STUDIES ON PSEUDORABIES (INFECTIOUS BULBAR PARALYSIS, MAD ITCH)

I. HISTOLOGY OF THE DISEASE, WITH A NOTE ON THE SYMPTOMATOLOGY

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Pseudorabies has now been reported as occurring in Hungary (Aujeszky, 1902, and many others), Brazil (Carini and Maciel, 1912), United States (Shope, 1931, 1932), Denmark (Bang, 1932) and Holland (Burggraaf and Lourens, 1932). Although in this disease nervous symptoms are prominent, usually no lesions other than vascular congestion have been found in the nervous system. Fölger (1932) found hemorrhages and less often perivascular infiltration in cattle, but not in cats or rabbits. Among others, Bertarelli and Melli (1913), Sangiorgi (1914) and Fölger have failed to detect cellular inclusions. In rabbits and guinea pigs, the extraordinarily rapid course of the malady, measured in hours, presumably allows little time for the development of gross pathological changes. Nevertheless characteristic lesions, identical whether the Aujeszky virus or Shope's Iowa strain ("mad itch") is employed, are usually present. In the following pages a description of these lesions is given.

Technique

The source of virus consisted of the supernatant fluid from a 10 per cent suspension of brain tissue of rabbits succumbing to intracerebral inoculation; no growth of visible bacteria occurred on culture. The experimental animals were killed when moribund, or obtained immediately after death, and their nervous systems subjected to full examination by neuropathological methods.
Lesions in the Rabbit

After Intradermal, Subcutaneous and Intramuscular Inoculation.—Locally, after 12 hours, there was a lively inflammatory response indistinguishable from that following the introduction of normal brain emulsion similarly prepared; polymorphonuclear leucocytes, lymphocytes and a few macrophages were present, but no cellular inclusions were seen. From 16 hours onward changes became progressively more marked and greatly surpassed those due to normal brain, which gradually subsided. Occasional inclusions (see below) were present in connective tissue and capillary endothelial nuclei. At 24 to 30 hours cellular infiltration was more intense; large numbers of polymorphonuclear leucocytes, many karyorrhectic or ingested by macrophages, marked the centre of the lesion, while at the periphery lymphocytes and eosinophils were numerous. Muscle fibres stained poorly, with diminished striation. Inclusions were rather more numerous. From 40 hours onwards extensive necrosis of muscle or connective tissue was evident; even where a minimal amount of virus had been introduced by scarification, a tiny necrotic focus could be found in the subepithelial tissues. Necrosis was thus not dependent upon the trauma of biting and scratching which followed later. Inclusions, though not numerous, could now be detected also in lymphocytes, macrophages, epidermal cells bordering a scratch, and occasionally in sarcolemmal nuclei. The local lymph glands were acutely inflamed.

The finest nerve twigs and nerve endings participated, of course, in this local inflammation. By the 40th hour definite signs of inflammation were visible in the nerve leaving the inoculated area. Lymphocytes, a few polymorphonuclear leucocytes and occasional macrophages occurred in the connective tissue sheath, around small vessels and in small foci between the nerve fibres. Careful search revealed scanty inclusions in the nuclei of the sheath of Schwann. During the period of the developed disease similar changes were sometimes found in the upper part of the sciatic nerve (after inoculation into the calf); lower down, though variable in degree, they were now much more marked, and their development was possibly assisted by trauma and secondary infection.

During the incubation period no changes were discernible in the spinal ganglia and segments of the spinal cord corresponding to the site of inoculation. About the time when itching commenced, i.e. 1 to 2 hours after virus could first be detected in the ganglia, early lesions affecting a few cells were apparent. In animals surviving a further 10 or 12 hours, nearly every cell of the corresponding ganglia was affected (Fig. 2) and lesions had appeared on the opposite side. The sequence of events was best seen in sections stained with phloxin-methylene blue after sublimate-formol or Zenker-formol fixation. In brief, oxychromatic degeneration of the nuclei resulted in the formation of intranuclear inclusions of the general type of those in herpes, yellow fever, etc.; cytoplasmic degeneration and necrosis followed.

The earliest changes, in the form of definite increase of oxyphilic material grouped in deep red, granular aggregates around the nucleolus, were met with in
ganglion cells exhibiting normal contour of nucleus and body and well preserved nucleolus and Nissl substance; these masses were connected by fine threads with similar smaller masses near the nuclear membrane, but most of the nucleus was empty (Fig. 1 B). Rather later the nucleolus disappeared (Figs. 1 C, and 3). In two cases at an early stage considerable enlargement occurred in some nucleoli, chiefly of the acidophilic constituent; this appearance was not constant. The nucleus might now become completely filled with pale pink, finely particulate material surrounding small, deep red aggregates and sometimes fragments of the basophilic part of the nucleolus; most of the scanty basophilic chromatin was, however, marginated on the nuclear membrane (Fig. 1 D and E, Fig. 3). In a few cells the included material was less definitely acidophilic and assumed a mauve tint; sometimes, also, it appeared homogeneous rather than particulate. In the rabbit the former variation occurred only in the spinal ganglia.1 Up to this point the Nissl substance, Golgi net, neurofibrils and mitochondria remained intact, but as the nucleus became completely filled with acidophilic substance they underwent disintegration; the mitochondria persisted in a swollen condition, and the fragmenting Golgi apparatus was visible for some time after the Nissl substance had disappeared. Progressive shrinkage of the nucleus, wrinkling and fading of the nuclear membrane and increasing acidophilia of the cytoplasm now heralded complete destruction of the cell (Fig. 1 F). Meanwhile rather similar, but often more obviously granular masses appeared in the nuclei of the capsule cells; here nuclear enlargement and margination of basophilic chromatin were often more pronounced, and the inclusion was always clearly acidophilic (Fig. 1 E, F, G, Fig. 3). With exceptionally long survival of the animal (over 12 hours after itching began), evidence of cellular reaction in the form of polymorphonuclear infiltration with commencing lysis of the necrotic nerve cells might be present; a large proportion of the polymorphonuclear leucocytes were karyorrhectic. Occasionally, too, neuronophagia by proliferated capsule cells was seen (Fig. 1 G).

In the posterior horn of the cord similar lesions appeared perhaps slightly later; nerve cells, glial cells of the grey matter and of the posterior root entry zone all were affected. At a very late stage scanty infiltration with polymorphonuclear leucocytes might be seen. In the anterior horns, glial cells showed inclusions much less commonly; only once were inclusions seen in anterior horn nerve cells.

In the posterior nerve roots nuclear changes in the Schwann cells were more common than in the proximal part of the nerve supplying the site of inoculation.

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1 Saguchi (1930) has recently pointed out that the chromophilic state of neurons is characterized by overproduction of "nucleonephelium." Normally acidophilic, this substance may in the chromophilic state of the cell become basophilic and, dissolved in the nuclear juice, impart diffuse basophilia to the whole nucleus. This phenomenon is more common in the guinea pig, in which animal, as will be seen later, a much greater proportion of affected nuclei, even when fixed in Zenker's fluid, fail to show clearly acidophilic inclusions.
Although virus was usually present in small amount, no lesions were apparent in the cord and spinal ganglia at higher and lower levels, or in the brain stem, cerebellum or cerebrum. Their absence from these regions possibly accounts for previous failures to detect lesions in the central nervous system.

**Intracerebral Inoculation.**—At the site of inoculation, hemorrhage with some polymorphonuclear and eosinophil exudation but no massive necrosis was present; with an incubation period of about 28 hours, microglial reaction was very slight. The most prominent lesion was marked infiltration of the overlying meninges with polymorphonuclear leucocytes, lymphocytes, eosinophils and a few macrophages; many of the infiltrating cells were fragmented. With subdural inoculation (or leakage of inoculum into the meninges) meningitis was more intense and widespread; fibrinous exudation and necrosis occurred, the latter especially if the incubation period was slightly prolonged. In these cases inflammatory cells might extend along the perivascular spaces as deeply as the first or second layer of cortical nerve cells. A few polymorphonuclear leucocytes might overflow into the superficial nervous tissue.

Characteristic nuclear changes were found in almost every mesothelial cell of the pia-arachnoid, and in a large proportion of the subpial glial cells; in both types of cell they occurred beyond the limits of meningeal infiltration, and in many regions were the only abnormalities present (Figs. 1 H, and 4). They were seen less often in lymphocytes and macrophages of the meningeal exudate, in capillary endothelium, in adventitial cells of veins and arteries and in ependymal cells. Involvement of nerve cells and deeper glial cells depended largely on their proximity to a surface. Early nuclear inclusions, usually without cytoplasmic alterations, were not infrequent in superficial cortical neurons, in those of the fascia dentata and in Purkinje cells of the cerebellum (Fig. 5), while very superficial nuclei (e.g. the ganglion basale opticum) might show a majority of cells involved; adjacent glial cells suffered similar change. Deeply placed nerve cells, except those immediately adjacent to the site of inoculation, were mostly perfectly preserved, though some of the brain stem nuclei exhibited occasional lesions. Finally, inclusions were sometimes seen in the epithelioid cells of distant nodules due to infection with *Encephalitozoon cuniculi.*

Early inclusions were occasionally present in cells of the Gasserian ganglion. No definite changes were noted in the spinal cord.

**Lesions in Other Organs.**—Pathological changes commonly seen outside the nervous system were petechial hemorrhages in the thymus, and areas of intense congestion, hemorrhage and edema in the lungs. Edema of the lungs might also occur without marked congestion, or be of much more extensive distribution than the sanguineous areas. The hemorrhagic areas varied from spots a millimetre or two in diameter to areas occupying the whole of a lobe or even of several lobes; they were frequently present in animals dying of the disease, though probably not so frequently as in Shope's earlier cases.

When considered in relation to the virus content of the organ, the microscopical
changes in the lungs were somewhat confusing. In the first place the lung might appear normal, and yet on occasion contained virus. Secondly, in the absence of any macroscopic abnormality, the alveolar walls over large or small areas might show a marked excess of polymorphonuclear leucocytes; occasionally in association with this there occurred a few foci in which the alveolar epithelium had proliferated and undergone desquamation to mingle with extravasated polymorphonuclear leucocytes, often karyorrhectic. In one such case virus was not detected in the organ; in a second (infected with Aujeszky virus), not only was virus present, but occasional nuclear inclusions were found in the epithelial cells. (The latter observation was not repeated in a large series of lungs.) Thirdly, great congestion and alveolar hemorrhages, with or without serous or serofibrinous exudate, often existed in the absence of polymorphonuclear excess; again virus might or might not be present. Fourthly, combinations of these appearances obtained, once more with the inconstant presence of small quantities of virus; numerous polymorphonuclear leucocytes passed out into the exudate, which in the most marked instances completely filled the alveoli and small bronchi of the affected areas. The conclusion that some of the animals had, by dying, escaped pneumonia seemed unavoidable, but the exact rôle played by the virus was uncertain. More especially was this the case since in a few control animals peribronchial cuffing and even some leucocytic bronchial exudate were encountered, indicating a degree of spontaneous respiratory infection.

To summarize, in the rabbit subcutaneous, intradermal or intramuscular inoculation leads to local inflammation and necrosis followed by ascending infection of the corresponding peripheral nerve. With the onset of itching, nerve cells in the spinal ganglia and posterior horn of segments of the spinal cord corresponding to the site of inoculation undergo degeneration; acidophilic material accumulates in the nucleus to produce an inclusion of the type seen in herpetic encephalitis, cytoplasmic degeneration follows, and ultimately necrosis occurs. Similar lesions appear later in the posterior horn and spinal ganglia of the opposite side, and occasionally with long duration of symptoms in the anterior horns, but not at higher or lower levels of the nervous axis. The nerve cell degeneration is primary, and cellular reaction occurs only during the last stages of the malady. Nuclear inclusions are found also in a variety of cells in the local lesion, in sheath of

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2 These remarks refer chiefly to experiments with the Iowa strain of virus which, in contradistinction to the Hungarian strain, does not appear in the blood in any quantity, and is inconstanty present in the lungs, spleen, etc. Even after intravenous inoculation this statement holds good.
Schwann cells of the peripheral nerve and nerve roots, in capsule cells of the spinal ganglia and in glial cells of the grey and white matter of the spinal cord.

Intracerebral inoculation is followed by the development of nuclear inclusions in mesothelial cells of the pia-arachnoid, in subpial glial cells and in superficially placed nerve cells. Though a variable degree of meningeal infiltration obtains, the nerve and glial cell lesions are clearly primary, and in places occur in the absence of any cellular reaction.

With inoculation by any route, at autopsy hemorrhages are found with some frequency in the thymus, and congestion, hemorrhage or edema, or all three changes, in the lungs. In the absence of macroscopic alterations in the lungs, the alveolar walls may exhibit marked excess of leucocytes, sometimes associated with focal proliferation of the alveolar epithelium. Virus may be present in macroscopically normal, and absent from congested and edematous lungs. Occasionally characteristic nuclear inclusions are present in proliferating alveolar epithelium.

Lesions in the Guinea Pig

Subcutaneously inoculated animals alone have been examined. Morbid changes in the nervous system are essentially the same as in rabbits, with minor differences indicative perhaps of a slightly greater degree of resistance. The visceral changes are similar to those in the rabbit.

Nuclear changes in the spinal ganglion cells developed rather more slowly. Their intensity was not wholly dependent on the duration of symptoms; in animals surviving 24 and 27 hours respectively from the onset of itching, ganglion cell destruction had progressed no further than in rabbits or other guinea pigs dying in 8 hours, and was still unilateral. A greater proportion of cells showed inclusions typical except that they were not definitely acidophilic. Capsule cells contained inclusions less frequently. Cellular reaction and neuronophagia were more pronounced; the former was, however, very variable in amount. In the most marked instance, large numbers of polymorphonuclear leucocytes, many fragmented, with some eosinophils and lymphocytes infiltrated the spinal ganglia, posterior nerve roots and posterior root entry zone; fewer were present in the tip of the posterior horn, posterior and lateral columns, and the perivascular spaces of grey and white matter. Early microglial reaction might be evident.
In the anterior horns chromatolysis and swelling of motor neurons was the rule; on occasion vacuolation also obtained. In one animal in which symptoms lasted for 56 hours, specific nuclear inclusions were present in many anterior horn nerve cells (Fig. 6), in glial cells along the line of exit of the anterior roots and in fewer numbers in the white matter generally. In cases of similarly protracted duration specific nuclear changes might exist also in the brain stem.

Lesions in the Monkey

Three monkeys (M. rhesus) died within 6 to 9 days of the intracerebral inoculation of Iowa virus; another was infected with the Aujeszky virus. The clinical and experimental data will be considered in a future paper. A fifth monkey died of pulmonary tuberculosis towards the end of the incubation period (4th day). The histological picture differed in important particulars from that in rodents.

The lesion in the monkey consisted in widespread, primary degeneration of nerve cells, which showed various changes accompanied by increase of intranuclear acidophilic material, or appeared as shrunken, eosinophilic, necrotic structures with or without pyknotic or fragmented nuclear remains (Fig. 7). Similar changes accompanied by clasmatodendrosis occurred in fewer neuroglial cells, while others showed some enlargement of the cell body. In many regions no other changes were present. Where from greater severity, or, as experience with the 4 day animal suggested, from longer duration of the infective process, a larger proportion of nerve cells was involved, early diffuse microglial reaction and occasional small focal collections of these cells were observed. With still more intense lesions a few polymorphonuclear leucocytes, often fragmented, appeared in the nervous tissue. In such areas a proportion of the neurons exhibited degenerative changes (swelling, solution of the Nissl substance, vacuolation, shrinkage and basophilic impregnation, impregnation of the pericellular Golgi net, etc.) without specific nuclear changes. It could not be decided whether all the necrotic elements had passed through an inclusion-bearing stage or not. Evidence of neuronophagia was occasionally present.

Stained with phloxin-methylene blue the nerve cell inclusions appeared as (a) aggregates of comparatively few, coarse, pale pink granules or of many finer granules, (b) irregular, deeper pink masses like those described in the rabbit, (c) multiple deep pink spherules recalling the bodies in experimental poliomyelitis (Covell, 1930, Hurst, 1931) and, to a less extent, those in Borna disease. Combinations of these were encountered. Their formation was accompanied by early fragmentation of the nucleolus and margination of the basophil chromatin. Glial cell inclusions were less common and mainly of the first variety; they were present chiefly in the grey matter, and only rarely in neuroglia or oligodendroglia of the white matter or in ependymal cells. Inclusions were never, as in the rabbit, present in mesodermal elements of the vessels or meninges.

Meningeal infiltration with polymorphonuclear leucocytes and lymphocytes was intense only over the site of inoculation and more marked over the remainder of the
inoculated hemisphere than on the opposite side, where it might be wholly wanting. It was much less pronounced in passage animals than in the monkeys infected with rabbit brain. Only once did the infiltration extend to the brain stem and upper part of the spinal cord. Where the process was less marked, the cells collected chiefly in the walls of the meningeal veins and in the depths of the Sylvian fissure, superior temporal sulcus and cingular sulcus.

Save for slight polymorphonuclear and lymphocytic cuffing in severely affected cortical areas, perivascular cuffing was marked in only two situations, in the tissues around the third ventricle along the vessels entering the anterior and posterior perforated spaces, and in the grey matter surrounding the ventricular system of the brain stem. Occasionally a few infiltrated vessels occurred in the white matter of the hemispheres. In one animal perivascular infiltration was wholly wanting.

Distribution of Lesions.—At the site of inoculation, edema, limited necrosis and hemorrhage were accompanied by reparative changes without specific inclusions in the newly formed tissue. Lesions in the cortex were not determined primarily by adjacency to the inoculated area, and varying degrees of change obtained in gyri equidistant from this.

No major subdivision of the cerebral cortex appeared immune from attack, and there was no tendency to selective involvement of any cortical layer. On the whole, changes were much more marked at the frontal and occipital poles, and at the vertex than at the base: once, however, the vertical cortex was most severely damaged. At the vertex, lesions were usually most intense in the vicinity of the cingular sulcus, on the lateral surface in the island of Reil and neighbouring cortex. Here the proportion of cells affected commonly reached 20–50 per cent of the total. In all but one animal the basal surface of the frontal lobes and the anterior temporal cortex (superior and middle temporal gyri) were most severely damaged; just posterior to the temporal pole from 80–100 per cent of the nerve cells had perished or were severely injured. In two animals extensive destruction continued into the pyriform area, cornu Ammonis and dentate gyrus, with almost complete disorganization of structure. The findings in the 4 day animal suggested an earlier affection of the lower frontal and temporal than of the remaining cortex. Lesions were more intense on the inoculated side.

Compared with the cerebral cortex, the optic thalamus and globus pallidus were lightly affected, while the caudate nucleus and putamen escaped damage or exhibited only mild changes. The hypothalamic region and tissues around the third ventricle were affected with moderate severity; here perivascular infiltration and focal microglial proliferation, relative to the amount of nerve cell involvement, were more marked than elsewhere.

In the midbrain and pons perivascular infiltration was marked in the grey matter around the aqueduct of Sylvius and in the floor of the fourth ventricle. Isolated nerve cells, or occasionally the majority of cells in a particular nucleus, showed specific changes with focal microglial reaction in the surrounding tissue. Nerve cells not showing nuclear inclusions were always perfectly preserved. Changes
were distinctly more frequent in the dorsal than in the ventral regions. Similar lesions occurred in diminishing intensity through the medulla to the upper cervical segments of the cord. The cerebellum, lower cord, Gasserian and spinal ganglia and sciatic nerves were intact.

The other viscera showed no definite abnormality, except the salivary glands in which a certain number of acini manifested the results of marked secretory over-activity.

In the monkey, therefore, the essential lesion following intracerebral inoculation is widespread, primary degeneration of nerve and glial cells, chiefly in the cerebral cortex (of both sides), and attaining its maximum in the first and second temporal gyri, pyriform area, cornu Ammonis and island of Reil. The absence of nuclear inclusions from mesodermal elements in the meninges and elsewhere suggests that in this animal the attack of the virus is directed solely against ectodermal structures, which is far from being the case in the rabbit. Cellular reaction in the cerebral cortex is clearly secondary to the nerve cell necrosis. Perivascular infiltration is most marked in the grey matter around the third and fourth ventricles, where nerve cell damage is relatively slight. Meningeal infiltration may be wholly absent from the uninoculated hemisphere, and is much less pronounced in passage animals than in those infected with foreign (rabbit) nervous tissue; evidently in the latter non-specific factors play a part in its production. No significant lesions develop in other viscera.

*Lesions in the Cow*

From the scanty material available it appears that lesions in the cow approximate more closely to those in the monkey than to those in the rabbit.

Through the courtesy of Dr. Shope, a few pieces of brain from the cow furnishing the original strain of mad itch virus were available for examination.

At all levels of the cord, brain stem and basal ganglia, and in one part of the cerebral cortex, pathological changes were present. Perivascular infiltration, of moderate degree, with large and small lymphocytes and occasional large mononuclears was associated with areas of semidiffuse microglial proliferation in the grey matter; isolated polymorphonuclear leucocytes and lymphocytes were present in the nervous substance. In the white matter smaller compact microglial foci were of rare occurrence. The majority of nerve cells were normal or in a condition of mild chromatolysis; a few were acutely necrotic or, very rarely, undergoing neu-
ronophagia. In a minority nuclear inclusions were visible; the condition of the material was not ideal for fine cytological study. At one level of the medulla scanty meningeal infiltration with mononuclear elements was noted.

Lesions in the Pig

Intracerebral Inoculation.—In three pigs dying 3 1/2, 4 and 5 days after intracerebral inoculation the histological appearances differed considerably from those hitherto described. Vascular and interstitial changes were most conspicuous, and damage to nerve cells was relatively slight.

Two animals received rabbit brain as inoculum; in the brain of one of these meningitis was everywhere intense. In the second, and in that of an animal infected with pig brain, meningitis though fairly generalized was definitely less marked; it was severe only over the base and upper medial surfaces of the hemispheres and over the cerebellum. In all, the infiltrating cells were largely lymphocytes with a notable number of eosinophils, a few large mononuclears and rare polymorphonuclear leucocytes and plasma cells.

In the nervous tissue, the congested vessels of both grey and white matter frequently showed cuffing with a single layer of similar cells. In some parts of the cortex, particularly in the areas of most intense meningitis, perivascular infiltration was more marked and often associated with diffuse proliferation of microglia in the surrounding tissues (Fig. 8); microglial proliferation and cellular infiltration also occurred in the superficial cortical zone immediately beneath the pia-arachnoid. Some large foci of unusually densely packed microglial cells, with lymphocytes and eosinophils, occurred independently of the vessels; many of these cells were karyorrhectic. In stained sections such foci were readily visible to the naked eye. Smaller foci, more comparable with those commonly seen in virus infections, occurred occasionally in the white matter. Identical vascular and interstitial changes obtained around the whole ventricular system and in the choroid plexuses.

By comparison with the foregoing the nerve cell changes were slight, and relative to those in the monkey, insignificant. Only in the densest tissue foci did some neurons manifest severe degenerative phenomena culminating in death and neuronophagia. Elsewhere they exhibited mild swelling and chromatolysis of such general distribution as to suggest the uniform action of a toxin rather than that of a virus. Neuroglial nuclei were often swollen and hydropic.

The surprising feature in the pig was, however, the complete absence of typical nuclear inclusions such as were found in all other animals. In some swollen glial nuclei scanty oxyphilic material was sometimes present. It was not possible to deny that this might have been the homologue of the typical inclusions present in other species, but it could hardly be demonstrated as a convincing example of this type of nuclear degeneration.

Distribution of Lesions.—Edema was pronounced in a wide zone around the site of inoculation. The relatively slight cortical changes were as marked in the occip-
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In the frontal region there was the olfactory cortex was most heavily involved. The caudate nucleus, putamen and optic thalamus suffered less severely than the globus pallidus. The hypothalamic region and tissues around the third ventricle were markedly affected.

In the brain stem the dorsal region suffered more than the ventral; in both, extensive areas of diffuse microgliosis or more circumscribed foci might be observed. In the cerebellum similar foci involved all layers of the cortex. In the spinal cord foci occurred in both grey and white matter. Finally, in one case, mononuclear and eosinophilic infiltration extended into the optic nerve, and in two cases, along the fifth nerve as far as the Gasserian ganglion.

Subcutaneous Inoculation.—The pig differs from other animals in that, after subcutaneous inoculation, it passes through a mild febrile illness unaccompanied by itching, and only rarely develops nervous symptoms (Shope, 1931, 1932). Yet the local lesion is almost as pronounced as in other animals, and definite changes are detectable in the nervous system.

On the sixth day after inoculation in a pig recovering without having shown nervous symptoms, foci of necrosis in the corium were associated with extensive edema, fibrinous exudate and polymorphonuclear infiltration; around this area was a wide zone in which mononuclears gradually replaced polymorphonuclear leucocytes as the predominant cell type. The vessels were surrounded by many layers of similar cells; the large arteries and veins exhibited pronounced inflammation with cellular infiltration of all their coats and considerable proliferation of their endothelial linings (Fig. 9). The nerve bundles were often buried in cellular exudate and inflammatory cells lay between the individual fibres. Again no definite nuclear inclusions were demonstrated.

The corresponding spinal ganglia showed heavy polymorphonuclear and mononuclear infiltration, together with ganglion cell degeneration sometimes culminating in acute necrosis with neuronophagy by capsule cells or lysis by polymorphonuclear leucocytes. Infiltration continued along the nerve root into the cord, where microglial foci in grey and white matter and perivascular infiltration obtained together with mild chromatolysis of the nerve cells. At other levels of the cord, in the brain stem and in the cerebral hemispheres many vessels were cuffed with a single layer of lymphocytes, and occasionally foci of inflammatory cells occurred in the meninges. Meningeal infiltration was rather more marked over the cerebellum, with some microglial reaction in the nervous tissue immediately subjacent. Everywhere in the brain the nerve cells were well preserved.

Lesions in Other Organs.—In one of the two intracerebrally inoculated pigs in which the general viscera were examined, the cervical lymph glands were enlarged and showed macroscopic hemorrhages at the periphery. Microscopically, many eosinophils infiltrated the lymphoid nodules, particularly at the periphery of the
gland, and numerous extravasations of blood were present in the relatively acellular zone between the lymphoid tissue and the capsule; the capillary endothelium was obviously swollen. Many eosinophils were present in the spleen, again mainly in the lymphoid nodules. In the second pig, numerous polymorphonuclear leucocytes and eosinophils occupied the acellular zone; no hemorrhage or endothelial swelling was noticed.

In the subcutaneously injected animal, frank necrosis with considerable focal infiltration of polymorphonuclear leucocytes occurred in the same situation and in the processes of poorly cellular tissue penetrating towards the centre of the gland. In this animal foci of necrosis obtained also in the lymphoid nodules of the spleen. The heart showed acute myocarditis; the muscle bundles were separated by edema and numerous polymorphonuclear leucocytes were present in the tissue.

In the pig after intracerebral inoculation, meningeal, perivascular and tissue infiltration is far more pronounced than in the rabbit, cow or monkey. On the other hand, nerve cell damage is comparatively slight. Surprisingly enough no definite intranuclear inclusions are demonstrable.

After subcutaneous injection local inflammation and necrosis occur. Heavy cellular infiltration in the corresponding spinal ganglia is accompanied by nerve cell degeneration and occasionally necrosis. No inclusions are seen in either situation. In the spinal cord lesions are less severe. In the brain mild perivascular infiltration is evident.

The involvement of the lymphatic system constitutes a further difference in reaction in the pig.

Symptomatology of the Disease in the Rabbit Considered in the Light of Histological and Experimental Findings

Cause of Itching.—As already mentioned, 24 hours after subcutaneous or intramuscular injection, inflammation is already well advanced locally, and inclusions are present in connective tissue and other cells. Within 40 hours necrosis is evident, and lesions are extending proximally along the peripheral nerve leaving the damaged area. Itching does not begin for 50 hours or more (with the Iowa virus). During the period of irritation a saline extract of the local lesion inoculated into a fresh animal evokes no immediate symptoms. If inoculation is practiced in the calf muscles, provided that care be taken to prevent leakage into the overlying skin, itching is not local but is referred to the terminal distribution of the sciatic nerve, and perhaps to the pos-
terior part of the flank of the affected side. These facts suggest that the symptom is not due to irritation of nerve endings at the site of inoculation.

On the other hand, after subcutaneous or intramuscular injection, itching commences about the time when lesions can first be demonstrated in the corresponding spinal ganglia and segments of the spinal cord, and about 2–3 hours after virus can first be detected there. Although virus can later be detected in smaller amount at higher levels of the cord, no lesions are ordinarily present at these levels, and no itching occurs in the corresponding peripheral area. In about half the cases, intravenously inoculated animals itch at some point (unrelated to the site of venepuncture), and lesions are then present in the corresponding spinal ganglia. After intracerebral injection early lesions are occasionally present in the Gasserian ganglion; once an animal so inoculated scratched the face violently, but unfortunately the ganglion was not examined. In short, very early lesions, or scanty lesions affecting only occasional cells, may appear in spinal ganglia or cord without signs of peripheral irritation; otherwise there seems to be perfect correlation between the two phenomena.

Cause of Respiratory Symptoms and of Death.—Towards the end of the illness following subcutaneous or intramuscular injection, breathing is usually rapid and shallow, and death takes place from respiratory failure. After intravenous inoculation about half the animals do not itch; these may manifest unrest, convulsions, general subsultus, and rapid or laboured breathing, or may die suddenly without symptoms having been observed. Shope records similar lack of symptoms with nasal inoculation. Visible pathological lesions in the lungs are by no means always present when definite respiratory distress has been noted.

Now virus is invariably present in the medulla at the time of death, though usually in low concentration, giving a long incubation period in the passage animal. It has previously been remarked that no lesions are demonstrable here. In those intravenously inoculated animals which do not itch, no lesions are found in the central nervous system. These facts suggest that the virus may have reached the medulla only shortly before death, a suggestion supported by the data given in Table I. In the guinea pig symptoms may exceptionally en-
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duration for as long as 30–50 hours; then early changes are present in the medulla. We may probably infer that the medullary centres of the rabbit are peculiarly susceptible to the virus, and that only a minimal amount acting for a short time is necessary to arrest their function. Under suitable conditions, therefore, e.g. after intravenous inoculation, death may immediately follow infection of the medulla before specific lesions have time to develop. On these grounds it seems that the name "infectious bulbar paralysis" introduced by Marek (1904) ideally designates the malady in rabbits.

DISCUSSION

The virus of pseudorabies is capable of exciting lesions in a wide range of animal hosts; in all it exhibits definite predilection for the nervous system. Yet while it may be grouped with the neurotropic viruses, it does not behave as a strict neurotrophe since in some animals...
at least it produces specific changes in non-nervous tissues. Thus, in the rabbit, inclusions may be found in a great variety of cells, a probable indication of ability on the part of the virus to parasitize cells derived from any embryonic layer. In these respects the organism bears considerable resemblance to the herpes virus.

Comparative study of the lesions evoked in different animal species affords a striking example of differences in host reaction to a single infective agent. In the rodent, the monkey and the cow, nerve cell degeneration is clearly primary and independent of vascular and interstitial inflammation, though in all reactive changes occur sooner or later. In the cow and the monkey nuclear inclusions do not occur, as in the rabbit, in other cells besides nerve and glial cells. In the pig the meningeal and vascular reaction is dominant. In this animal, in the absence of clear cytological evidence of direct virus attack on the comparatively intact nerve cells of the brain, it is difficult to assess the relative parts played by the virus and by impaired nutrition consequent upon these other factors. The amount of nerve cell degeneration in the spinal ganglia of the subcutaneously inoculated pig was undoubtedly too great to be attributed solely to vascular and interstitial lesions; again, however, no nuclear inclusions existed as a mark of the activity of the virus. (Whether the porcine cell is capable of reacting in the particular manner necessary to develop intranuclear inclusions is not known. There is no a priori reason to suppose that it is not, but such bodies have never been convincingly demonstrated in the pig.) In the pig, too, the obvious involvement of the lymphatic system constitutes a further difference in reaction to the infection. It would appear that, from clinical and pathological viewpoints alike, pseudorabies in the pig is very different from the disease in most animal species.

During the incubation period of the disease following inoculation into the leg of the rabbit, the extending chain of nuclear inclusions along the branches of the sciatic nerve indicates a route followed by the virus. Moreover, from the presence of these inclusions in the cells of the sheath of Schwann, and the variable degree of inflammatory

\*In a future publication evidence will be presented that the virus can spread also by other channels; the distribution of lesions described above leaves no doubt, however, that the nervous route is the most favourable.
reaction in the neural connective tissue sheath, it appears likely that the virus exists interstitially in the nerve and is not only passing along the axis-cylinders. On the other hand, at a time when early lesions appear in the corresponding spinal ganglia, and slightly later when inclusions are becoming relatively numerous in the posterior nerve root between the ganglion and cord, inclusions in the upper part of the sciatic nerve are still very few in number; a possible explanation, for which there is at present no supporting evidence, is that virus also travels, and travels more quickly, by the axis-cylinders and becomes liberated interstitially at the level of the ganglion. Although in cases of protracted duration, and therefore more especially in the guinea pig, nuclear inclusions may be found in the anterior horn nerve and glial cells, their appearance here follows by a definite interval their development in the spinal ganglia, posterior roots and posterior horn. This may indicate a greater susceptibility of the sensory neurons or, if the virus can traverse the axis-cylinders, that the direction of the nervous impulses may affect its speed of progression. After intracerebral inoculation in the rabbit, the distribution of inclusions in nerve and glial cells is largely explicable by assuming a meningeal spread of the infection. In the monkey too, the relative severity of the lesions closely parallels the quantitative distribution of dyestuffs injected into the cisterna magna (Hurst, 1932); if, however, in this animal the virus spreads by the meninges, it does not necessarily leave any trace, since in the uninoculated hemisphere severe nerve cell destruction may occur in the temporal lobe, etc., in the complete absence of meningeal infiltration. Reference has already been made to the prominence of meningitis in the pig.

Intracerebral lesions in the rabbit differ sufficiently from those of herpes to permit of their differentiation. In herpes, meningitis may be pronounced, but the nuclear inclusions in mesodermal elements are much less numerous, and the subpial glial cells are not affected with the same frequency or over as wide an area as in pseudorabies. Eosinophils and lymphocytes play (at the time of death) a more, and polymorphonuclear leucocytes a less important part in the infiltration. Involvement of the nervous tissues is more marked, with abundant necrosis of nerve cells and much more cellular infiltration around the vessels and in the tissues. Nuclear inclusions, while of the same gen-
eral type as in pseudorabies, tend to be more coarsely granular and more often fill and distend the nucleus.

SUMMARY

The histology of pseudorabies differs materially in various animal species. In the rabbit, subcutaneous, intradermal or intramuscular inoculation leads to local inflammation and necrosis. The infection ascends the peripheral nerve (possibly both interstitially and by the axis-cylinders) to the corresponding spinal ganglia and segments of the spinal cord, where primary degeneration of nerve and glial cells takes place. The nerve cell changes are probably responsible for the cardinal symptom of the disease, itching. Death ensues soon after virus reaches the medulla, before visible changes have been produced here. Intracerebral inoculation is followed by characteristic lesions in the meninges, in subpial glial cells and in superficially placed nerve cells. Morbid changes in the lungs are not necessarily related to the presence of virus, but specific lesions may be present. Intranuclear inclusions bearing some resemblance to those in herpetic encephalitis, yellow fever, etc., occur in cells derived from all embryonic layers.

The disease in the guinea pig resembles closely that in the rabbit and is modified only by the slightly greater resistance of the animal.

In the monkey after intracerebral inoculation, widespread degeneration and necrosis of cortical nerve cells are accompanied by the appearance of specific nuclear alterations in nerve and glial cells, but not in cells of mesodermal origin. No lesions are found in other viscera.

In the spontaneous disease in the cow lesions approximate more closely to those in the monkey than to those in the rabbit.

In the pig vascular and interstitial lesions predominate, nerve cell degeneration is relatively slight and typical inclusions are not observed. These differences probably explain the benign course of the malady following subcutaneous inoculation in this animal. The lymphatic system, too, participates in the reaction to the virus.

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EXPLANATION OF PLATES

PLATE 20

FIG. 1. A. Normal spinal ganglion cell of rabbit. The nucleus contains a prominent nucleolus and a variable amount of granular, weakly acidophilic material enclosing one or more rather definite, more strongly acidophilic masses ("nucleonephellum" of Saguchi). The peripheral cytoplasmic zone free from Nissl bodies is a normal feature.

B-G. Successive stages of degeneration of spinal ganglion cells following subcutaneous inoculation of pseudorabies virus; acidophilic nuclear degeneration leads to formation of intranuclear inclusions of the type seen in herpetic encephalitis.

B. Definite increase of acidophilic intranuclear material grouped chiefly around the nucleolus leaving most of the nucleus empty. The Nissl substance is perfectly preserved.

C. More pronounced increase of acidophilic material. The nucleolus has disappeared. The Nissl bodies are still normal.

D. The nucleus is filled with finely granular, feebly acidophilic material enclosing more strongly acidophilic aggregates. The nucleolus has disappeared and the Nissl bodies are disappearing.

E. The nucleus is completely filled with acidophilic material; fragments of the nucleolus are present. The Nissl substance has entirely disappeared. Inclusions are seen in the nuclei of some capsule cells.

F. Acidophilia of the cytoplasm and a shrunken nucleus indicate death of the nerve cell. Inclusions are present in some of the capsule cells.

G. Neuronophagia by proliferated capsule cells, some bearing nuclear inclusions, an unusual picture in the rabbit. A few karyorrhectic polymorphonuclear leukocytes are present.

H. Nuclear inclusions in mesothelial cells of the pia-arachnoid and subpial glial cells following intracerebral inoculation in the rabbit. Pyknotic remains of infiltrating leukocytes are also seen.

Drawn from preparations stained with phloxin-methylene blue after sublimate-formol or Zenker-formol fixation.
PLATE 21

FIG. 2. Spinal ganglion of rabbit showing great majority of nerve cells in various stages of the degeneration described in the text. The darker cells are necrotic. Sublimate-formol: phloxin-methylene blue. × 172.

FIG. 3. Early changes in one spinal ganglion cell of rabbit (above): later stage (below) with intranuclear inclusion in a capsule cell. Sublimate-formol: phloxin-methylene blue. × 1000.


FIG. 5. Inclusions in Purkinje cell of cerebellum and adjacent glial cells. Intracerebral inoculation in rabbit. Sublimate-formol: phloxin-methylene blue. × 1165.

PLATE 22

FIG. 6. Nuclear inclusion in anterior horn cells of guinea pig (above and to left). Zenker-formol: iron alum hematoxylin and eosin. × 455.


(Hurst: Studies on pseudorabies. 1)