INVASION OF THE MOUSE NERVOUS SYSTEM BY SCRAPIE AGENT

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THE demonstration by Sigurdsson (1954) and Sigurdsson and Palsson (1958) of the occurrence of slow virus infections in sheep has been followed by the demonstration that such conditions may occur in other species. Recently Gajdusek, Gibbs and Alpers (1967) have succeeded in transmitting the human disease kuru to chimpanzees and there are reports that scrapie, another slow infection, may emerge when multiple sclerosis (MS) material is inoculated into sheep or mice (Palsson, Pattison and Field, 1965; Field, 1966). Scrapie, indeed, since it can now be readily passed in mice (Chandler, 1961) has become the paradigm of slow virus infections. The transmissible agent of scrapie has a number of remarkable properties such as marked resistance to heat, formalaldehyde (Pattison, 1965*a*, *b*), ultraviolet irradiation (Alper, Haig and Clarke, 1966) and appears to be very small perhaps containing no nucleic acid (Alper *et al.*, 1966; Alper, Cramp, Haig and Clarke, 1967). In view of these remarkable attributes the localization of scrapie agent in the central nervous system after peripheral inoculation has been studied to see in what way it might differ from classical "neurotropic" viruses.

MATERIALS AND METHODS

Mice (Swiss BSVS) of both sexes were inoculated i.m. into the anterior thigh region of the hind limb or s.c. in the foot pad. Others were inoculated in the fore-limb either i.m. in the posterior brachial compartment or s.c. on the dorsum of the paw. A 5th group was inoculated i.p. with the same material. In each case 0·1 ml. of a 10^{-1} sterile saline suspension of mouse adapted scrapie brain was used as inoculum. Individuals from all 3 groups were killed 92, 119, 126 and 131 days after inoculation when they were yet clinically normal. Altogether 34 mice were systematically examined. Brains and spinal cords were fixed in formol ammonium bromide for 36–48 hr. and astrocytes stained by the method of Cajal, since hypertrophy of these cells is an early and reliable sign of the development of scrapie (Hadlow, 1965; Pattison, 1965*b*; Savage and Field, 1965). Coronal sections of the brain (20 μ) were made at hypophyseal level and the spinal cord was sectioned both longitudinally and transversely at various levels.

RESULTS

The investigation arose from the chance observation that mice inoculated intraperitoneally showed well marked hypertrophy of astrocytes in the grey matter of the spinal cord at a time when there was yet no reaction in the brain (Fig. 1). The reaction appeared in the vicinity of the incoming small blood vessels and a striking feature was the clear delimitation of the changes to the grey matter

whilst the astrocytes of the white matter appeared normal. Animals inoculated in the fore limb showed astroglial hypertrophy in the cervical cord whilst the lumbar region was unaffected (Fig. 2a, b). Conversely animals inoculated in the hind limb showed lumbar cord changes whilst the cervical cord remained unaffected. In 2 animals, killed 121 days after inoculation in the hind limb, astroglial hypertrophy was limited to the lumbar ipsilateral ventral horn of grey matter, the opposite anterior horn remaining unaffected (Fig. 3a, b).

DISCUSSION

The earliest sign of reaction in the central nervous system following peripheral inoculation of scrapie infected nervous tissue occurs in the segments of spinal cord associated with the inoculation site. Histological examination of the intervening main nerve trunks has revealed no abnormality. The site of injection thus determines the " settling " of the agent in much the same way as was noted by early workers with poliomyelitis virus and the same problem of route of invasion of the central nervous system is posed. Although scrapie agent has never been found in the blood of an affected animal, the early widespread dissemination in body tissues (Eklund, Kennedy and Hadlow, 1965) makes it nevertheless probable that it does spread by the blood stream. In Eklund's experiments mice were inoculated s.c. (no site mentioned) and the earliest astrocytic response was noted in the spinal cord. More recently Eklund, Kennedy and Hadlow (1967) have inoculated mice s.c. in the left thigh and studied dissemination of the scrapie agent in the various tissues by titration of activity in producing disease on intracerebral inoculation into other mice. They point out that hypertrophy and proliferation of astrocytes was by far the most conspicuous microscopic change observed in sick mice, especially early in the course of the disease. Whilst no extensive histological examination was made, they point out that: " in the few mice examined pronounced astrocytic response was detectable first in the spinal cord, in which all levels were affected. " (Eklund *et. al.*, 1967). The present work analyses the earliest astrocytic response and shows it localized to the neuraxis segments corresponding with the inoculation site whether i.m. or s.c. Such localization may be associated with the stimulation of peripheral inoculation resulting in increased blood supply to the corresponding segments of the neuraxis (German and Trask, 1938; Field, Grayson and Rogers, 1951) or to some other factors as yet unrecognized. Local reaction to the inoculum does not however appear marked, but if " viraemia " occurs within a short time of inoculation, may be sufficient to establish the agent preferentially in the corresponding segments of the neuraxis.

SUMMARY

When mouse adapted scrapie brain is inoculated intramuscularly into the fore- or hind-limb of a mouse the earliest scrapie reaction is found in the corresponding segments of the neuraxis and appear to originate in the ipsilateral grey matter. It is suggested that an early blood dissemination of the scrapie agent occurs and that invasion occurs selectively under the influence of the peripheral stimulation.

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EXPLANATION OF PLATES

FIG. 1.—Longitudinal section through thoracic cord of Mouse (SM/350/3) inoculated i.p. with 0·1 ml. 10^{-12} scrapie mouse brain suspension and killed after 121 days when it appeared clinically normal. White matter on left and grey to right. Small vessel entering the latter. As it approaches the grey matter astrocytes in its vicinity become enlarged, especially their sucker feet which impregnate well. In the grey matter itself there is astroglial hypertrophy. (Normally astrocytes stain only very faintly). Cajal. ×280.

FIG. 2.—Mouse (SM/437/4) inoculated s.c. on dorsum of fore paw and killed after 126 days when clinically normal.

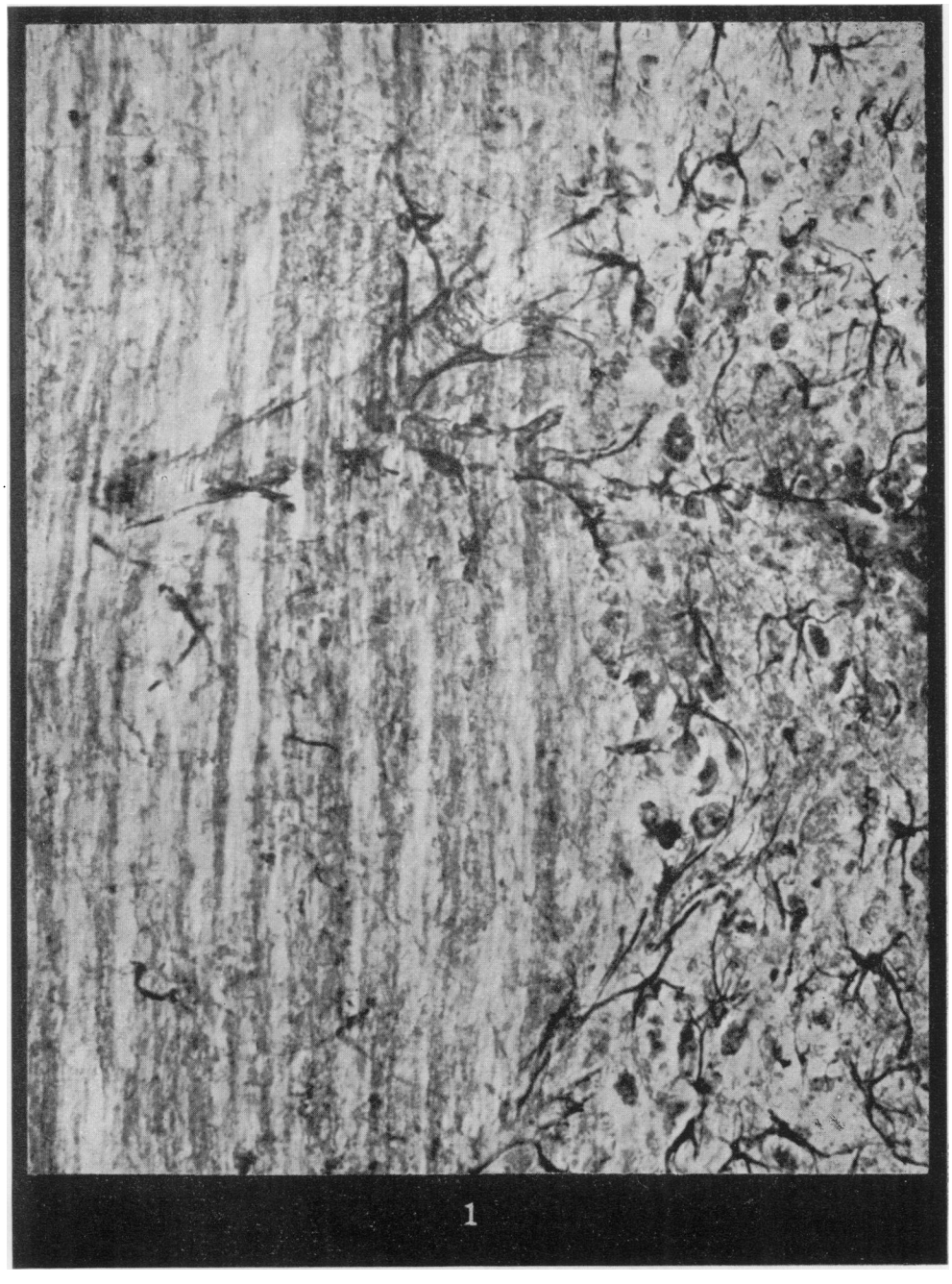
(a) Transverse section through cervical cord showing astrocytic hypertrophy in grey matter.

(b) Transverse section through lumbar cord showing no astrocytic reaction. Cajal. ×55.

FIG. 3.—Mouse (SM/436/5) inoculated s.c. in the left hind foot pad and killed at 126 days when clinically normal.

(a) Lumbar cord showing astroglial hypertrophy in the grey matter on the left side.

(b) Lumbar cord showing non-stimulated astroglia on the right side. Cajal. ×90.



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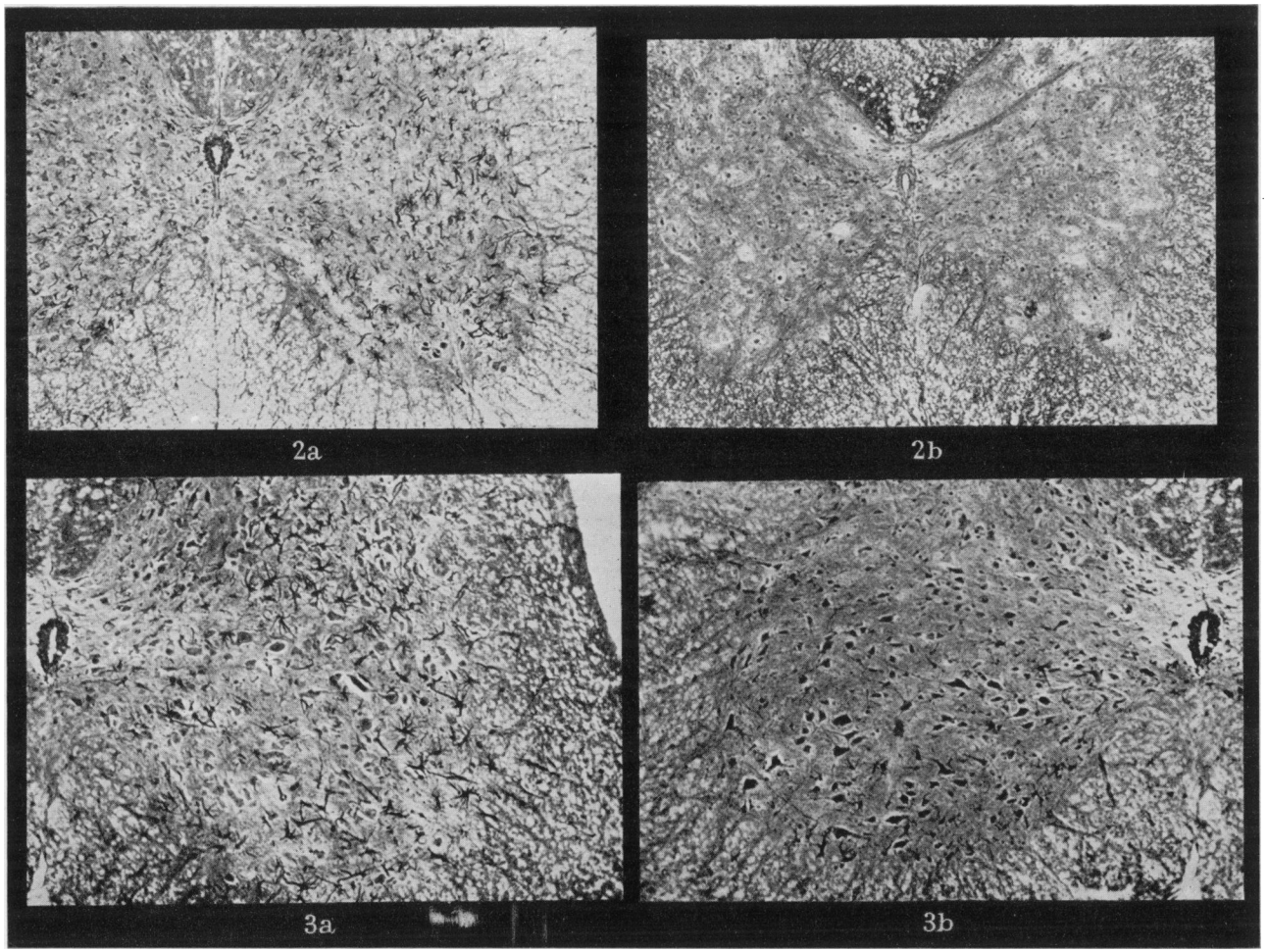

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