STUDIES ON PSEUDORABIES (INFECTIOUS BULBAR PARALYSIS, MAD ITCH)

III. The Disease in the Rhesus Monkey, Macaca mulatta

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Plates 35 and 36

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It has already been noted (Hurst, 1933) that the monkey (*Macaca mulatta*) is susceptible to intracerebral inoculation of either the Aujeszky or the Iowa strain of pseudorabies virus. The details of infection in this species appear of sufficient theoretical importance to warrant the brief description which is the object of the present communication.

EXPERIMENTAL

In England in 1930, using a strain of Aujeszky virus,[1] I was unable by intracerebral or intramuscular inoculation to secure infection of a *rhesus* monkey. Remlinger and Bailly (1933) also recorded failure with another species—*Inuus ecaudatus*.

With the Iowa strain of pseudorabies virus, and with an Hungarian strain,[2] intracerebral injection at Princeton (1932) was successful in each of 5 cases. Inoculation by other routes met with less success. Intrasciatic inoculation accompanied by mild trauma (Hurst, 1930), and often by ligature of the nerve between the site of deposition of infective material and the point of entry of the needle (to prevent leakage), gave very inconstant results. Of 12 animals so inoculated with highly virulent rabbit virus, 2 only developed symptoms; the remainder were inoculated a second time in the nerve of the opposite

[1] Obtained through the kindness of Mr. I. A. Galloway.

[2] Obtained by Dr. R. E. Shope from Professor Aujeszky.

side, when one more animal became infected. The survivors, refractory to 2 intraneural inoculations and having throughout shown a normal temperature curve, possessed no antiviral substances in the serum. A 13th monkey injected on each of two occasions with monkey virus developed no symptoms, but was later found to resist intracerebral inoculation of rabbit virus and to possess neutralising bodies in the serum. Intramuscular and intravenous inoculation of massive doses (3 cc. and 5 cc. respectively of a 10 per cent suspension[3]) evoked neither symptoms nor immunity.

Returning to England (1934), I inoculated 2 more monkeys intracerebrally; both showed a temperature reaction without definite

TABLE I

Results of Inoculating Pseudorabies Virus into Monkeys Not Previously Tested for Immunity to B Virus

Route of inoculation	No. of monkeys inoculated	Developed nervous symptoms Killed for histology or when moribund	Temperature reactions No definite nervous symptoms Later immune serologically	No apparent illness Later immune serologically	No apparent illness Later not immune serologically
Intracerebral	7	5	2	—	—
Intrasciatic	13	2	—	1	9
Intrasciatic (reinoculation)	11	1	—		
Intramuscular	1	—	—	—	1
Intravenous	2	—	—	—	2

nervous symptoms, and later possessed neutralising bodies in the serum. These results are summarised in Table I.

At this point Sabin made the interesting observation of a partial antigenic relationship between the pseudorabies and B viruses (1934 *a*) and found that 1 of 13 apparently normal monkeys was immune to the latter (1934 *b*).

It therefore seemed desirable to ascertain whether immunity to B virus (Sabin and Wright, 1934) is an important factor in influencing the course of pseudorabies in the monkey.

Incidence of B Virus-Immune Monkeys.—As stated, Sabin found

[3] 1 cc. of 10 per cent suspension of the virus used represents 1 to 10 million intracerebral infecting doses for the rabbit.

TABLE II

Results of Inoculating Aujeszky Virus into Monkeys Tested for Immunity to B Virus

No.	0.5 cc. serum neutralised B virus	Route of inoculation	Result	Remarks
	M.I.D.			
6-16	>500	Intracerebral	T 4–12, R	Serum neutralized Aujeszky virus after, but not before experiment. Characteristic residual lesions in brain when killed
6-17	>500	"	+5, D 8	—
6-19	>500	"	T 2–9, R	As 6-16
6-20	>500	Intravenous	Nil	Later, no neutralising antibodies to Aujeszky virus
6-21	>500	"	"	As 6-20
6-22	>500	Intradermal	"	As 6-20. Later succumbed to intracerebral inoculation (+3, D 9)
6-18	<10	Intradermal	Nil	As 6-20
6-25	<10	"	"	As 6-20. Later succumbed to intracerebral inoculation (+5, D 9)
6-26	<10	"	"	As 6-20. Later succumbed to intracerebral inoculation (+4, K 5)
6-27	<10	Intracerebral	+6, D 10	—
6-28	<10	"	+4, D 8	—
6-29	<10	"	+4, D 9	—
6-36	<10	Intracisternal	T 4–10, R	As 6-16
6-37	<10	"	+7, K 8	—
6-38	<10	Intrasciatic	Nil	As 6-20
6-39	<10	"	+20, K 21	—
6-40	<10	"	T 7–9, R	As 6-16
6-41	<10	"	+13, K	—
6-42	<10	"	Nil	As 6-20
6-43	<10	"	T 5–7, R	As 6-16
6-44	<10	"	T 6, K	Nervous system virulent
6-45	<10	"	T 8, K	As 6-44
6-49	<10	"	T 8–10, K	As 6-44
6-50	<10	"	T 8–10, R	As 6-16
6-51	<10	"	Nil	As 6-20
6-52	<10	"	T 8–9, K	As 6-44

T 4 = temperature on 4th day. No definite nervous signs.

+5 = typical nervous disease on 5th day (confirmed by histological examination).

K 6, D 6 = killed or died on 6th day.

R = recovered.

only 1 of 13 monkeys immune to B virus. It was, therefore, surprising to find that of the first 7 monkey sera tested, 6 contained abundant antibodies to this virus; the animals had all been obtained at one time from the dealer, and it is probable that they represented a single consignment from India. Of a second group of 5 monkeys and a third group of 14 obtained at later dates, not one furnished a neutralising serum.

Pseudorabies in Monkeys Tested for Immunity to B Virus.—The results of the renewed investigation are set forth in Table II. Although the observations are not sufficiently numerous to be of statistical value, they suggest that in the presence of immunity to B virus intracerebral inoculation of pseudorabies leads often to a non-fatal instead of a fatal illness. On the other hand, intracisternal inoculation of 1 cc. of 10 per cent pseudorabies virus is not invariably fatal even in monkeys possessed of no immunity to B virus. Recent results of intrasciatic inoculation (0.2 cc. of 10 per cent emulsion), always in animals not immune to B virus, were distinctly better than those on previous occasions. Intradermal inoculation (2 cc. of 10 per cent suspension distributed in ten areas) was without effect whether the monkeys were immune to B virus or not; as compared with areas inoculated in the same animals with normal rabbit brain no difference in macroscopic reaction was noted, and the temperature curve was only slightly disturbed immediately after the injection, presumably as a result of injection of much foreign protein. Intravenous inoculation (5 cc. of 10 per cent suspension prepared with fresh normal rabbit serum to eliminate the possibility of any toxic effect of the brain extract (Dold and Ogata, 1911, 1912)) was negative in 2 B virus-immune animals. Although infected animals were kept in cages closely adjacent to normal monkeys which picked their affected brothers, no contact infection ever resulted.

Symptomatology

Within 50 to 90 hours of intracerebral inoculation, 9 of 12 animals showed rises of temperature of from 1.5° to 3.5°F. lasting until nervous symptoms had reached an advanced stage. 3 cases which developed nervous symptoms and died (or were killed at an advanced stage) remained afebrile throughout. In all, the temperature fell to a subnormal level when death appeared imminent. On a number of occasions too numerous to be coincidental, the temperature became

subnormal about half way through the period of nervous symptoms, to rise again within 12 to 24 hours; this drop was accompanied by no amelioration of the clinical condition, and occurred both in cases showing nervous symptoms, and later dying, and in those suffering only from pyrexia and subsequently recovering. Nervous symptoms appeared at times ranging from 3 to 7 days after inoculation.

After successful intrasciatic inoculation the clinical course was very similar; the temperature usually rose on the 5th–8th day, with nervous signs supervening in most cases on the 11th–15th day; in one case, however, not until the 20th day.

Though not dramatic, the onset of nervous symptoms was sufficiently abrupt. From a state of normal vivacity and inclination for food the animal lapsed into one of apathy, but not somnolence, and could be roused only by sustained stimulation; often it was no longer aggressive on disturbance and paid no attention to food or to a cage mate. In many instances within the next 12 hours or so epileptiform fits ensued in increasingly rapid succession. A fixed stare and twitching of the eyelids, ears and mouth, often heralded by characteristic cries, preceded tonic and later clonic spasms first of the contralateral face and tongue, then of the contralateral arm, and finally of the whole musculature. Between the convulsions coarse lateral nystagmoid movements, sometimes associated with jerks of the head to the contralateral side, often persisted together with fibrillary twitches in the general musculature. "Tasting movements" of the jaws unassociated with the usual picking and eating of sebum were in some monkeys almost continuous. Almost as frequent were forced movements of greater or less extent. In their mildest form the head slowly wandered to one side, usually that of the inoculation, to be brought back by a sudden jerk; when more severe the head and body were forcibly twisted until the animal was compelled to complete the rotation around its vertical axis and thus return to its original position. When recumbent it rolled over and over on the floor of the cage. Some cases manifested constant, almost rhythmic, rolling movements of the head, which was successively flexed on the chest, rotated to one side, extended and returned to its former position; or sliding movements of the arm across the body, with flexion at the elbow and extension of wrist and fingers, recurred frequently. Mild, moderate or marked salivation was present. The pupils were small and reacted briskly to light.

In severe cases, with the passage of a further 24 or 48 hours the monkey was semicomatose; more or less generalised muscular twitching continued or status epilepticus supervened. The pupils were now dilated and insensitive to light. There was great general weakness but no localised paralyses, except once double ptosis.

The foregoing clinical picture was not invariable. Fits might mark the onset and the animal thereafter remain free, to die suddenly a few days later. Twitching of the face might be the only spasmodic element. Sometimes great general weakness without local paralyses or fits obtained from the onset. Animals presenting only fever later showed unmistakable residual lesions (see below) in the

characteristic situations; of course, it is realised that in these cases rare fits might have occurred during periods when the animals were not under observation. Such animals made an apparently complete recovery except in one case in which the monkey manifested a change in temperament, becoming extremely tame and docile though prior to the experiment it had been just the reverse. Once nervous symptoms were definite, recovery never occurred.

Distribution of Virus

Four monkeys, one dying of tuberculosis on the 4th day and the others sacrificed with advanced nervous symptoms on the 6th, 6th and 9th days respectively after intracerebral inoculation, showed no virus in the defibrinated blood when 10 cc. amounts were injected subcutaneously into rabbits. The cerebrospinal fluid in 2.5–5.0 cc. amounts was likewise uninfective. The lungs, livers, spleens, kidneys, adrenals, salivary glands and cervical lymph glands in doses of 1 cc. of 10 per cent suspensions all proved innocuous. Both cerebral hemispheres of each case were virulent, as were, with long incubation periods denoting the presence of a minimal amount of virus, the lumbar cords. The sciatic nerves were not virulent.

Four other intracerebrally infected monkeys were bled daily from the beginning of the experiment, and defibrinated blood in amounts of 2–3 cc. inoculated subcutaneously into rabbits with uniformly negative results.

Table III illustrates the spread of virus in the nervous systems of monkeys infected by the intrasciatic route. The cerebrospinal fluid appears to take no part in its dissemination. The lumbar cord is the first part of the nervous system to become infective, and the cervical cord and medulla are virulent before the cerebral cortex. At a given period lesions in the lumbar cord are more advanced than in the cortex, yet at the same time are always minimal in intensity (see below). When the characteristic cortical disease is present the lumbar cord may be no longer infective, especially in cases of slow evolution, though sections show slight lesions indicative of the previous activity of the virus. These facts are interpreted as indicating that the cells of the lumbar cord are relatively insusceptible to the virus, which does not readily gain a foothold here and tends soon to die out. Parts of the cerebral cortex representing sites of election of severe lesions (see below) become infective before areas seldom or never affected

(*e.g.*, the lateral occipital cortex), and judging by the duration of the incubation period in passage animals the latter when virulent contain only small amounts of virus. In a single case the motor cortex cor-

TABLE III

Distribution of Pseudorabies Virus in the Nervous System of Monkeys Following Left Intrasciatic Inoculation

No.	Days after inoculation	Symptoms	Presence of virus in											
			Cerebrospinal fluid 2.5–5.0 cc.	Lumbar cord	Cervical cord	Medulla	Right anterior frontal cortex	Right motor cortex	Right temporal cortex	Right occipital cortex	Left anterior frontal cortex	Left motor cortex	Left temporal cortex	Left occipital cortex
6-44	6	Temperature 1°F. above previous level	0	+5	0	0	0	0	0	0	0	0	0	0
6-45	8	Temperature rising sharply from previous level	0	+4	+4	+3	0	0	0	0	0	0	0	0
6-49	10	Pyrexia for 3 days; no nervous symptoms	0	+8	+4	+3	+4	0	0	0	0	0	0	0
6-52	9	Pyrexia for 2 days; no nervous symptoms	0	+4	+4	+3	+8	0	+3	0	+4	0	0	0
P 12	15	Pyrexia for 2 days; early nervous symptoms	–	0	–	+11	0	+3	+3	0	+5	0	+4	0
P 7	12	Nervous symptoms for 24 hrs.	0	+3	+5	+5	+3	+4	+3	+5	+3	+5	+3	+8
6-39	21	" "	0	0	–	+3	–	–	+2	–	–	–	+3	–

\+ = development of pseudorabies in passage rabbit with incubation period in days.

0 = no take.

– = not tested.

The areas of cortex chosen for passage were as follows: (*a*) anterior frontal—frontal pole including part of basal surface; (*b*) motor cortex—upper part including leg area and part of medial surface of hemisphere to cingular sulcus; (*c*) temporal pole—including pyriform area and anterior extremity of cornu Ammonis; (*d*) occipital cortex—smooth lateral surface anterior to occipital pole.

responding to the inoculated nerve was virulent before that of the opposite side (*cf.* observations in poliomyelitis; Hurst, 1930), but in view of the complexity of the nerve paths from the lumbar cord to

other parts of the cortex examined it is not perhaps to be expected that a clear cut unilateral distribution would obtain at an early stage of cortical involvement.

Histological Examination

1. Lesions Following Intracerebral Inoculation.—The essentials of the histological picture following injection by this route have already been described (Hurst, 1933), and certain points regarding the distribution of the lesions alone require emphasis.

Greater experience permits more definite statement of the parts of the nervous system chosen for the major attack. These are in order of frequency and of severity of affection (*a*) the cornu Ammonis or the pyriform area, or often both together, (*b*) parts of the cerebral cortex bordering on the Sylvian fissure, particularly the lower lip of the fissure and the anterior part of the island of Reil, (*c*) much less frequently and less severely other parts of the cerebral cortex, especially the basal surface of the frontal lobe, parts of the temporal cortex not mentioned above, and the lips of the cingular sulcus. The basal ganglia and brain stem suffer to a variable but never considerable degree; vascular and tissue infiltration are here more conspicuous than is extensive nerve cell destruction. The cerebellum is usually intact. In the cerebrum the attack of the virus appears to be on the selected areas as areas, and not on a particular cell type—thus in the cornu Ammonis the greater part of the pyramidal cell band may be necrotic and the end-plate and fascia dentata spared, or the fascia dentata may suffer more than the pyramidal cells, or the cells of the end-plate may be chiefly affected. At a significantly early stage there may be no meningeal or vascular damage; the picture is then unequivocally that of a primary attack on the nerve and glial cells. After intracisternal inoculation the same distribution obtains.

Residual Lesions.—In the first day or two of the nervous disease, the absence of tissue and vascular reaction makes the detection of lesions in all but the most severely affected areas a matter of rather painstaking study with a high power of the microscope. Later, the secondary inflammatory reaction increasing rapidly in intensity causes the sites of election of nervous destruction to leap to the eye, as the series of photographs here reproduced demonstrates. In the most severely affected areas of non-fatal cases the tissues may ultimately be

reduced to porencephalic cavities, or where the injury has been rather less severe a dense glial-mesodermal scar may develop. Perivascular and meningeal infiltration persists for months after the initial injury. In these later stages definite macroscopic changes, previously absent, are also noted; the earliest is considerable congestion of the stricken areas first visible about 4 days after the onset of nervous symptoms. The following cases are typical.

M 6-16. 3 weeks after intracerebral inoculation in a monkey (serologically) immune to B virus. Pyrexia from the 4th–12th day had been accompanied by no definite nervous symptoms other than great general weakness. At death the serum neutralised pseudorabies virus.

At autopsy both temporal poles were distinctly soft to palpation. They looked pale, yellowish and opaque as contrasted with other areas of the brain, and the finest vascular markings were absent. On approaching the damaged area the larger vessels seemed to diminish in calibre, probably because a small amount of greyish translucent exudate obscured them in the sulci. A few brownish flecks probably represented old pial haemorrhages. On section after fixation in formol-saline, the grey matter of the cortex appeared to end on reaching the curve of the temporal pole, where it gave place to a wider, yellowish, opaque zone in which demarcation between the grey and the white matter proved impossible.

Photographs show far better than does a lengthy description the distribution of residual lesions in the brain (Figs. 1–4). The cortex of the temporal pole and the cornu Ammonis were in large part completely destroyed; the remaining structures consisted almost wholly of hyperplastic and infiltrated pre-existing and new-formed vessels; granular corpuscles and infiltrating lymphocytes and plasma cells (scanty); and many hypertrophied glial cells where the intensity of the destruction was slightly less. There was a similar scar at the anterior end of the island of Reil, and smaller areas in the lips of the Sylvian fissure and superior temporal sulcus. Elsewhere in the cortex and basal ganglia lesions were minimal; in the walls of the third ventricle and in the brain stem they were only slightly more severe.

M 6-36. 3 weeks after intracisternal injection. Pyrexia on the 4th–10th days after injection and transient nervous symptoms on the 8th day were the chief clinical features. At death the serum neutralised pseudorabies virus.

Residual lesions did not compare in severity with those in the preceding case; they were most intense in the pyriform area of the temporal lobe, especially on one side, slight around the Sylvian fissure, and of intermediate severity in the walls of the third ventricle. The brain stem was not examined.

M 6-06. 2½ months after intracerebral inoculation which was succeeded on the 3rd–10th days by pyrexia but no nervous symptoms other than general weakness. Later the temperament of this animal was quite altered; the serum contained neutralising antibodies.

On palpation both temporal lobes gave the impression of softening; microscopi-

cally severe residual lesions were seen. Anteriorly, the meninges over the temporal poles were thickened and infiltrated with mononuclear cells. In the subjacent cortex marked perivascular sheathing, diminution or absence of nerve cells, and increase of glia and microglia (chiefly granular corpuscles) marked the less affected parts, while the more affected areas were reduced to small porencephalic cavities separated by a scanty framework of glial-mesodermal scar tissue (Fig. 5). A similar much smaller area lay in the anterior part of the island of Reil, and still less severe lesions in the cortex forming the lower lip of the Sylvian fissure. There was some residual meningeal infiltration in one part of the cingular sulcus.

Farther back all structures of the cornu Ammonis were wanting except in its most posterior part behind the level of the thalamus; the descending horn of the lateral ventricle was greatly dilated and separated from the meninges by a narrow strip of sclerotic tissue only slightly exceeding 1 mm. in width (Fig. 6). No other lesions were noted.

M 6-05. 4 months after intracerebral injection. No definite pyrexia or nervous symptoms followed the inoculation, but the serum later contained neutralising antibodies. Residual lesions were less extensive than in the preceding animal.

Macroscopically there was some shrinkage in the cornu Ammonis and adjacent regions. The chief destruction was of the pyramidal cell band of the cornu Ammonis, which over more than half of its extent including the whole expansion within the curvature of the fascia dentata was completely replaced by a glial-mesodermal scar. The fascia dentata was intact. Less severe changes (diminution in the number of nerve cells, increase of glia and residual perivascular cuffing) were present in parts of the temporal cortex anterior to the cornu Ammonis. Meningeal infiltration persisted in the Sylvian fissure and superior temporal sulcus.

2. Lesions Following Intrasciatic Inoculation.—The distribution of lesions following intrasciatic inoculation is of importance; the 8 cases examined histologically were sufficiently similar to obviate the necessity of separate description.

At the site of inoculation in the sciatic nerve wholly non-specific degenerative and reparative changes proceeded. In no case at the time of autopsy were nuclear inclusions present.

According to the period at which the monkey was killed, the spinal ganglia corresponding to the inoculated nerve showed either occasional necrotic nerve cells, with chromatolysis and mild degenerative changes in a proportion of the remainder, or else moderate mononuclear interstitial infiltration (with perhaps a few polymorphonuclear leucocytes or plasma cells) and foci of proliferated capsule cells replacing vanished neurons. The ganglia of the opposite side presented less severe lesions or none at all; those examined from higher levels were normal.

In the anterior horn cells of the lumbosacral cord of some cases, axonal reaction attributable to ligature of the inoculated sciatic nerve had to be distinguished from lesions resulting from virus activity. The latter consisted in necrosis of occasional nerve cells and their replacement by phagocytic cells; a few focal collections of microglial cells, particularly in the posterior and anterior horns of the inoculated side; moderate or mild mononuclear cuffing of the larger vessels; and in some cases, but not in all, slight mononuclear infiltration in the meninges.

In the dorsal and cervical cord at different levels and in different cases lesions might resemble the above in severity or be almost or wholly non-existent.

Above the foramen magnum lesions were wholly comparable with those following intracerebral inoculation. The outstanding nervous destruction was limited to the regions already indicated, and in many cases the degenerative process in the nerve cells again progressed to necrosis in the complete absence of meningeal infiltration, or of any abnormality, inflammatory or otherwise, of the blood vessels.

Finally, histological examination supported the belief that the spread of virus is from below upwards. For in cases in which early lesions were present in the lumbar cord and ganglia, the temporal cortex, etc., were still free from change. While when the temporal cortex was strewn with recently necrotic cells or cells bearing nuclear inclusions, but before tissue, vascular and meningeal reaction had appeared here, the lumbar cord and ganglia were the seat of definite reactive inflammation, many or all the dead nerve cells had been removed, and nuclear inclusions had disappeared.

To summarise, the histological findings agree with the experimental evidence in suggesting an upward spread of the virus, after intrasciatic inoculation, presumably by the nervous path. Although virus enters by the lumbar cord and might be expected to produce its maximal effects here, the striking localisation of the most severe lesions to the temporal lobe, etc., is once more observed, and must be regarded as characteristic of the action of pseudorabies virus in the monkey. The virus evidently possesses relatively little affinity for the neurons of the spinal ganglia, cord, brain stem, cerebellum and basal ganglia. On the contrary certain areas of the cerebral cortex are peculiarly vulnerable to its action, and the result is a selective distribution of lesions as insusceptible of facile explanation as that in poliomyelitis or louping-ill.

3. Lesions Following Intradermal Inoculation.—It has been recorded (Hurst, 1933) that in pseudorabies in the monkey nervous system nuclear inclusions are seen only in nerve and (less often) glial cells, and never in mesodermal cells of the vessels and meninges. In the rabbit, on the other hand, nuclear inclusions occur in cells derived

from all three embryonic layers (Hurst, 1934). If the virus be introduced into the skin of the rabbit or pig a focus of necrosis accompanied by much local multiplication of virus results.

In the present work monkeys were inoculated intradermally in many areas with pseudorabies virus, and with normal rabbit brain for purpose of control; daily from the 1st–6th days skin was excised for histological examination. In no instance were the appearances with pseudorabies virus different from those with normal rabbit brain. Inclusions were never seen. In the disease provoked by intracerebral or intrasciatic inoculation virus and lesions are absent from the general viscera. There is, therefore, no cytological or other evidence that in the monkey the pseudorabies virus can attack any but neuro-ectodermal cells.

DISCUSSION

Two points of interest emerge from this study. The first concerns the different behaviour of the virus of pseudorabies in various animal species. While in the rabbit it is strongly neurotropic, its activities do not end here; in this animal it is capable of multiplying and producing specific lesions in a great variety of cells derived from all three embryonic layers. In other words, it can be described as pantropic (Hurst, 1934). In the monkey, on the other hand, it appears to have no affinity for any cells other than nerve and glial, and in its behaviour under experimental conditions resembles purely neurotropic viruses like rabies and poliomyelitis. In future, in qualifying a virus by some term indicative of its cellular affinities, it will be necessary to specify clearly the animal species on which observations have been carried out.

Most of the common domestic and laboratory mammals exposed to pseudorabies virus readily contract the disease and die; two stand out as possessed of greater resistance, *viz.* the pig and the monkey. In stating that the virus is less virulent towards these species, or alternatively that they are less susceptible to its action, it is well to realise that in the two instances the mechanism of resistance is wholly different.

Inoculated subcutaneously the pig develops a definite local lesion (Hurst, 1933), associated presumably as in the rabbit[4] with great

[4] Unpublished observations.

multiplication of virus; only small amounts are necessary to infect by the skin (Köves and Hirt, 1934). During the course of the infection virus is present in the blood and blood-rich organs (Köves and Hirt); lesions may be present in the lymph glands, spleen and heart (Hurst). Virus invades the nervous system where, however, there is no great outfall of nerve cells (Hurst), with the consequence that definite nervous symptoms are rare (Shope, 1931) occurring probably in less than 10 per cent of the cases (Köves and Hirt); in the absence of the characteristic biting and scratching, the disease is often wrongly diagnosed, if indeed without regular temperature readings it is diagnosed at all. Almost always the pig recovers and is subsequently immune; Köves and Hirt place the mortality rate at less than 5 per cent. But the nasal secretions become infective (Shope, 1934, 1935) as does the urine (Köves and Hirt), and the pig transmits the disease to its fellows and to other animals. In short, in the pig the virus exhibits multiple tissue affinities; the animal very easily contracts the disease, but survives because its nervous system is relatively resistant and is not overwhelmed unless a considerable dose of virus be given directly into the brain.

By way of contrast, large doses of highly virulent rabbit brain introduced intradermally into the monkey evoke no more reaction than does normal brain tissue; intramuscular and intravenous inoculation are probably equally fruitless. No infection follows, so that subsequently the animal is not immune. But if virus be brought into sufficiently intimate contact with nervous tissue, as by intrasciatic inoculation, it may invade the nervous system to set up a disease accompanied by clinical symptoms and fatal in many cases. During this disease virus does not circulate in the blood and the animal does not infect a cage mate; no virus or lesions are present in the general viscera. In brief, in the monkey the nervous system is probably less resistant to infection than in the pig, but since the virus behaves in this species as a strict neurotrope it does not readily reach the susceptible tissue, destruction of which is incompatible with life. Another factor in the resistance of the monkey is, no doubt, the relative insusceptibility of the lower regions of the nervous axis; were the highly susceptible neurons in this instance those of the anterior horns of the cord as they are in poliomyelitis, instead of those of the cerebral cortex not easily reached from the periphery, intramuscular and intradermal

deposition of virus, even though not followed by local multiplication, might be more effective in producing nervous infection.

The last observation is the second point worthy of comment. Although in pseudorabies in the monkey the most severely affected areas of the cortex are roughly those underlying the most deeply coloured parts of the meninges after intracisternal inoculation of dyestuffs (Hurst, 1932), we cannot explain the present findings solely, if at all, on the basis of dissemination of the virus by the cerebrospinal fluid. Not only was the virus never found in the fluid, even at an early stage after intrasciatic inoculation, but the correlation between the distribution of lesions in pseudorabies and that of colouring matter introduced into the fluid is far from complete if the nervous system as a whole is considered. We know already of two virus diseases of the central nervous system in which, for reasons at present obscure, a particular type of nerve cell is involved far more severely than others; these are poliomyelitis in man and the monkey, and louping-ill in the monkey (Hurst, 1931; Findlay, 1932), with respectively motor neuron and Purkinje cell involvement far surpassing that of other cell types. In the present instance certain cortical areas seem to be selectively attacked, though within these areas more than a single cell type is included in the destruction. Whatever the underlying cause, this distribution of lesions appears at present to be highly characteristic of pseudorabies in the monkey.

CONCLUSIONS

In the monkey (*M. mulatta*) the virus of pseudorabies, pantropic in the rabbit, behaves as a strict neurotrope. Infection, usually fatal, readily follows intracerebral and intracisternal inoculation of rabbit virus, and often intrasciatic inoculation; the symptomatology of the ensuing disease is described. In a limited number of experiments no infection resulted from intradermal, intramuscular or intravenous inoculation. Nerve and glial cells are primarily attacked by the virus, but no cytological or other evidence of susceptibility of non-nervous tissue or of growth of virus outside the nervous system was obtained. Certain cortical areas, of which the principal are the pyriform area, cornu Ammonis, island of Reil, lower lip of the Sylvian fissure and basal surface of the frontal lobe, are affected far more severely than are other parts of the nervous axis; the reasons for this elective dis-

tribution of the most severe lesions, seen alike after intracerebral and intrasciatic inoculation and analogous perhaps to that in poliomyelitis and louping-ill, are not obvious. Other areas of the nervous system are relatively insusceptible to the action of the virus. Cases showing clinically only a febrile reaction without definite nervous symptoms may later exhibit marked residual lesions at the sites of election. The blood and cerebrospinal fluid play no apparent rôle in disseminating the virus, which, after intrasciatic inoculation, spreads upwards by the nervous path.

Some suggestion was received from the experiments that in monkeys possessed of immunity to B virus (Sabin and Wright, 1934) pseudorabic infection is less likely to prove fatal than in animals not so immune, but the observations made were insufficiently numerous to be of statistical value.

The sera of 6 out of 26 monkeys were found to contain antibodies neutralising B virus; these 6 monkeys were all included in one batch of 7 received at one time from the dealer.

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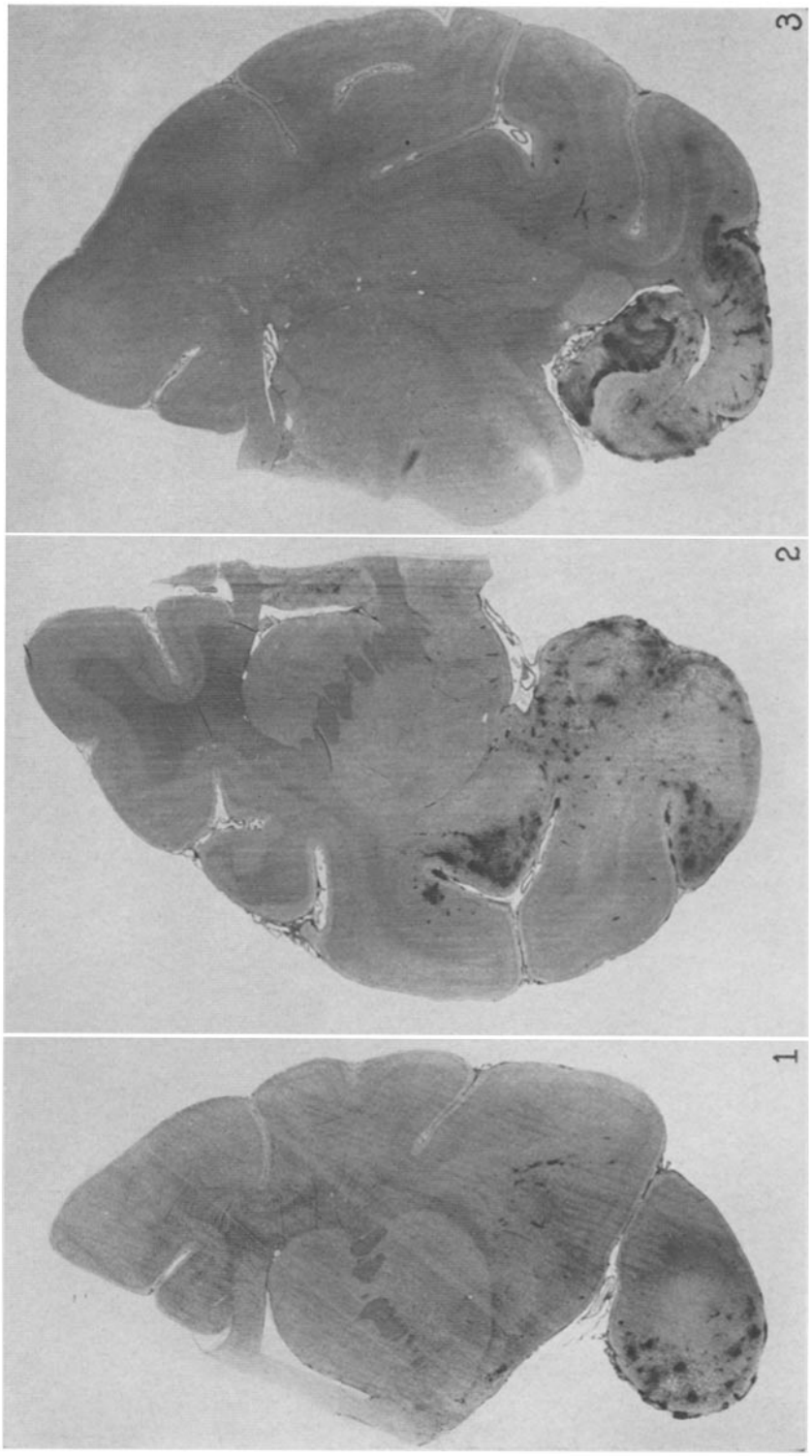
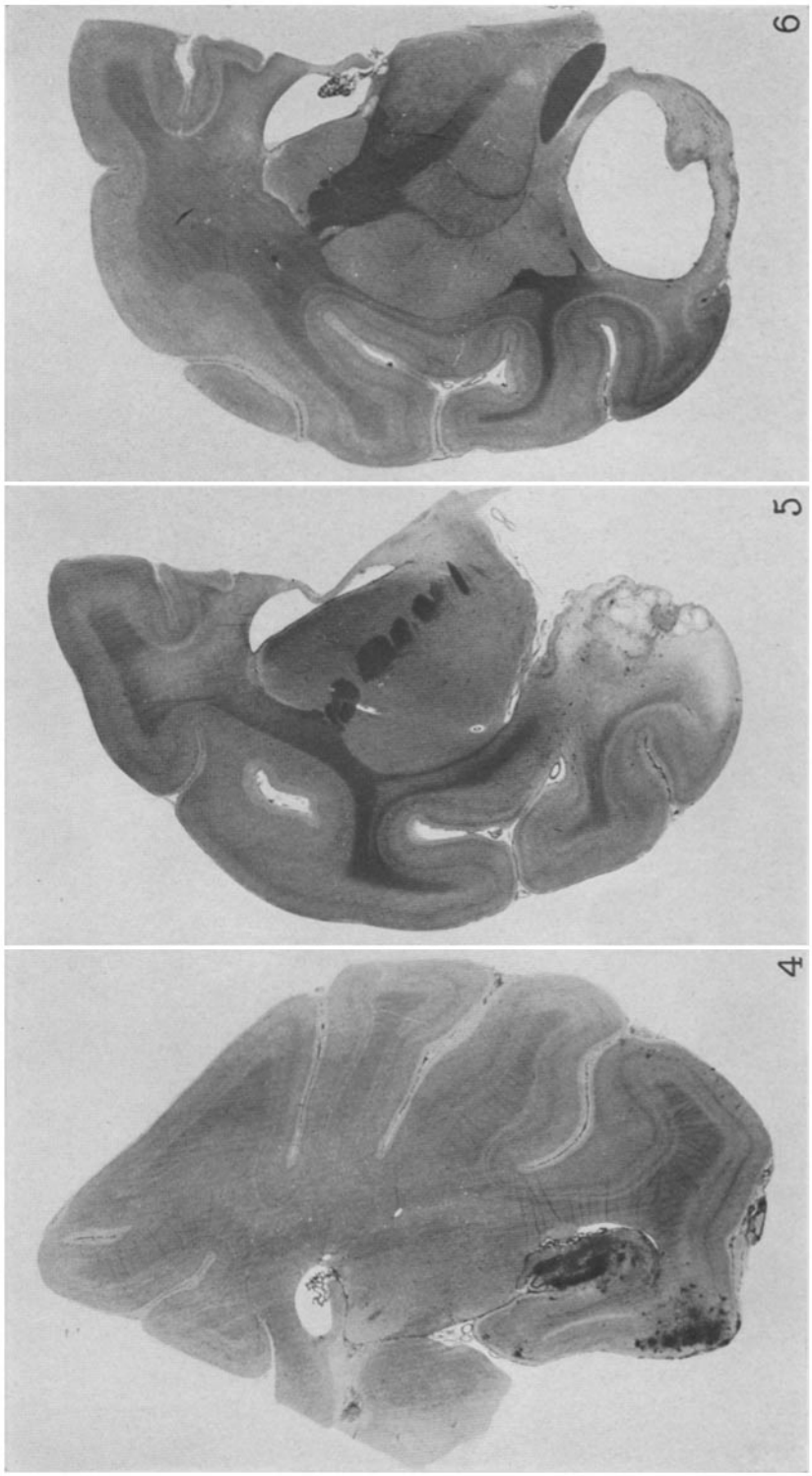
EXPLANATION OF PLATES 35 AND 36

FIGS. 1 to 4. Residual lesions in the brain of M 6-16. The intensely stained areas represent tissue and perivascular infiltration at the sites of maximal damage. The distribution is outlined in the text. Haematoxylin and Van Gieson. × about 3¼.

FIGS. 5 and 6. Residual lesions in the brain of M 6-06. The former shows the porencephalic softening at the temporal pole, the latter the shrinkage of cerebral tissue with complete absence of the cornu Ammonis and compensatory dilatation of the lateral ventricle. Haematoxylin and Van Gieson. × about 3¼.



(Hurst: Studies on pseudorabies. III)



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