	A. 48, M. 3829	3	Immediate	A few hours	Normal saline	7	5
A. 50, M. 3839	15/4/18	A. 49, M. 3835	5	"	"	33% glyc. sol.	5	5
A. 55, M. 3848	16/4/18	A. 50, M. 3839	5	Some hours	1 day	"	8	2½
A. 62, M. 3845	23/4/18	A. 52, Sh. 3839 b	4	3	1 day	"	5	6
A. 64, M. 3854	7/5/18	A. 55, M. 3848	2½	A few hours	2 days	"	7	5
A. 66, M. 3860	17/5/18	A. 65, Sh. 3855	1	A few hours	1 day	"	6	8
A. 72, M. 3873	30/5/18	A. 66, M. 3860	8	Immediate	A few hours	Normal saline	11	6
A. 78, M. 3890	15/6/18	A. 72, M. 3873	6	"	"	"	10	3
A. 87, M. 3900	27/6/18	A. 78, M. 3890	3	"	"	"	7	4
A. 100, M. 3912	7/7/18	A. 87, M. 3900	4	"	3 hours	"	9	4
A. 110, M. 3925	19/7/18	A. 100, M. 3912	4	"	A few hours	"	11	4*
A. 117, M. 3937	2/8/18	A. 110, M. 3925	4	"	"	"	23	3
A. 124, M. 3952	17/8/18	A. 110, M. 3925	4	"	15 days	33% glyc.	17	5
A. 129, M. 3967	27/8/18	A. 117, M. 3937	3	"	A few hours	Normal saline	10	4

\* Perhaps would have recovered.

M. = Monkey. Sh. = Sheep.

APPENDIX III. *Details of the Successful Inoculations in Individual Monkeys (Macacus rhesus).*

A. 8, M. 3783, became ill on the ninth day. During the first two days its movements were violent and incoördinate, and it showed apparent weakness in the legs. On the third day it was quieter, but markedly weak and very clumsy in its movements. On the sixth day, when it was killed, both legs were markedly paretic, whilst the right arm seemed absolutely paralysed and useless.

A. 9, M. 3785, became ill on the twelfth day, showing slight weakness in the legs. Its condition was much the same on the two following days. On the fourth day the right arm seemed decidedly paretic and clumsy, whilst the legs were markedly paretic and dragged after the body on movement. The body swayed on moving and movements were clumsy. On the fifth day the animal was ataxic and frequently fell over, whilst its movements were clumsy and the neck seemed weak. Its condition during the next three days was much the same. On the ninth day, in addition, the head was somewhat retracted; the muscles of both arms and legs still possessed some tone. On the eleventh day, when the animal was killed, it was much exhausted and no movements could be detected in the arms.

A. 10, M. 3786, after an incubation period of fifteen days, showed an anxious expression and seemed drowsy. It reeled like a drunken man; the arms and legs seemed paretic. On the second day of illness it showed marked ataxia and the neck seemed weak. On the third day it was lying on its side; the right arm was apparently paralysed; the left arm and both legs could be moved a little, but some tone still remained in the limbs. The animal was killed on this day.

A. 14, M. 3805, after an incubation period of ten days, became markedly ataxic whilst the hind limbs were apparently paretic. Next day it uttered sharp cries from time to time, and its head was frequently buried on the chest. On being startled it reeled and swayed about in a convulsive way, injuring itself. The hind limbs and right arm were weak. On the third day the animal was quite prostrate; it showed slight convulsive movements of the left arm and left leg, and the head was slightly retracted. The temperature was subnormal. It died on this day.

A. 33, M. 3803, after an incubation period of twelve days, had an anxious expression and showed clumsy movements. There was no definite paresis. On being disturbed, convulsive seizures, without loss of consciousness, occurred. On the second day there was marked incoördination and apparently some rigidity, whilst attacks of convulsive movements occurred from time to time. There was marked incoördination and an occasional twitch of the limbs. Partial paresis of the hands was manifested when it tried to feed itself. The same intense incoördination and general convulsive movements occurred during the next two days. On the fifth day it was lying on its side at the bottom of the cage; both hind limbs seemed useless but rather rigid than flaccid, whilst both arms were paretic. On the sixth day, when it was killed, the animal was still prostrate, and there was paresis of all four limbs which were rather flaccid.

A. 48, M. 3829, became ill on the eleventh day and showed the usual very anxious expression. Its movements were decidedly incoördinate. During the next two days the incoördination was greatly increased and an internal squint was present on the third day of illness when the animal was killed.

A. 49, M. 3835, became ill on the seventh day, showing a frightened expression and being nervous. One arm and leg seemed rather spastic. On the third day of illness it uttered peculiar staccato cries, was very "jumpy," and showed exaggerated incoördinated movements. Next day its condition was worse, and on the fifth day, when it was killed, it was prostrate on the bottom of the cage with the head markedly retracted.



A. 50, M. 3839, showed a frightened expression on the fifth and sixth days after its inoculation. On the third day of illness it uttered barking noises; its movements were exaggerated but there was no definite incoördination. Next day, in addition, there was slight incoördination, and on the fifth day of illness the animal was prostrate on the bottom of the cage, though it could partly raise itself. Its movements were much exaggerated, during which it injured itself. It was killed with chloroform on this day.

A. 55, M. 3848, did not use the left arm on the seventh day after its inoculation. Next day its movements were very shaky and rather incoördinate. This may be considered as the first day of illness. On the second day the animal was intensely shaky, trembling all over as if from paralysis agitans, and was rather incoördinate in its movements; it died during the night.

A. 62, M. 3845, on the fifth day after inoculation appeared to show slight incoördination. Next day its movements were distinctly ataxic, and there appeared to be some weakness in one arm and leg. On the third day it swayed on movement, but the apparent weakness of the arm and leg had disappeared. On the fourth day it was much the same. On the fifth day the hind limbs seemed paretic and movements were very shaky, jumpy, and incoördinate. The animal fell from time to time on the bottom of the cage, and showed violent incoördinated movements, almost convulsive in character. The animal was killed with chloroform.

A. 64, M. 3854, after an incubation period of seven days, seemed jumpy when disturbed. Two days later it looked anxious, and there was marked incoördination of movement. On the fourth day it was lying prostrate on the bottom of the cage. It moved the arms and legs but the movements were incoördinate and paretic. Next day the animal could not sit up or even hold its head up, and the head was slightly retracted. There were slight irregular muscular contractions in the right arm, both legs and the tail. Some tone was still retained in the limbs. Chloroform was administered.

A. 66, M. 3860, after an incubation period of six days, had an anxious expression and its movements were excessive and there was slight incoördination. Next day, in addition, there was weakness of the hind limbs. From the third to the fifth day it was much the same, but on the sixth day the right arm was apparently paretic. On the seventh day it was worse, but could still jump about and feed. On the eighth day it was prostrate on the floor of the cage, and there seemed to be almost complete paralysis. On the eighth day the condition was worse, though the limbs were not absolutely flaccid, and still possessed some tone. The animal was killed by chloroform.

A. 72, M. 3873, after an incubation period of eleven days, had an anxious expression. For the next two days its condition was the same, but on the fourth day there was ptosis of both eyelids, slight incoördination of movement, and dragging of the left leg. On the fifth day its movements were markedly incoördinate and jumpy, and there were slight twitchings of the left arm. The right arm and leg during the day became paretic. On the sixth day it was prostrate on the bottom of the cage, and the right side seemed paralysed. This was found not to be a true complete paralysis, as there was some resistance to passive movement. The knee-jerks and elbow-jerks were present. The animal was killed with chloroform.

A. 78, M. 3890, after an incubation period of ten days, had a slightly anxious expression and seemed more excitable than its healthy fellow. Next day it was markedly ill and apparently intensely sleepy. There was definite ptosis on both sides, but more decided on the left side. It swayed on movement and struggled violently. On the third day it was prostrate, could not raise its head or sit up. There was no head retraction; the limbs still possessed tone. Chloroform was administered.

A. 87, M. 3900, after an incubation period of seven days, had an anxious expression and was rather jumpy. Next day its movements were very incoördinate and it had two convulsions after being disturbed. On the third day its condition was worse, and towards evening it was prostrate on the bottom of the cage, and when disturbed, violent and irregular con-

vulsive movements occurred. On the fourth day it was still prostrate; it could only move the arms and tail a little, but some tone was still present in them. Chloroform was administered.

A. 100, M. 3912, after an incubation period of nine days, had a slightly anxious expression. Next day its countenance was still slightly anxious-looking, and there was decided incoördination and exaggeration of movements. On the third day it had fallen to the bottom of the cage, and could only raise itself partly and then struggled round with convulsive movements. The head became retracted. On the fourth day the animal was still prostrate, uttered occasional barking noises and had head retraction. The limbs were rather rigid and there was no flaccid paralysis though there was probably some paresis. Chloroform was administered.

A. 110, M. 3925, after an incubation period of eleven days, had an anxious expression and walked somewhat "gingerly." Next day there was marked incoördination, and it presented a somnolent appearance at times. There was slight tremor of the arms and occasional tremors of the head, limbs and hands. Next day the head was somewhat depressed on the chest and would gradually sink lower and lower as if the animal were dropping off to sleep, when it would overbalance and struggle incoördinately round. There were slight muscular twitchings, and the eyes were occasionally turned to the left; there was drooping of the eyelids. On the fourth day the monkey seemed distinctly, though slightly, better. There was an inward and upward squint of the right eye, but no ptosis of either lid. It was not now somnolent. Its movements were distinctly incoördinate and violent. There was no definite paresis. The animal looked as though it might possibly recover. Chloroform was administered to obtain the virus.

A. 117, M. 3937, after an incubation period of twenty-three days, was found prostrate on the bottom of its cage. On being disturbed it struggled round with intense incoördinate movements, during which it injured itself. It uttered short sharp barking noises from time to time. It could not sit up or stand, but could grip hold with its hands and feet. On the second day of illness it was quite prostrate but conscious; the right arm seemed paralysed, but it kicked vigorously with both hind legs and the left arm. On the third day of illness, when chloroform was administered, the right arm was severely paralysed though there was still some slight tone in the biceps and very slight tone in the flexors of the forearm. The left arm was markedly paretic and rather rigid, whilst the legs were rather rigid and paretic. The knee and back muscles seemed also paretic. The temperature was subnormal.

A. 124, M. 3952, after an incubation period of seventeen days, was noticed to be slightly "nervous." Next day the left arm and leg were weak, and the animal was still "nervous." On the third day the left arm seemed quite paralysed whilst the left leg and the hind-quarters were weak. It did not seem able to see things properly. On the fourth day it was having convulsions at frequent intervals, lying on the floor of the cage between times. On the fifth day, that in which the animal was killed, the left arm seemed completely paralysed, but it could grip with the right hand and both feet. The temperature was subnormal.

A. 129, M. 3967, after an incubation period of ten days, appeared "nervous" with incoördination of movement and some paresis of the left arm. Next day there was intense incoördination, during which the animal injured itself against the sides of the cage; some of its muscular movements might be described as contortions. On the third day of illness the left arm appeared to be completely paralysed, and there was indefinite weakness of the right arm. Convulsions, lasting a few seconds, occurred from time to time. Incoördination was intense. On the fourth day the animal was found prostrate on the bottom of its cage apparently dead. Its eyes still seemed to recognise its surroundings, however, whilst there were slight indications of movement at the ends of the extremities. The temperature was markedly subnormal.

APPENDIX IV. *Death of a Monkey in Twelve Days from Pathogenic Infection without Co-existent Evidence of Encephalo-myelitis.*

A. 11, M. 3801, was inoculated on February 12th, with material from A. 8, M. 3783, which was killed on this date. A day later A. 14, M. 3805, received an inoculation containing the same virus with the addition of serum from the monkey contributing the virus. A. 14 developed encephalo-myelitis, as proved by final histological examination. A. 11, nine days after inoculation, developed symptoms resembling those of other animals infected with this disease. It died four days later. Histological examination showed a picture obscured by widespread miliary abscesses in which micrococci were detected. There was no histological evidence of encephalo-myelitis. As portions of the brain, from increased intracranial pressure, protruded through the trephined hole, and the surface wound, in consequence, had re-opened after the animal became ill, complicating septic infection was expected. It was, however, believed at the time that the hernial protrusion had been caused by the congestion of the brain due to the development of encephalo-myelitis, and that lesions of this disease would be detected as well as those of the secondary infection; such, however, was not the case. The interesting speculation arose as to whether histological lesions were really present but were overlooked or masked, or whether the pyogenic infection had in some way destroyed the virus of encephalo-myelitis. As against the first of these views, it may be noted that the inoculations made from A. 11 were negative; somewhat in favour of the latter, and as tending to show that active virus was originally injected into A. 11, is the successful result with the same material, used on the following day, in A. 14.

APPENDIX V. *Failure in Monkeys of Certain Intracerebral Inoculations of Human Material.*

Intracerebral injections of emulsions from portions of the brain and (or) spinal cord from human cases failed as follows:

A. 27, M. (*Macacus rhesus*) 3809 (from Case 28, J. M., Narrabri, dying on the sixth day), inoculated with the glycerine emulsion of the upper cervical cord treated with serum of Case 30, O'M, 1917 series, a supposed recovered case. Inoculation made ten days after patient's death.

A. 28, M. (*M. rhesus*) 3810, inoculated as in A. 27, M. 3809, but the glycerine emulsion was treated with a normal serum. Inoculation ten days after patient's death.

A. 29, M. (*M. rhesus*) 3819, inoculated with the same material as A. 27, M. 3809, and A. 28, M. 3810, but without treatment with serum, and twenty-eight days after the death of the patient.

A. 37, M. (*M. rhesus*) 3811 (from Case 6, R. M., Broken Hill, dying on the fifth day of the disease), inoculated with a glycerine emulsion of portions of the brain and spinal cord after transmission through the post. Inoculation eight days after patient's death.

A. 38, M. (*M. rhesus*) 3812 (from Case 7, G. B., Broken Hill, dying on the fourth day of the disease), inoculated with a glycerine emulsion of portions of the brain and spinal cord after transmission through the post. Inoculation eight days after patient's death.

A. 39, M. (*M. rhesus*) 3830 (from Case 18, E. C., Broken Hill, dying on the eighth day of the disease), inoculated with similar material to A. 37, M. 3811, and A. 38, M. 3812. Inoculation eleven days after patient's death.

A. 40, M. (*M. rhesus*) 3828 (from a supposed case in Brisbane). The glycerine emulsion of the brain and spinal cord was inoculated a week or ten days after the patient's death.

A. 41, M. (*M. rhesus*) 3831 (from Case 54, G. S., Narrandera, dying on the 12th day of the disease). Small portions of the brain and spinal cord were transmitted through the post in glycerine, and inoculations made five weeks after the patient's death.

A. 44, M. (*Macacus cynomolgus*) 3825 (from Case 37, A. C. F., Boggabri, dying on the third day of the disease, post-mortem next day). A glycerine emulsion of portions of the brain and spinal cord was inoculated two days after the patient's death.

A. 45, M. (*M. rhesus*) 3837, was inoculated with the same material as A. 44, M. 3825, twenty-nine days after the patient's death.

*Analysis of the above unsuccessful results.*

CASE 28. As regards the experiments contingent on Case 28, J. M., Narrabri, of the two monkeys used for the inoculations, one monkey (A. 28, M. 3810) was two months later successfully inoculated with the disease from A. 50, M. 3839, under the designation of A. 55, M. 3848, on 16/4/18. It would therefore appear that this monkey was susceptible to the disease and that it had not been protected against the later introduction of the virus by having suffered from a mild but unrecognised attack after the first inoculation. Only a very partial post-mortem examination was allowed on this human case, the material obtained being curetted from the upper cervical cord through a small incision in the back of the neck. Failure in the inoculation may be attributed to this particular area of the spinal cord either not containing the virus (though showing the characteristic lesions), or containing the virus in a subinfective amount. The treatment of the emulsions by sera, one of which was normal, cannot be considered as the cause of failure, inasmuch as A. 9, M. 3785, and A. 10, M. 3786, were inoculated with emulsions also treated with sera, and these monkeys "took." It is of course possible that the serum of the supposed recovered case of the previous year might have protected A. 27, M. 3809, had the virus been present. The normal serum, however, should not have protected A. 28, M. 3810. The inoculations were made ten days after the patient's death, and this period of glycerinisation may have been responsible for the failures. As regards A. 29, M. 3819, inoculated with the glycerinated material untreated by serum, twenty-eight days after the patient's death, failure may have again occurred either because of the absence of the virus originally in the material used or on account of the long period during which the virus had been exposed to the influence of glycerine.

*Conclusions.* Failure of inoculations from Case 28, J. M., Narrabri, may be attributed either to the absence, relative or complete, of the virus in the material obtained, or to the length of time, ten to twenty-eight days, during which the glycerinated material was kept.

*Broken Hill and Narrandera Cases.* As regards the four cases in which the material was transmitted by post, we did not take this material ourselves, and it is possible that portions of cerebral tissue containing the virus were not selected. The material came in blocks in glycerine during the warm period of the year, and exposure to the late summer high temperature may have destroyed the virus. The inoculations were made in two instances eight days after death of the host, in one eleven days, and in one five weeks. As the patients died on the 4th, 5th, 8th and 12th days of the disease the virus should have been still present in the brain or cord of two of these cases at least, when death occurred.

*Conclusions.* Failure in these four cases may be attributed to the exposure of the glycerinated material during transit for several days to the high temperature of late summer—a condition inimical to the keeping qualities of vaccinia virus for instance—or to the period of exposure to glycerine after death of the patient, which was eight days or longer; or possibly to infective material not having been selected.

*Brisbane Case.* As regards the Brisbane case, the material was obtained by a colleague, Dr Bradley, so that portions likely to contain the virus were selected. The glycerinated material was necessarily kept at late summer temperature during transit, and was inoculated a week or ten days after the death of the patient.

*Conclusions.* Failure may be attributed to the elevated temperature during transit and the long period before the inoculation was made.

CASE 37. As regards the material from Case 37, A. C. F., Boggabri, the first monkey inoculated was *M. cynomolgus*. We have not so far obtained a successful result in the few inoculations we have made into this species of monkey. The patient died on the third day of the disease; the material was taken next day, and the monkey (A. 44, M. 3825) was inoculated on the succeeding day. A. 45, M. (*M. rhesus*) 3837, was inoculated twenty-nine days after the patient had died; that it was not immune to the virus was shown by a successful inoculation six weeks later (A. 72, M. 3873).

*Conclusions.* Whilst the failure of A. 45, M. 3837, to "take" might be attributed to the length of time that had elapsed after the patient had died, there seems no explanation, except perhaps the species of monkey employed, for the failure of A. 44, M. 3825. The material used in the glycerine emulsion was obtained from the frontal, parietal, occipital and temporo-sphenoidal regions of the cerebrum, and from the cerebellum, pons, medulla, and cervical, dorsal and lumbar regions of the cord—that is from parts which have been successfully employed in other cases. The child died on the third day of the disease before the virus could be expected to have died out. A post-mortem was made on the day after death—a period which allowed successful results in other cases. The first inoculation was made only two days after death—that is, after a period which gave success in other cases.

#### APPENDIX VI. *Failure in Monkeys of Certain Intracerebral Inoculations of Brain and Spinal Cord from Monkeys.*

A. 17, M. 3836, failed to develop the disease when inoculated on April 11th with material from A. 8, M. 3783, which material, when used on February 13th, conveyed the disease to A. 14, M. 3805. That A. 17 was not immune was shown by its successful inoculation later as A. 55, M. 3848. The inference is that the virus died out during its storage as a glycerine emulsion for two months.

A. 18 and A. 19. These two experiments are discussed under the sections dealing with the influence in monkeys of the treatment of the emulsion of the virus with various sera. Though the serum used in the case of A. 19 may have afforded protection, the "normal" human serum used in A. 18 cannot be expected to have done so in this case. The source of the supposed virus used in the experiments on A. 18 and A. 19 was A. 9, M. 3785, which had an unusually long incubation period of eleven to thirteen days, and a prolonged illness of ten or eight days. It is possible that, by the time this monkey was killed, the virus causing its disease had died out, or was only present in subinfective amount. This would explain the failure in both monkeys.

A. 20 and A. 21. The failures in these monkeys are easily explained, inasmuch as there was no histological evidence that the source of the virus, A. 11, M. 3801, had the disease at the time of its death.

A. 116. The failure in this monkey, when its fellow, A. 117, "took," is fully discussed in the section dealing with the influence of various sera on the virus. The evidence suggests that it was protected against infection by the serum of A. 89, Sh. 3902, which had had, and perhaps still had at the time of its death, the disease in question. Another explanation of the failure is that the strain employed had undergone a phase of weakening in virulence, through which the virus, on this account alone, failed to infect certain individual monkeys. Still another explanation is that the monkey was naturally immune, or had been rendered artificially immune by a previous intracerebral injection of material capable of causing immunity but not of producing the disease.

A. 132. This monkey is the same animal as A. 116. It failed to take when inoculated with material two days old from A. 129, M. 3967, which might reasonably have been expected to convey the disease. It is reasonable to suppose that artificial immunity had been

established in this monkey by the inoculation of the virus combined with a (presumed) immune serum, referred to under A. 116, or possibly by the first intracerebral inoculation.

*Summary.* Reasonable explanations are forthcoming for the failures of all these inoculations.

#### APPENDIX VII. *Failure of Intraperitoneal Inoculations.*

Intraperitoneal inoculations failed in animals as follows:

A. 2, M. (*Macacus rhesus*) 3777. Swabbings of the naso-pharynx were made from several contacts of Case 26, J. C. B., Narrabri, and the swabs were emulsified in a glycerine solution. The patient had died on January 15th, a day before these swabs were taken; the glycerine emulsion was inoculated on January 17th.

A. 6, M. (*M. rhesus*) 3781. From Case 27, A. B., Narrabri, dying on the third day of the disease. Post-mortem examination next day. Inoculation made two days after the patient's death with material from the brain and spinal cord.

A. 24, M. (*M. rhesus*) 3798. From Case 28, J. M., Narrabri, dying on the sixth day of the disease. Post-mortem examination next day. A glycerinated emulsion of the upper part of the cervical cord inoculated three days after the patient's death.

A. 67, Sh. 3861. From A. 65, Sh. 3855, dying with typical lesions of this disease. The glycerine emulsion of the brain and spinal cord inoculated two days after death.

*Analysis of Results.* From A. 2, M. 3777, inoculated from the swabbings of contacts of a case of the disease, nothing is to be learned.

The failure of A. 6, M. 3781, is important, inasmuch as A. 8, M. 3783, inoculated intracerebrally on the same day, and A. 9, M. 3785, and A. 10, M. 3786, inoculated intracerebrally on the next day—all took.

The failure of A. 24, M. 3798, must be considered in connection with the failures of A. 27, M. 3809, A. 28, M. 3810, and A. 29, M. 3819, injected intracerebrally. The period after death of the host at which the inoculation took place in this monkey was only two days. Even had infective material been present it is possible that, as in the case of A. 6, M. 3781, the monkey might not have taken.

A. 67, Sh. 3861, failed to take intraperitoneally, whilst the same material inoculated on the same day into A. 66, M. 3860, took intracerebrally. That this sheep was not naturally immune is shown by its successful inoculation six weeks later (A. 89, Sh. 3902).

*Conclusions.* A. 6, M. 3781 and A. 67, Sh. 3861, show that infective material injected intraperitoneally may fail to convey infection, whilst the same material injected intracerebrally may be successful.

#### APPENDIX VIII. *Failure of Intrasciatic Inoculations.*

A. 4, M. 3779, was inoculated in this way with an emulsion of the throat swabbings from the contacts of a case. The animal was *Macacus cynomolgus*—a species in which, in the few inoculations made into it, we have been so far unsuccessful in producing the disease. Further, we do not know as yet whether the virus is frequently present, or present at all, in the naso-pharynx of contacts or cases.

A. 5, M. 3780, was injected with an emulsion which "took" on the same date by intracerebral inoculation in the case of A. 8, M. 3783, and a day later by the same route in A. 9, M. 3785, and in A. 10, M. 3786.

#### APPENDIX IX. *Failure of Pasteur-Chamberland F. filtrates.*

All these inoculations were negative. To enable the material to pass through the filter it was necessary to dilute it to a considerable extent.

A. 26, M. 3807, need not be further considered inasmuch as the inoculation of other material from the same case failed to "take," and hence the presence of the virus in the material as used was not established.

A. 12, M. 3802, was inoculated with a filtrate from the ten times diluted emulsion of A. 8, M. 3783, on the day of this animal's death. As A. 14, M. 3805, inoculated next day with the same emulsion, diluted with an equal amount of monkey serum, developed the disease, the virus was manifestly present in the emulsion before its filtration.

A. 54, M. 3846, was inoculated with a filtrate obtained from an emulsion from A. 50, M. 3839, on the day of this animal's death. As A. 55, M. 3848, inoculated next day with the untreated emulsion, developed the disease, the virus was manifestly present in the original emulsion.

*Conclusion.* The diluted virus has been proved in two cases not to pass through the pores of a Pasteur-Chamberland F. filter, at least in sufficient quantity to produce infection in monkeys by the intracerebral injection of about 1 c.c. of filtrate.

#### APPENDIX X. *Failure of Cerebro-spinal Fluid to Cause Infection.*

A. 1, M. 3776, was unaffected by the intracerebral injection of cerebro-spinal fluid taken from a fatal case on the second day of illness and injected two days later.

A. 32, M. 3800, inoculated intracerebrally four days afterwards with cerebro-spinal fluid taken from a fatal case on the third day of illness, failed to take.

M. 3689, was inoculated intraperitoneally and intrathecally with cerebro-spinal fluid from Case 28, F. R., of the 1917 series.

#### APPENDIX XI. *Failure of a Pasteur-Chamberland F. Filtrate of Faeces.*

A. 31, M. 3779, received an intracerebral injection of a Pasteur-Chamberland F. filtrate of faeces obtained from Case 36, J. K., Boggabri. It remained unaffected. As failures have resulted from the use of such Pasteur-Chamberland F. filtrates obtained from material known to be virulent, the failure in this case teaches us nothing. Attention may also be called to the fact that the monkey used was *Macacus cynomolgus*, a species to which we have so far not been able to convey the disease.

*Summary.* The failure of this filtrate leaves open the question as to whether the virus may or may not be present in the faeces.

#### APPENDIX XII. *Failure of a "Noguchi Culture."*

An attempt was made to grow the virus from monkey material according to Noguchi's method for spirochaetes (*Jnl. Exp. Med.*, xvi, 1912, p. 621).

A diffuse cloud appeared in one of the original cultures which otherwise remained sterile, and this material was inoculated a month after the death of the monkey from which it was obtained. The animal remained perfectly well, and was later successfully inoculated with further material.

*Summary.* Failure resulted from the inoculation of a first generation of a presumed Noguchi culture a month after the death of the monkey from which it was made.

#### APPENDIX XIII. *Failure in Monkeys of Intracerebral Inoculations from the Nasopharyngeal Swabs of Contacts and of a Case.*

A. 3, M. 3778, was inoculated with an emulsion in glycerine solution of the nasopharyngeal swabs of several contacts of a case, and A. 30, M. 3797, with a similar swabbing from an actual case at the height of the disease. These experiments were conducted on the same animal, which happened to be *Macacus cynomolgus*, a species to which, in the few experiments we have made, we have so far been unsuccessful in conveying the disease. Apart from this, the method adopted was not one that entailed any concentration of the virus. These experiments, therefore, neither prove nor disprove the possible presence of the virus in the nasopharynx of contacts or cases.

APPENDIX XIV. *Failure in a Monkey of the Intracerebral Inoculation of Brain and Spinal Cord from a Horse.*

A. 42. This failure throws no light on the present disease. A horse died at Narrabri from a nervous complaint, which has not been shown to be connected in any way with human encephalo-myelitis.

APPENDIX XV. *Failure of Intracerebral Inoculation of an Emulsion of Fowl Ticks (Argas persicus).*

A. 43. The failure in this monkey throws no light upon the disease in question. The reason why an inoculation was made of an emulsion of fowl ticks is discussed in the section on the possibility of the occurrence of an intermediate (invertebrate) host of the virus in our full official report. It was thought possible that the human encephalo-myelitis might be due to some parasite transmitted by fowl ticks, such as the spirochaete producing the spirochaetosis of these birds.

The failure of the monkey does not support any such contention, though it does not necessarily exclude it.

APPENDIX XVI. *Failure in a Monkey and a Sheep after the Introduction of Horse Serum into the Spinal Canal and Introduction of the Virus into a Vein.*

The animals thus dealt with comprise A. 71, M. 3872, and A. 76, Sh. 3877. The virus in each instance was obtained from monkeys. In the case of A. 71, M. 3872, the virus had been kept for nine days, and, though no positive results were obtained from other inoculations of this virus, there is every reason to consider that it was present when the monkey yielding it was killed. In the case of A. 76, Sh. 3877, the virus was a day old, and had produced a successful result by intracerebral inoculation on the previous day in A. 72, M. 3873. The amount of the virus introduced, about 0.5 c.c., may have been too small to produce infection by this route in either animal. Further, as regards the monkey inoculation, the virus may have died out during the nine days in which it was kept in an ice-chest; and, as regards the sheep inoculation, the sheep employed is shown, under the designation A. 93, to have been naturally immune to the disease. These experiments, therefore, neither prove nor disprove the possibility of causing infection by the method employed.

APPENDIX XVII. *Table of Sheep, Calf and Horse Inoculations. Positive Results with Death.*

No.	Source of virus	Date of inoculation	Incubation period in days	Days of illness at death
A. 52, Sh. 3839 b	A. 49, M. 3835	16/4/1918	3	3
A. 65, Sh. 3855	A. 55, M. 3848	7/5/1918	6	3
A. 80, Sh. 3892	A. 72, M. 3873	17/6/1918	7	5
A. 81, Sh. 3893	A. 72, M. 3873	17/6/1918	12	4
A. 89, Sh. 3902	A. 78, M. 3890	27/6/1918	5 or 7	30 (death adventitious?)
A. 91, Sh. 3904	A. 78, M. 3890	27/6/1918	6	1
A. 92, Sh. 3905	A. 78, M. 3890	27/6/1918	4 (or more)	5 (or less)
A. 98, Sh. 3910	A. 92, Sh. 3905	6/7/1918	5	5
A. 103, Sh. 3915	A. 87, M. 3900	7/7/1918	9 (or 7)	2 (or 4)
A. 105, Sh. 3917	A. 87, M. 3900	7/7/1918	7 (or less)	3 (or more)
A. 108, Sh. 3920	A. 98, Sh. 3910	16/7/1918	6	1
A. 109, Sh. 3921	A. 103, Sh. 3915	18/7/1918	9 (possibly 6)	1 (possibly 4)
A. 121, Sh. 3941	A. 110, M. 3925	2/8/1918	5 or 7	7 or 5
A. 57, Calf 3848 b	A. 50, M. 3839	25/4/1918	5	3
A. 95, Horse 3908	A. 78, M. 3890	28/6/1918	9	3



APPENDIX XVIII. *Summary of Successful Inoculations in Sheep, a Calf and a Horse.*

The foregoing table (Appendix XVII) indicates the source of the virus (whether from a monkey or a sheep), the date of inoculation, the incubation period and the duration of the illness. In those cases in which previous inoculations had been made, details will be found in the section dealing with sheep reinoculations.

A. 52, Sh. 3839 *b*, after an incubation period of three days, became slightly sick and seemed to have difficulty in getting its head down to nibble grass. Next day the animal was restless; its legs seemed somewhat rigid; there was stiffness in the neck, and the head was turned to one side; the lower jaw quivered, the ears twitched and the animal tended to walk in a circle, and took fits during which the head was thrown back and it pawed the air. It presented much the same symptoms on the next day and was unconscious on the third day of illness when it died—exactly six days after the inoculation.

A. 65, Sh. 3855, became ill after an incubation period of six days. The symptoms consisted of shallow and fairly rapid respirations, some stiffness of the neck, quivering of the nostrils and a somewhat slow gait. On the second day it was very sick, would jump round, fall on the ground and struggle, and had convulsive movements. It died on the morning of the third day of illness.

A. 80, Sh. 3892, after an incubation period of seven days, became ill, holding its head down and lying down most of the time. Next day its lips were quivering and the head was turned to one side. On the fourth day it was dribbling from the mouth; there was no apparent paresis. On the fifth day it was lying on its side with the head thrown slightly back and had frequent convulsive movements. At this stage it was killed.

A. 81, Sh. 3893, became ill on the twelfth day. It ran holding its head high and breathing quickly. The ears and lips were twitching slightly. Its temperature was 106° F. Next day, in addition, the animal tended to circle round holding its head high. On the fourth day it was lying on its side with the head somewhat retracted. It manifested convulsive movements from time to time and twitched all over.

A. 89, Sh. 3902, showed, on the fifth day after inoculation, rapidity of breathing, protrusion of the tongue, and running from the nose. Next day it was still breathing quickly. On the seventh day it fell down on one occasion and struggled, and later held its head high and made "champing" movements of the head, lips and jaws. On the eighth day it seemed well again. Twenty-one days after the inoculation it was bled, and it was bled again thirteen days later. Two days after the last bleeding it apparently had respiratory trouble, became worse next day, and died thirty-eight days after the inoculation. Typical histological changes were present.

A. 91, Sh. 3904, on the second and third days (excluding the day of inoculation) seemed ill, the symptoms suggesting some respiratory trouble following the anaesthetic. On the fourth and fifth days it was better. On the sixth day it was not feeding; it turned slowly in a circle and then fell on its side with retraction of the head and trembling of the lips and nostrils. Convulsions then supervened and the animal seemed unconscious. The respirations were highly irregular. It died an hour after the manifestation of these grave signs.

A. 92, Sh. 3905, on the fourth to the sixth day after inoculation seemed ill, and on the last of these days had a mucous discharge from the nose. On the seventh day its temperature was 105° F. and the respirations were sometimes rapid. When placed on its legs it moved backwards and to one side with a stiff and jerky gait. It was lying down most of the time with the ears twitching. Next day it was much the same. There was a mucous discharge

from the nostrils and very irregular respirations, and occasionally convulsive movements. The temperature reached 106° F. It died early next day.

A. 98, Sh. 3910, became ill on the fifth day. On the sixth day the temperature was 107.4° F. It was drowsy and the breathing was rapid. On the eighth day it tended to circle towards one side and ran into objects, and was breathing rapidly. On the ninth day it showed convulsive movements all day, and died during the night.

A. 103, Sh. 3915, had a temperature of 107° F. on the sixth day but did not show definite symptoms until the ninth day. Its temperature now was 104°. It showed occasional twitchings and was breathing rapidly, and died on the tenth day.

A. 105, Sh. 3917, had a temperature of 106° F. on the fifth day. On the seventh day it was dribbling from the mouth and hardly moved. On the eighth day it had a convulsion and there seemed to be weakness in the hind legs. On the ninth day there were continuous twitchings of the nose and mouth, and it died during the following night.

A. 108, Sh. 3920, six days after inoculation, became sick and had rapid respirations. The same evening it exhibited "staggers," and died during the night.

A. 109, Sh. 3921, between the second and eighth days showed occasionally rapid breathing, but no other definite symptoms. It became definitely ill on the ninth day. The head was retracted, the animal circled and showed twitchings of the lips. These were followed by convulsive movements and death.

A. 121, Sh. 3941, on the fifth day after inoculation, was breathing fast. Next day it was not taking its food and seemed weak and the temperature was raised. Convulsive movements appeared on the seventh day after inoculation and occurred again the following day. On the ninth day it had intense convulsive movements lasting for about an hour, together with fine quiverings of the ears and eyelids, twitchings of the head and working movements of the jaws, and very irregular breathing. The animal seemed unconscious during the convulsive attacks. When these had passed off the animal could only stand by leaning against some support, and in walking it swayed from side to side dragging one hind leg a little. Next day the head was retracted and the back markedly arched. Respirations were irregular and there was continual grinding of the teeth. On the eleventh day after inoculation it was lying on its side. The head was slightly retracted; the tail and back legs moved on stimulation. The animal died in the afternoon.

A. 57, Calf 3848 *b*, first showed symptoms five days after inoculation, when it kept its head dependent, inclined to "go" in the front legs, and was restless. Next day there was a clear discharge from the nostrils. On the third day of illness the animal was walking about, the legs were weak and the gait tended to be circular. General convulsive seizures then developed, accompanied by rigidity of the limbs and muscular tremors, whilst the animal lay on its side with the head markedly retracted and the back arched. It was possibly unconscious. After the convulsions had continued for about twenty minutes the animal became fairly quiet, but died an hour later. Typical lesions were found on histological examination.

A. 95, Horse 3908, on the ninth day after inoculation had two seizures, during which it walked round towards the left. Next day its head was depressed, and on moving it tended to "go" towards the left side and threatened to fall on this side. There were twitchings of the facial muscles, staring eyes and apparently partial blindness. On the third day (of illness) the animal was lying on its right side with the head drawn to the left and manifested irregular movements of the left fore and hind limbs. The nostrils were working and the mouth at times was drawn to the left side. Later, intense and repeated convulsions developed alternating with short quiescent periods, and at this stage the animal was killed. Histological examination showed typical lesions.

APPENDIX XIX. *Sheep showing no Symptoms after an Intracerebral Inoculation of Brain and Spinal Cord from Human Cases.*

A. 35. On February 26th this sheep was inoculated intracerebrally with material from a human case which infected A. 33, M. 3803, when similarly inoculated on February 13th. Later this sheep, under the designation A. 65, was successfully inoculated from a monkey. This shows that the failure in the first inoculation was not due to natural immunity, and the inference therefore is that the virus had died out during the thirteen days of its storage in an ice-chest between the time of the successful inoculation of the monkey and its use on this sheep.

A. 46. This happens to be the same sheep as A. 35, and as is thereunder indicated it was finally successfully inoculated under the designation A. 65, so that it clearly possessed no natural immunity to the disease. The first inoculation was made on April 16th with material from Case 37, A. C. F. (J.). As previous inoculations of this material on March 15th and April 11th into monkeys were both failures there is no evidence that the virus was present in the materials used at the time of any of these inoculations.

*Comment.* There are reasonable explanations for these failures.

APPENDIX XX. *Sheep Showing no Symptoms after the Intracerebral Inoculation of Brain and Spinal Cord from Infected Monkeys.*

A. 53 and A. 84. The failure in these experiments is discussed in the section dealing with reinoculations. In these animals later inoculations were successful, and reasonable explanations are given for the initial failures.

A. 63 (93), A. 69 (94), A. 83, A. 101, A. 104. These experiments are also discussed in the section dealing with reinoculations. The evidence indicates that these sheep were naturally immune to the disease.

A. 75. Death occurred on the fifth day, apparently from lung trouble. No histological lesions of encephalo-myelitis could be detected though convulsive movements on the fourth day suggested the possibility of a "take." As the incubation period in some sheep has been apparently as short as three days, it is possible that this animal really did have the disease, but died before recognisable histological changes developed.

A. 96. The inoculation in this sheep was made with material preserved for over a month in glycerine in an ice-chest and obtained from a monkey. In the fresh state, this virus conveyed the disease to a monkey, a sheep and a horse. Failure may be attributed either to the length of time the material was preserved or to natural immunity.

A. 106. This animal died from post-anaesthetic lung trouble five to six days after inoculation. No evidence of the disease was detected histologically, though it might or might not have had time to develop. This sheep may have been naturally immune.

A. 111. Since the same material, as was used in this experiment, was successful on the same day in conveying the disease to A. 110, M. 3925, the failure is to be attributed to a natural immunity.

A. 122. The successful results in A. 117, M. 3937, and in A. 121, Sh. 3941, on the same date with the same material, indicate that natural immunity is the explanation of the failure in A. 122.

*Summary.* In seven of these sheep, natural immunity seems clearly to be the explanation of the failures. In two, death occurred so early, from complications, that the disease, if about to develop, may not have had time to manifest itself or produce recognisable lesions. In one, the length of storage of the virus may have destroyed its activity. In the other two, reasonable explanation of the failures are given elsewhere.

APPENDIX XXI. *Sheep showing no Symptoms after the Intracerebral Inoculation of Brain and Spinal Cord from Infected Sheep.*

A. 68, A. 114, and A. 115. These three animals are all discussed in the section dealing with re-inoculations, where it is shown that they were naturally immune.

A. 126. This animal failed to take definitely whilst its fellow, A. 125, showed symptoms, possibly due to encephalo-myelitis, and recovered. A. 126 may have enjoyed natural immunity.

APPENDIX XXII. *Failure in a Sheep of an Intracerebral Inoculation of Dried Brain and Spinal Cord from a Positive Monkey.*

A. 77, Sh. 3878, received an intracerebral injection of emulsified dried brain and spinal cord from a positive monkey eight days after this monkey's death. The material had been dried in Petri dishes in an incubator.

As many sheep are immune to intracerebral inoculation of the virus, the failure in this case neither proves nor disproves the possibility of the virus resisting desiccation for eight days at incubator temperature.

APPENDIX XXIII. *Failure in Sheep of a Berkefeld Filtrate from a Positive Monkey.*

A. 119, Sh. 3939, and A. 120, Sh. 3940, received intracerebral injections of a Berkefeld filtrate and remained unaffected, whilst inoculations on the same date of the unfiltered emulsion were successful in conveying the disease to A. 117, M. 3937, and A. 121, Sh. 3941.

One of these sheep, A. 119, Sh. 3939, received a further inoculation twenty-six days later, which was followed a week later by symptoms suggesting slight encephalo-myelitis from which the animal recovered. There is reason therefore to think that this sheep was not naturally immune.

The inference to be drawn from these experiments is that the virus did not pass through the pores of the Berkefeld filter, at least in sufficient quantity to induce infection.

APPENDIX XXIV. *Failure to Convey the Disease to a Sheep by Intranasal Swabbing.*

A. 70, Sh. 3865, had the inside of the nostrils vigorously swabbed with an emulsion of the brain and spinal cord of A. 64, M. 3854 and was unaffected thereby. A. 69, Sh. 3864, inoculated intracerebrally with the same material, on the same date, failed to develop the disease. A. 71, M. 3872, seven days later had an injection of horse's serum into the spinal canal and a small quantity of the material from this monkey (A. 64) introduced into a vein. It also remained well. These were the only three animals receiving inoculations from A. 64, M. 3854, and none of them developed the disease. Only one was inoculated intracerebrally, the only route by which we have so far obtained infections, and the animal so inoculated was a sheep, a species which we have found frequently to be immune to the virus introduced by this route. That A. 70 was not immune to the virus was shown later, under the designation A. 92, Sh. 3905, when the disease was successfully conveyed to it.

*Comment.* The result of this experiment neither proves nor disproves the possibility of infection occurring through the nose.

APPENDIX XXV. *Sheep Reinoculations.*

For various reasons—economy of expensive animals and testing for natural immunity being the most important—a number of sheep were reinoculated. The animals so dealt with were fourteen in number. They may be divided into those which "took" after reinoculation

and those in which reinoculations were unsuccessful. Unless otherwise stated, the inoculation was intracerebral and the material used an emulsion of portions of the brain and spinal cord. There were six in the first and eight in the second group.

(a) *Successful Reinoculations.*

A. 23, Sh. 3816—A. 52, Sh. 3839 *b*. The first inoculation was made on February 26th with material from A. 11, M. 3801. It was afterwards found by histological examination that this monkey did not show the lesions of encephalo-myelitis. The later successful result by the inoculation on April 16th can therefore be explained.

A. 35, Sh. 3817—A. 46, Sh. 3839 *c*—A. 65, Sh. 3855. The first inoculation was made on February 26th with material from Case 38, G. H. From this human case A. 33, M. 3803, had been successfully inoculated on February 13th. The second inoculation was made on April 16th with material from Case 37, A. C. F. (J.); A. 44, M. 3825, was unsuccessfully inoculated on March 15th and A. 45, M. 3837, on April 11th with the same material. The successful result of the inoculation on May 7th from a monkey, A. 55, M. 3848, may be explained in connection with the first inoculation by the view that the glycerinated virus may have died out in the interval of a fortnight that elapsed between the successful inoculation in the monkey and the unsuccessful one in this sheep. As regards the second inoculation there was no evidence by the two monkey inoculations that the virus was present in the material used.

A. 53, Sh. 3863—A. 91, Sh. 3904. The first inoculation was made with material which, though originally infective, as shown by positive results in A. 50, M. 3839, and A. 52, Sh. 3839 *b*, had been conserved in the ice-chest for a month. The later positive result is therefore best explained by supposing that the first virus had died out during the period of its storage.

A. 67, Sh. 3861—A. 77, Sh. 3878—A. 89, Sh. 3902. The first inoculation on May 17th was an intraperitoneal one of an emulsion from a positive sheep, A. 65, Sh. 3855. The second experiment was on June 7th, and consisted of the intracerebral injection of dry brain material from A. 66, M. 3860. The third inoculation was made on June 27th from A. 78, M. 3890. A few days after the last inoculation it seemed sick and presented a few slight symptoms, possibly indicating a mild form of the disease. On July 18th blood was taken under an anaesthetic and it was bled again similarly on July 31st. It died on August 4th, apparently from lung trouble as a result of the anaesthetic, but histological examination showed the lesions of encephalo-myelitis, possibly in an early stage of resolution. Serum from the blood taken on the first occasion may have protected A. 111, Sh. 3922. It certainly seems as though the second sample of blood, when mixed with the virus before injection, protected A. 116, M. 3936.

In connection with the first injection, which was into the peritoneum, we possess as yet no evidence that this is a successful route for introducing the virus. As regards the second inoculation which was with dry brain material, the drying may reasonably be considered as having destroyed the virus. This animal cannot therefore be considered to have been proved to be naturally immune to the disease before the third inoculation with its delayed successful result.

A. 70, Sh. 3865—A. 92, Sh. 3905. The first experiment, performed on May 20th, consisted in swabbing the nose with an emulsion from A. 64, M. 3854. The successful result, therefore, of the intracerebral inoculation on June 27th can be explained by the first procedure not having been a reliable method for obtaining infection.

A. 84, Sh. 3896—A. 109, Sh. 3921. The first inoculation was made with tissues from the frontal and occipital regions only of the monkey yielding the virus. The general emulsion of the brain and spinal cord of this monkey gave positive results in A. 78, M. 3890; A. 80, Sh. 3892; and A. 81, Sh. 3893. The second inoculation, which was successful, was with the

mixed emulsion of brain and spinal cord from an infected sheep. The failure of the first inoculation may be explained by absence, relative or complete, of virus in the material used.

(b) *Unsuccessful Reinoculations.*

A. 63, Sh. 3853—A. 76, Sh. 3877—A. 93, Sh. 3906—A. 115, Sh. 3934. The first inoculation was made on May 3rd from a positive monkey, A. 62, M. 3845. The second experiment, performed on May 31st, consisted of lumbar puncture with the intraspinal injection of 2 c.c. of antimeningococcal serum, together with an injection into a vein of 0.5 c.c. of virus from A. 66, M. 3860. The third experiment, on June 27th, consisted of the intracerebral injection of a saline emulsion of the medulla of A. 78, M. 3890. The companion sheep, A. 92, Sh. 3905, similarly injected at the same time, gave a positive result. The fourth inoculation was made on July 29th from a positive case in a sheep, A. 109, Sh. 3921. It is clear that the material used for the third inoculation contained the virus at the time it was used, while it is almost certain that the virus was also present in the material used for the first and fourth inoculations though there were no control animals to prove this conclusively. The technique of the second operation cannot be considered, as yet, a proved method of obtaining infection. These results suggest that the sheep has a natural immunity to the virus, which even the actual introduction of the virus into the brain cannot break down.

A. 68, Sh. 3862—A. 86, Sh. 3879—A. 90, Sh. 3903. The first inoculation was on May 17th with material from a positive sheep, A. 65, Sh. 3855. The second inoculation was on June 7th with material from A. 75, Sh. 3876. A. 75, Sh. 3876, died four days after inoculation and histological examination of its brain and spinal cord was negative. The third inoculation was on June 27th with virus from A. 78, M. 3890, which "took" in the companion sheep A. 89, Sh. 3902; A. 91, Sh. 3904; and A. 92, Sh. 3905. As regards the first inoculation we know from A. 66, M. 3860, that the virus was present on the date of inoculation. We also know, as indicated above, that the virus was present in the material used for the third inoculation. This animal would seem, therefore, to have possessed a natural immunity to the disease.

A. 69, Sh. 3864—A. 94, Sh. 3907. The first inoculation was made on May 20th with material from A. 64, M. 3854. The second inoculation was made on June 27th and consisted of an emulsion of the frontal and occipital regions of the brain of A. 78, M. 3890. As regards the first inoculation we have no proof—by means of a control successfully-inoculated animal—that the virus was present in the material used, but inasmuch as this monkey presented the typical histological appearances of the disease, there is every reason to suppose that the virus was present. As regards the second inoculation, we have no proof that the virus is consistently present in the frontal and occipital areas of the brain, though histological examinations of affected animals would suggest that it is so, at least frequently. This sheep therefore also seems to have possessed a natural immunity.

A. 83, Sh. 3895—A. 114, Sh. 3933. The first inoculation was made on June 17th with material from A. 72, M. 3873, companion sheep, namely, A. 80, Sh. 3892 and A. 81, Sh. 3893, giving positive results. The second inoculation was made on July 29th from a positive sheep, A. 109, Sh. 3921. A companion animal of this last inoculation, which was also a previously inoculated animal, likewise remained unaffected, so that there was no control successful case for the second inoculation. The results of these two inoculations seem to show again a natural immunity in this animal.

A. 101, Sh. 3913—A. 125, Sh. 3948. The first inoculation was made with material which successfully conveyed the disease to A. 100, M. 3912; to A. 103, Sh. 3915; to A. 105, Sh. 3917; and probably to A. 102, Sh. 3914, which recovered. The virus was therefore evidently present in the material used on A. 101, Sh. 3913. The second inoculation was with material obtained from a positive sheep, A. 121, Sh. 3941. Decomposition was commencing in the carcase when the tissues were obtained for inoculation purposes. On the fourth and fifth days the inocu-

lated animal showed slight symptoms suggestive of encephalo-myelitis but recovered. Inoculations of the same material on the same day into another sheep, a calf, and a monkey (*Macacus cynomolgus*) were negative. The results of these experiments again suggest a natural immunity, not perhaps complete or varying from time to time in degree.

A. 104, Sh. 3916—A. 126, Sh. 3949. The same remarks apply to A. 104, Sh. 3916, regarding the first inoculation, as to the above-mentioned A. 101, Sh. 3913, the successful experiment in A. 103, Sh. 3915, being an exact counterpart. The second inoculation was also with the same material as was used on the above A. 125, Sh. 3948, but no illness resulted. Natural immunity to the virus, as evidenced by the first inoculation, seems to be again evident in this animal.

A. 119, Sh. 3939—A. 130, Sh. 3968. The first inoculation was with a Berkefeld filtrate of material which, unfiltered, conveyed the disease to A. 117, M. 3937, and to A. 121, Sh. 3941. After the second inoculation, with monkey material that successfully conveyed the disease to A. 129, M. 3967, an illness, probably encephalo-myelitis, developed, from which the sheep recovered. The inference drawn from these experiments is that the virus was not present, or was only present in subinfective amount, in the filtrate used.

A. 120, Sh. 3940—A. 131, Sh. 3969. These were parallel inoculations to those in the previous case. A possible mild attack of encephalo-myelitis followed the second inoculation. The same comment applies.

*Inferences drawn.* As regards the six sheep in which the final inoculations were successful, there seem to be valid reasons why the first were unsuccessful.

As regards the unsuccessful reinoculations, in the cases of the first three sheep the presence of a natural immunity to the introduction of the virus into the brain seems to be reasonably established. As regards the fourth sheep, there appears to be evidence of a natural immunity, perhaps not complete or varying in degree from time to time. The last two sheep suggest that the virus is held back, completely or to a large degree, by the pores of a Berkefeld filter.

#### APPENDIX XXVI. (a) *Calves showing Symptoms of Illness, possibly due to Encephalo-myelitis, after Intracerebral Inoculation of Material from the Brain and Spinal Cord of a Positive Monkey or a Positive Horse.*

A. 88. On June 27th this calf was inoculated intracerebrally with material from a monkey which successfully conveyed the disease on the same date to A. 87, M. 3900; A. 89, Sh. 3902; A. 91, Sh. 3904; A. 92, Sh. 3905; and A. 95, Horse 3908.

On July 1st the calf was sick and staggered, being especially weak in the hind legs. In the afternoon shivers were noticed in the hindquarters. On July 2nd it was very sick. It kept its head down, tended to walk in a circle, was very jerky on its legs, and stiff in the hindquarters and drowsy. In the evening it had convulsive movements, during which it bellowed, jumped in the air, fell down and struggled, and appeared to be unconscious. On July 3rd its hind legs swayed on movement, and it seemed weak and fell down at times. Next day it was better and could get up by itself from the ground, and was apparently almost normal in behaviour. On July 5th it could walk about, but swayed slightly on movement. On July 7th it was in much the same condition, though it had occasional convulsive seizures, and was not inclined to "play." It swayed a little on movement and there were occasional twitchings in one hind leg. On July 12th it was lying down and seemed drowsy. On July 14th it developed diarrhoea, which continued for about a fortnight, but from which it eventually recovered.

Considering the success of the material used in other animals, and the nature of the symptoms manifested, there seems little doubt that this calf had a mild form of encephalo-myelitis, from which it recovered.

A. 107. On July 10th this calf was inoculated intracerebrally with material from A. 95, the successfully inoculated horse. It seemed ill two days later, and on July 13th seemed very

sick. On July 14th it was weak and "wobbly" on the legs but otherwise normal. On July 16th it was better and thereafter showed no signs of illness until August 14th, when it received another inoculation (*vide infra* A. 127).

It is possible that the slight symptoms shown were due to a mild attack of encephalomyelitis from which the animal recovered.

(b) *Calf showing no Symptoms after Intracerebral Inoculation (a second inoculation) of Brain and Spinal Cord from a Positive Sheep.*

A. 127. This calf is the same animal as has just been referred to under the designation A. 107. The second inoculation was made on August 14th and the calf showed no symptoms which could be attributed to encephalomyelitis as the result of this inoculation.

It is possible that the first inoculation had rendered the animal immune to the later introduction of virus.

APPENDIX XXVII. *Failure to Convey the Disease to Dogs.*

Inoculation into A. 34, Dog 3806, failed on February 14th, while a monkey, A. 33, M. 3803, inoculated the day before with the same material developed the disease.

A. 16, Dog 3813, was inoculated on February 21st with material from A. 8, M. 3783, and failed to take, whilst A. 14, M. 3805, inoculated on February 13th with the same material developed the disease. During the eight days that elapsed between these inoculations, the virus may possibly have died out.

A. 56, Dog 3847, was inoculated from A. 50, M. 3839, unsuccessfully, whilst A. 55, M. 3848, inoculated on the same day with the same material developed the disease. This dog was the same animal as A. 16.

A. 97, Dog 3909. This was a third attempt to inoculate the animal shown as A. 16 and A. 56. This time fresh material from a positive sheep was used for the intracerebral inoculation. The result was again negative.

*Comment.* It is clear that in three of these inoculations, if not in all, the material used contained the virus. The dog has therefore not been shown to be susceptible to the disease.

APPENDIX XXVIII. *Failure to Convey the Disease to a Kitten by Intracerebral Inoculation.*

A. 79, Kitten 3891, received an intracerebral inoculation of an emulsion of brain and spinal cord from A. 72, M. 3873, and remained perfectly well afterwards, whereas A. 78, M. 3890, inoculated on the same day with the same material developed the disease.

APPENDIX XXIX. *Failure to Convey the Disease to Rabbits.*

A. 58, Rabbit 3849, was inoculated from A. 50, M. 3839, and failed to take. A. 55, M. 3848, inoculated with the same material, the day before, developed the disease.

A. 73, Rabbit 3874, inoculated from A. 66, M. 3860, failed to take, whilst A. 72, M. 3873, inoculated on the same day, developed the disease.

*Summary.* Two rabbits inoculated with material shown to contain the virus did not contract the disease.

APPENDIX XXX. *Doubtful Results in Guinea-pig Inoculations.*

Two guinea-pigs, A. 60, Gp. 3851, and A. 74, Gp. 3875, were inoculated. In the case of the first the material was obtained from a monkey the day after the same material conveyed the disease to A. 55, M. 3848, and A. 57, Calf 3848*b*. In the case of the second guinea-pig, the material used gave a positive result when inoculated on the same day into A. 72, M. 3873.



A. 60, Gp. 3851, six days after the inoculation was very sluggish and its hind legs seemed slightly weak, and on the following day it hardly moved at all. It died on the third day of illness. Histological examination showed some slight changes which might or might not be interpreted as evidence of a very mild form of encephalo-myelitis. The second guinea-pig, A. 74, Gp. 3875, remained apparently well until nearly a month after the inoculation, when its head was somewhat retracted and it could not raise itself up or walk. It died during the first day of this illness. Histological examination again showed some slight changes though these were probably not due to encephalo-myelitis.

*Summary.* One guinea-pig showed symptoms and histological lesions which might or might not be attributable to a very mild form of encephalo-myelitis. The second guinea-pig probably gave a negative result.

APPENDIX XXXI. *Failure to Convey the Disease to a Hen by Intracerebral Inoculation.*

A. 118, Hen 3938, received an intracerebral inoculation of material from A. 110, M. 3925, and remained perfectly unaffected whilst a monkey and a sheep inoculated on the same day with the same material developed the disease. This inoculation was specially made into a hen on account of the possibility of the human disease being the same as the spirochaetosis of fowls so common in the affected districts.

*Summary.* Virulent material failed to convey the disease to a hen by intracerebral inoculation.

APPENDIX XXXII. *Table showing the various Animal Inoculations.*

(Unless otherwise stated, the inoculations were made intracerebrally and the material comprised portions of the cortex of the cerebrum, pons, medulla, and spinal cord. + means a successful result, - means an unsuccessful result, and a blank indicates that the inoculated animal died too soon to allow manifestations of the disease to appear, supposing the virus had been transmitted. M. = monkey, Sh. = sheep, in. = inoculated, d. = died, k. = killed. Numbers in brackets [e.g. (A. 33)] mean that the animal so referred to received other inoculations under such designations. Roman figures represent generations.)

CASE 26, J. C. B., Narrabri.

(Ill four days; cerebro-spinal fluid taken 14/1/1918; swabbings of contacts taken 16/1/1918; patient died 15/1/1918.)

- A. 1 (A. 33), M. 3776 ( - ), cerebro-spinal fluid (in. 15/1/18).
- A. 2 (A. 13), M. 3777 ( - ), nasopharyngeal swabs, intraperitoneal (in. 17/1/18).
- A. 3 (A. 30, A. 44, A. 51), M. 3778 ( - ) nasopharyngeal swabs (in. 21/1/18).
- A. 4 (A. 22, A. 31), M. 3779 ( - ), nasopharyngeal swabs, into sciatic nerve (in. 22/1/18)

CASE 27, A. B., Narrabri.

- A. 5 (A. 21), M. 3780 ( - ), into sciatic nerve (in. 29/1/18).
- A. 6 (A. 20), M. 3781 ( - ), intraperitoneal (in. 29/1/18).
- A. 7, M. 3782.
- A. 8, M. 3783 ( + ), (in. 29/1/8; ill 7/2/18; k. 12/2/18).
- A. 9, M. 3785 ( + ), (in. 30/1/18; ill 11/2/18; k. 21/2/18).
- A. 10, M. 3786 ( + ), (in. 30/1/18; ill 14/2/18; k. 16/2/18).

*From A. 8, M. 3783, (in. 29/1/18; k. 12/2/18).*

- II. A. 11, M. 3801 ( - ), (in. 12/2/18; d. with miliary abscesses 25/2/18).
- A. 12, M. 3802 ( - ), Pasteur-Chamberland F. filtrate (in. 12/2/18).
- A. 13 (A. 2), M. 3804.
- A. 14, M. 3805 ( + ), (in. 13/2/18; ill 22/2/18; d. 25/2/18).

- A. 15, Dog 3808.  
 A. 16, Dog 3813 ( - ), (in. 21/2/18).  
 A. 17 (A. 64), M. 3836 ( - ), material kept two months (in. 11/4/18).  
*From A. 9, M. 3785, (in. 30/1/18; k. 21/2/18).*  
 A. 18 (A. 27), M. 3823 ( - ), (in. 15/3/18).  
 A. 19 (A. 26), M. 3824 ( - ), (in. 15/3/18).  
*From A. 11, M. 3801, (in. 12/2/18; d. from military abscesses 25/2/18).*  
 A. 20 (A. 6), M. 3814 ( - ), (in. 27/2/18).  
 A. 21 (A. 5), M. 3815 ( - ), (in. 27/2/18).  
 A. 22 (A. 4, A. 31), M. 3818.  
 A. 23 (A. 52), Sh. 3816 ( - ), (in. 26/2/18).

## CASE 28, J. M., Narrabri.

(Ill six days; died 6/2/18; cervical cord and adjacent part of brain only used.)

- A. 24 (A. 29), M. 3798 ( - ), intraperitoneal (in. 9/2/18).  
 A. 25, M. 3799 (abscess in five days).  
 A. 26 (A. 19), M. 3807 ( - ), Pasteur-Chamberland F. filtrate (in. 15/2/18).  
 A. 27 (A. 18), M. 3809 ( - ), treated with serum (in. 16/2/18).  
 A. 28 (A. 43, A. 55 + ), M. 3810 ( - ), treated with serum (in. 16/2/18).  
 A. 29 (A. 24), M. 3819 ( - ), (in. 6/3/18).

## CASE 36, J. K., Boggabri.

(Ill five days; died 8/2/18.)

- A. 30 (A. 3, A. 44, A. 51), M. 3797 ( - ), nasopharyngeal swab (in. 9/2/18).  
 A. 31 (A. 4, A. 22), M. 3779 ( - ), filtrate of faeces (in. 9/2/18).  
 A. 32 (A. 41, A. 42), M. 3800 ( - ), cerebro-spinal fluid (in. 11/2/18).

## CASE 38, G. H., Wee Waa.

(Ill two days; died midnight 11-12/2/18.)

- A. 33 (A. 1), M. 3803 ( + ), (in. 13/2/18; ill 25/2/18; k. 2/3/18).  
 A. 34, Dog 3806 ( - ), (in. 14/2/18).  
 A. 35 (A. 46, A. 65 + ), Sh. 3817 ( - ), (in. 26/2/18).

*From A. 33, M. 3803 (k. 2/3/18).*

- II. A. 36 (A. 62 + ), M. 3834 ( - ), Noguchi culture (in. 4/4/18).

## BROKEN HILL CASES.

- A. 37 (A. 39, A. 66 + ), M. 3811 ( - ), case 6; patient ill five days; d. 13/2/18 (in. 21/2/18).  
 A. 38 (A. 47), M. 3812 ( - ), case 7; patient ill four days; d. 13/2/18 (in. 21/2/18).  
 A. 39 (A. 37, A. 66 + ), M. 3830 ( - ), case 18; patient ill eight days; d. 16/3/18 (in. 27/3/18).

## BRISBANE CASE.

- A. 40, M. 3828 ( - ), patient d. about ten days previously (in. 22/3/18).

## NARRANDERA CASE.

- A. 41 (A. 32, A. 42), M. 3831 ( - ), case 54, G.S.; ill twelve days; d. 22/2/18 (in. 27/2/18).

## HORSE.

- A. 42 (A. 32, A. 41), M. 3820 ( - ), horse d. 27/2/18 (in. 6/3/18).

## FOWL TICKS, from Boggabri,

- A. 43 (A. 28, A. 55 + ), M. 3827 ( - ), (in. 16/3/18).

*Acute Encephalo-myelitis*

## CASE 37, A. C. F. (J.), Boggabri.

(Ill three days; died 13/3/18.)

- A. 44 (A. 3, A. 30, A. 51), M. 3825 ( - ), (in. 15/3/18).  
 A. 45 (A. 72 + ), M. 3837 ( - ), (in. 11/4/18).  
 A. 46 (A. 35, A. 65 + ), Sh. 3839 c ( - ), (in. 16/4/18).

## CASE 32, L. B., Narrabri.

(Ill five days; died 15/3/18.)

- I. A. 47 (A. 38), M. 3826.  
 A. 48, M. 3829 ( + ), (in. 22/3/18; ill 2/4/18; k. 4/4/18).  
*From A. 48, M. 3829 (k. 4/4/18).*
- II. A. 49, M. 3835 ( + ), (in. 4/4/18; ill 11/4/18; k. 15/4/18).  
*From A. 49, M. 3835 (k. 15/4/18).*
- III. A. 50, M. 3839 ( + ), (in. 15/4/18; ill 20/4/18; k. 24/4/18).  
 A. 51 (A. 3, A. 30, A. 44), M. 3840.  
 A. 52 (A. 23), Sh. 3839 b ( + ), (in. 16/4/18; ill 19/4/18; d. 22/4/18).  
 A. 53 (A. 91 + ), Sh. 3863 ( - ), (in. 17/5/18).  
*From A. 50, M. 3839 (k. 24/4/18).*
- IV. A. 54 (A. 71), M. 3846 ( - ), Pasteur-Chamberland F. filtrate (in. 24/4/18).  
 A. 55 (A. 28, A. 43), M. 3848 ( + ), (in. 25/4/18; ill 3/5/18; d. 5/5/18).  
 A. 56 (A. 16, A. 97), Dog 3847 ( - ), (in. 25/4/18).  
 A. 57, Calf 3848 b ( + ), (in. 25/4/18; ill 30/4/18; d. 2/5/18).  
 A. 58, Rabbit 3849 ( - ), (in. 26/4/18).  
 A. 59, Rabbit 3850.  
 A. 60, G. pig 3851 (?), (in. 26/4/18; ill 2/5/18; d. 4/5/18).  
 A. 61, Kitten 3852.  
*From A. 52, Sh. 3839 b (d. 22/4/18).*  
 A. 62 (A. 36), M. 3845 ( + ), (in. 23/4/18; ill 29/4/18; k. 3/5/18).  
*From A. 62, M. 3845 (k. 3/5/18).*
- V. A. 63 (A. 76, A. 93, A. 115), Sh. 3853 ( - ), (in. 3/5/18).  
*From A. 55, M. 3848 (d. 5/5/18).*  
 A. 64, M. 3854 ( + ), (in. 7/5/18; ill 14/5/18; k. 18/5/18).  
 A. 65 (A. 35, A. 46), Sh. 3855 ( + ), (in. 7/5/18; ill 13/5/18; d. 14-15/5/18).  
*From A. 65, Sh. 3855 (d. 14-15/5/18).*
- VI. A. 66 (A. 37, A. 39), M. 3860 ( + ), (in. 17/5/18; ill 22/5/18; k. 30/5/18).  
 A. 67 (A. 77, A. 89), Sh. 3861 ( - ), intraperitoneal (in. 17/5/18).  
 A. 68 (A. 86, A. 90), Sh. 3862 ( - ), (in. 17/5/18).  
*From A. 64, M. 3854 (k. 18/5/18).*  
 A. 69 (A. 94), Sh. 3864 ( - ), (in. 20/5/18).  
 A. 70 (A. 92 + ), Sh. 3865 ( - ), nose swabbed (in. 20/5/18).  
 A. 71 (A. 54), M. 3872 ( - ), lumbar puncture, venous injection (in. 27/5/18).  
*From A. 66, M. 3860 (k. 30/5/18).*
- VII. A. 72 (A. 45), M. 3873 ( + ), (in. 30/5/18; ill 13/6/18; k. 15/6/18).  
 A. 73, Rabbit 3874 ( - ), (in. 30/5/18).  
 A. 74, G. pig 3875 (?), (in. 30/5/18; ill 25/6/18; d. 25/6/18).  
 A. 75, Sh. 3876 ( - ), d. in four days (in. 31/5/18; d. 4/6/18).  
 A. 76 (A. 63, A. 93, A. 115), Sh. 3877 ( - ), lumbar puncture, venous injection (in. 31/5/18).  
 A. 77 (A. 67, A. 89), Sh. 3878 ( - ), dried brain injected (in. 7/6/18).  
*From A. 72, M. 3873 (k. 15/6/18).*
- VIII. A. 78, M. 3890 ( + ), (in. 15/6/18; ill 25/6/18; k. 27/6/18).  
 A. 79, Kitten 3891 ( - ), (in. 15/6/18).

- A. 80, Sh. 3892 ( + ), (in. 17/6/18; ill 24/6/18; k. 28/6/18).  
 A. 81, Sh. 3893 ( + ). (in. 17/6/18; ill 29/6/18; k. 2/7/18).  
 A. 82, Sh. 3894 ( - , ? recovery), (in. 17/6/18; ill ? 29/6/18; d. 10/7/18).  
 A. 83 (A. 114), Sh. 3895 ( - ), (in. 17/6/18).  
 A. 84 (A. 109), Sh. 3896 ( - ), frontal and occipital only (in. 17/6/18).  
 A. 85 Sh. 3897 (death in 2 days), medulla only.  
     *From A. 75, Sh. 3876 (sheep histologically negative, d. 4/6/18).*  
 A. 86 (A. 68, A. 90), Sh. 3879 ( - ), (in. 7/6/18).  
     *From A. 78, M. 3890 (k. 27/6/18).*
- IX.** A. 87, M. 3900 ( + ), (in. 27/6/18; ill 4/7/18; k. 7/7/18).  
 A. 88, Calf 3901 (recovered ?), (in. 27/6/18; ill 2-7/7/18).  
 A. 89 (A. 67, A. 77), Sh. 3902 ( + , recovering ?), (in. 27/6/18; ill 2-4/7/18; d. 4/8/18).  
 A. 90 (A. 68, A. 86), Sh. 3903 (recovered ?), (in. 27/6/18; ill 2 and 3/7/18).  
 A. 91 (A. 53), Sh. 3904 ( + ), (in. 27/6/18; ill 3/7/18; d. 3/7/18).  
 A. 92 (A. 70), Sh. 3905 ( + ), medulla only (in. 27/6/18; ill 3/7/18; d. 6/7/18).  
 A. 93 (A. 63, A. 76, A. 115), Sh. 3906 ( - ?), medulla only (in. 27/6/18; ill? 2/7/18).  
 A. 94 (A. 69), Sh. 3907 ( - ), frontal and occipital only (in. 27/6/18).  
 A. 95, Horse 3908 ( + ), (in. 28/6/18; ill 7/7/18; k. 9/7/18).  
 A. 96, Sh. 3935 ( - ), (in. 29/7/18).  
     *From A. 81, Sh. 3893 (k. 2/7/18).*  
 A. 97 (A. 16, A. 56), Dog 3909 ( - ), (in. 2/7/18).  
     *From A. 92, Sh. 3905 (d. 6/7/18).*
- X.** A. 98, Sh. 3910 ( + ), (in. 6/7/18; ill 11/7/18; d. 15/7/18).  
 A. 99, Sh. 3911 (death in 2 days).  
     *From A. 87, M. 3900 (k. 7/7/18).*  
 A. 100, M. 3912 ( + ), (in. 7/7/18; ill 16/7/18; k. 17/7/18).  
 A. 101 (A. 125), Sh. 3913 ( - ), (in. 7/7/18).  
 A. 102, Sh. 3914 (recovered ?), (in. 7/7/18; ill 12-16/7/18).  
 A. 103, Sh. 3915 ( + ), emulsion plus serum of A. 82, Sh. 3894 (in. 7/7/18; ill 14/7/18; k. 17/7/18).  
 A. 104 (A. 126), Sh. 3916 ( - ), emulsion plus serum of A. 82, Sh. 3894 (in. 7/7/18).  
 A. 105, Sh. 3917 ( + ), emulsion plus normal sheep's serum (in. 7/7/18; ill 14/7/18; d. 16-17/7/18).  
 A. 106, Sh. 3918 ( - , death in 5 to 6 days), emulsion plus normal sheep's serum (in. 7/7/18; d. 12/7/18).  
     *From A. 95, Horse 3908 (k. 9/7/18).*  
 A. 107 (A. 127), Calf 3919 (recovered ?), (in. 10/7/18; ill on 13 and 14/7/18).  
     *From A. 98, Sh. 3910 (d. 15/7/18).*
- XI.** A. 108, Sh. 3920 ( + ), (in. 16/7/18; ill 22/7/18; d. 23/7/18).  
     *From A. 103, Sh. 3915 (k. 17/7/18).*  
 A. 109 (A. 84), Sh. 3921 ( + ), (in. 18/7/18; ill 25/7/18; d. 27/7/18).  
     *From A. 100, M. 3912 (k. 17/7/18).*  
 A. 110, M. 3925 ( + ), (in. 19/7/18; ill 30/7/18; k. 2/8/18, might have recovered).  
 A. 111, Sh. 3922 ( - ), emulsion plus serum of A. 89, Sh. 3902 (in. 19/7/18).  
 A. 112, Sh. 3925 (d. in four days), emulsion plus serum of A. 89, Sh. 3902.  
 A. 113, Sh. 3924 (d. in two days), emulsion plus serum of A. 89, Sh. 3902.  
     *From A. 109, Sh. 3921 (d. 27/7/18).*
- XII.** A. 114 (A. 83), Sh. 3933 ( - ), (in. 29/7/18).  
 A. 115 (A. 63, A. 76, A. 93), Sh. 3934 ( - ), (in. 29/7/18).  
     *From A. 110, M. 3925 (k. 2/8/18).*  
 A. 116 (A. 132), M. 3936 ( - ), emulsion plus serum of A. 89, Sh. 3902 (in. 2/8/18).  
 A. 117, M. 3937 ( + ), emulsion plus serum of A. 102, Sh. 3914 (in. 2/8/18; ill 25/8/18; k. 27/8/18).

- A. 118, Hen 3938 ( - ), (in. 2/8/18).  
 A. 119 (A. 130), Sh. 3939 ( - ), Berkefeld filtrate (in. 2/8/18).  
 A. 120 (A. 131), Sh. 3940 ( - ), Berkefeld filtrate (in. 2/8/18).  
 A. 121, Sh. 3941 ( + ), (in. 2/8/18; ill 8/8/18; d. 13/8/18).  
 A. 122, Sh. 3942 ( - ), (in. 2/8/18).  
 A. 123, Sh. 3943.  
 A. 124, M. 3952 ( + ), (in. 17/8/18; ill 3/9/18; k. 7/9/18).  
     *From A. 121, Sh. 3941 (d. 13/8/18).*
- XIII.** A. 125 (A. 101), Sh. 3948 (recovered ?), (in. 14/8/18; ill on 18 and 19/8/18).  
 A. 126 (A. 104), Sh. 3949 ( - ), (in. 14/8/18).  
 A. 127 (A. 107), Calf 3950 ( - ), (in. 14/8/18).  
 A. 128, M. 3951.  
     *From A. 117, M. 3937 (k. 27/8/18).*  
 A. 129, M. 3967 ( + ), (in. 27/8/18; ill 6/9/18; k. 9/9/18).  
 A. 130 (A. 119), Sh. 3968 (recovered?), (in. 28/8/18; ill 5-10/9/18).  
 A. 161 (A. 120), Sh. 3969 ( - ), (in. 28/8/18).  
     *From A. 129, M. 3967 (k. 9/9/18).*
- XIV.** A. 132 (A. 116), M. 3977 ( - ), (in. 11/9/18).