SPECIFICITY OF HUMAN BRAIN AND NERVE ANTIBODY AS SHOWN BY IMMUNOFLUORESCENCE MICROSCOPY

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WHILST the injection of brain with Freund's adjuvants leads to the production of experimental " allergic " encephalomyelitis (EAE), the substitution of peripheral nerve results in an experimental " allergic " neuritis (EAN). Considerable species differences exist. The guinea-pig has been found to develop both central and peripheral lesions with either antigen ; about half of the guinea-pigs injected with brain develop, however, lesions limited to the brain alone (Waksman and Adams, 1955 ; 1956). Of guinea-pigs receiving nerve, about one-third develop a purely peripheral disease.

In the course of a fluorescent antibody study of EAE in the guinea-pig (Ridley, 1962), an anti-human brain serum prepared in the rabbit was found to stain myelinated autonomic nerves in a section of adrenal medulla used as a control for brain staining. It was decided, therefore, to investigate further the specificity of this antibody to brain with respect to central and peripheral nervous systems. Conversely, the avidity of central and peripheral nerve myelin for antibody against human peripheral nerve has been examined.

MATERIAL AND METHODS

To prepare antiserum against human cerebral white matter, rabbits of about 2·5 kg. body weight were injected on 3 occasions at monthly intervals with a mixture of white matter and Freund's adjuvants known to be highly encephalitogenic for guinea-pigs. 0·5 ml. (75 mg. white matter) was injected into the pads of both hind feet. Sera were tested at intervals on an Ouchterlony plate and good lines against human brain (as well as kidney) were obtained from 6 weeks onwards—though the rabbits did not show encephalitis until nearly 4 months had elapsed.

To prepare antiserum against human sciatic nerve, the carefully cleaned nerve was emulsified with Freund's adjuvant to make a 40 per cent suspension (Heitmann and Mannweiler, 1957). Considerable difficulty was experienced in making a satisfactory suspension of nerve on account of its connective tissue stroma. One injection of 0·25 ml. into each foot pad sufficed to produce clinical signs at 4 weeks. At this stage the serum showed clearly defined lines on an Ouchterlony plate, when set up against sciatic nerve and human brain suspensions.

Conjugation of serum with fluorescein iso-thiocyanate (Borden Chemical Co., Philadelphia, U.S.A.) was carried out by the methods of Marshall, Eveland and Smith (1958) and Riggs, Loh and Eveland (1960).

Normal guinea-pig brain, spinal cord (with attached dorsal root ganglia), kidney, testis and cervical sympathetic ganglion were cut on a cryostat at 4 μ, fixed in absolute alcohol for 15 min. at room temperature and stained with conjugated serum for 10 min. at 37° in a moist atmosphere. Sections were then washed well in buffered saline and mounted in buffered glycerol. Control sections were stained with a similarly conjugated normal rabbit serum.

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RESULTS

Brain stained well and myelinated fibres showed up brightly. Fig. 1*a* shows a representative section taken coronally through the corpus striatum. Bundles of white fibres are seen cut transversely and amongst them groups of fibres are much more intensely stained than others. No difference of size or staining properties between these fibres could be seen by ordinary histological methods and the appearances suggest immunochemical differences within seemingly homogeneous bundles. An adjacent section stained with conjugated normal rabbit serum did not contain any fibres which stained (Fig. 1b).

The white matter of the spinal cord stained brightly (Fig. 2) and so did the heavily myelinated fibres of the sciatic nerve (Fig. 3). The superior cervical sympathetic ganglion showed well stained fibres but none of the ganglion cells took up the conjugated serum antibody (Fig. 4).

Adrenal gland did not stain but a small bundle of apparently myelinated fibres in the medulla did so (Fig. 5). Kidney and testis were negative.

Thus a rabbit anti-serum against human brain attaches specifically to some, but not all, white fibres in the brain, the white fibres of the cord, sciatic nerve and superior cervical ganglion of the guinea-pig. It did not attach to non-nervous tissues.

The serum prepared against human sciatic nerve had an altogether weaker affinity for nervous tissues. In preparing the following photographs, care was taken to make conditions as far as possible identical with those under which the previous ones were produced (exposure, development time, etc.) so that brightness might be some measure of the avidity of the serum for the tissue in each case.

Brain stained diffusely and non-specifically (Fig. 6). Spinal cord close to incoming dorsal nerve roots stained only moderately well (Fig. 7). The heavily myelinated fibres in the dorsal root ganglion (Fig. 8) and sciatic nerve (Fig. 9) appeared brighter but still much less intensely stained than with antibrain serum.

Thus a rabbit anti-serum against human sciatic nerve attaches specifically to

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EXPLANATION OF PLATES

FIG. 1*a*.—Transverse section through corpus striatum of normal guinea-pig stained with conjugated anti-human brain serum. Note bundles of white fibres stained specifically and amongst them groups of fibres with marked avidity for the antiserum. ×190.

FIG. 1*b*.—Control section stained with conjugated normal rabbit serum. ×190.

FIG. 2.—Transverse section of spinal cord of guinea-pig stained with conjugated antibrain serum. ×190.

FIG. 3.—Longitudinal section of sciatic nerve of guinea-pig stained with conjugated antibrain serum. ×190.

FIG. 4.—Superior cervical sympathetic ganglion of guinea-pig stained with conjugated human antibrain serum. ×190.

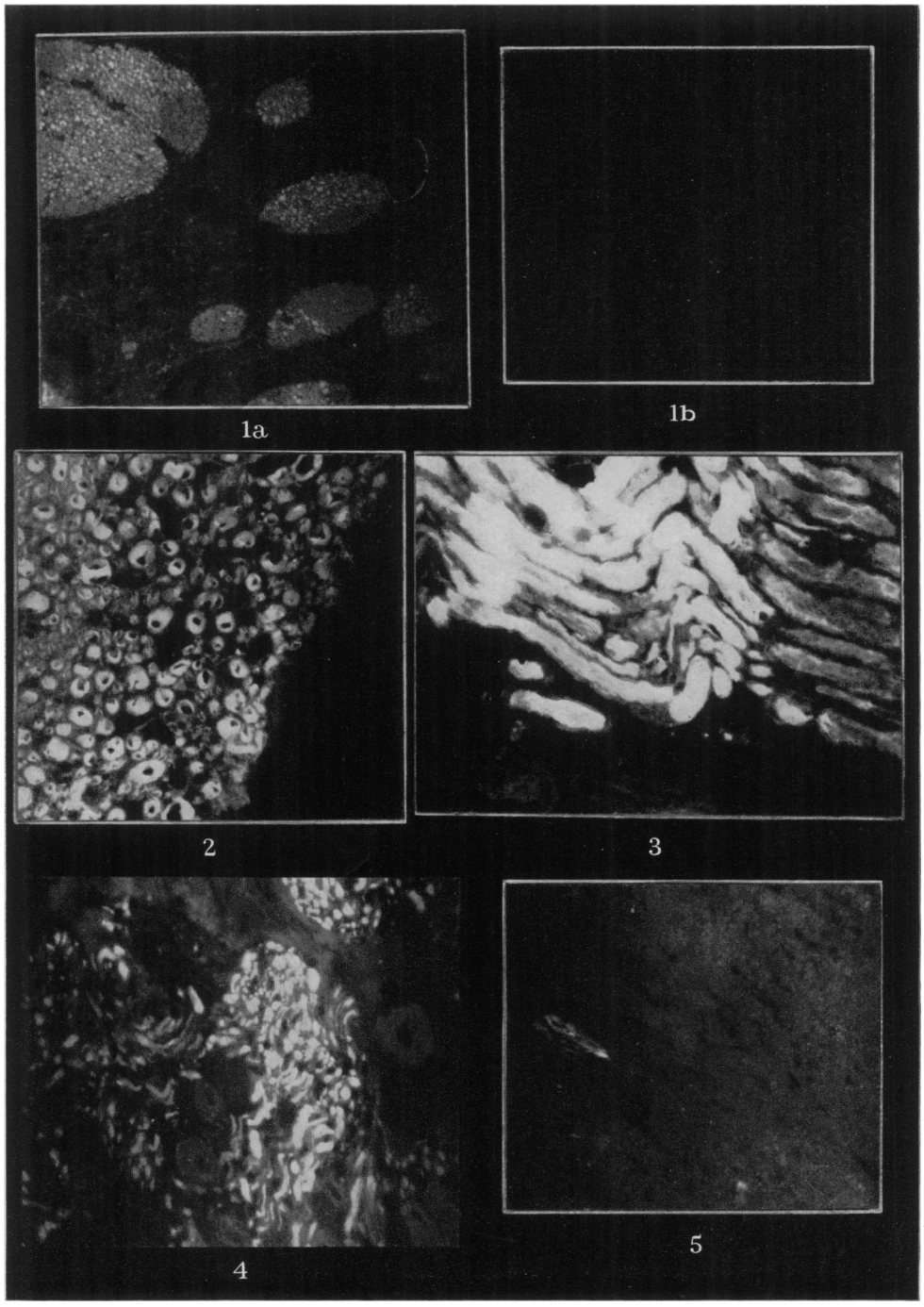
FIG. 5.—Guinea-pig adrenal gland stained with conjugated anti-human brain serum. Note small fluorescent bundle of autonomic fibres in medulla. × 190.

FIG. 6.—Transverse section of guinea-pig brain through region of corpus striatum stained with conjugated anti-human sciatic nerve serum. Diffuse, non-specific staining. ×230.

FIG. 7.—Transverse section of spinal cord posterior column stained with anti-human sciatic nerve serum, showing moderate fluorescence of myelin. ×230.

FIG. 8.—Dorsal root ganglion of guinea-pig strained with conjugated anti-human sciatic nerve serum. Note fluorescence of myelinated fibres but no staining of ganglion cells. ×230.

FIG. 9.—Longitudinal section of sciatic nerve of guinea-pig stained with conjugated anti-human sciatic nerve serum. ×230.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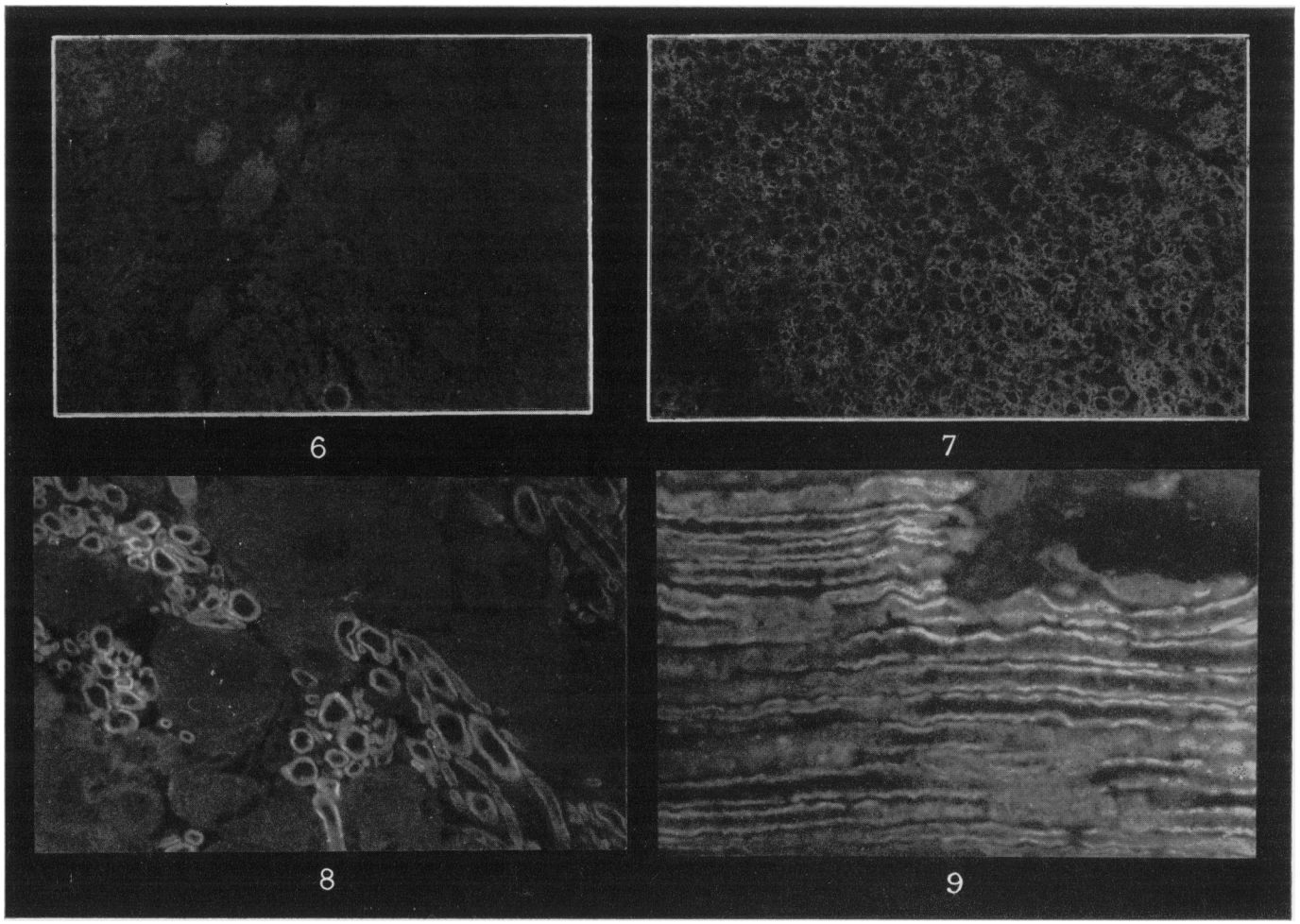

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guinea-pig sciatic nerve, nerve roots and to incoming longitudinal fibres of the cord but not to brain. However, the avidity is not so great as is that of an anti-brain serum.

DISCUSSION

Although the original rabbit brain-antiserum showed lines against saline extracts of both brain and kidney, binding was by central and peripheral myelin alone. This suggests that the myelin antibody is of the non-precipitating type and is further evidence that non-precipitating as well as precipitating antibody can be used as fluorescent tracers (Fothergill, 1962). Failure of the conjugate to stain kidney may be due to the low titre of this precipitating antibody which required 4 or 5 days to appear on Ouchterlony plates.

Staining of rabbit central nervous system myelin with conjugated serum from rabbits with EAE was reported by Sherwin, Richter, Cosgrove and Rose (1961), and they found that myelinated fibres and human spinal cord were also stained. These workers produced their antiserum with rabbit spinal cord. The present results confirm the non-species specificity of an antibrain serum and extend it from the central to the peripheral nervous system.

Beutner, Witebsky, Rose and Gerbasi (1958) obtained a positive staining of rabbit spinal cord with the serum of 1 of 4 rabbits immunized with the same material. Both Beutner *et al.* and Sherwin *et al.* noted that there was no relationship between staining power of the antiserum and the onset of experimental disease in the animal from which the serum came. This was also true in the present experiments, in which rabbits were found to have active sera long before clinical illness was apparent. It seems unlikely, therefore, that the myelin fixing antibody can be of immediate pathogenetic significance in the production of EAE.

The finding that anti-brain serum stains both central and peripheral nerve myelin, whilst anti-nerve serum is virtually limited in staining capacity to peripheral nerve, was unexpected but accords reasonably well with Waksman and Adams' (1956) investigation of the incidence of EAE and EAN in guinea-pigs when injected with central or peripheral nervous tissue. Summarizing their findings with nervous material obtained from several different species, they found that " the guinea-pig developed lesions with either type of antigen. However, among guinea-pigs receiving central antigen, about half developed disease limited to the central nervous system and none had peripheral lesions alone. Among those which received peripheral antigen, the reverse was the case, *i.e.* about one-third developed pure peripheral disease and almost none had central lesions only." So far as human nerve (? sciatic) was concerned, two-thirds of their guinea-pigs developed pure EAN and one-third both peripheral and central lesions. Whether the latter were more severe in the spinal cord than in brain is not indicated.

The significance to be attached to the general agreement between the myelin-binding properties of anti-nervous tissue serum and the distribution of lesions in animals which receive such tissue is difficult to assess. Whilst there is general agreement that no parallelism exists between the presence of complement-fixing antibody to nervous tissue and development of the disease, the claim that a specifically myelotoxic factor is demonstrable in the serum of rabbits with EAE has revived interest in a humoral pathogenesis (Bornstein and Appel, 1961). The possible relationship of such a factor to myelin-binding power, as shown by immunofluorescent studies, remains to be investigated.

SUMMARY

Rabbit anti-human brain serum attaches to guinea-pig myelin, both central and peripheral. Rabbit anti-human nerve serum combines only with peripheral nerve and nerve roots, and this combining power is less than that of the serum against brain.

The possible significance of these findings is briefly discussed.

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