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Cerebral Vascular Permeability and Cellular Infiltration in Experimental Allergic Encephalomyelitis

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Summary. Cerebral vascular permeability was measured in guinea-pigs developing experimental allergic encephalomyelitis (EAE). A sensitive double radioisotope method was used which allowed the permeability changes to be measured independently of alterations in cerebral blood volume. The onset of the disease was accompanied by a striking increase in vascular permeability, but there was no evidence that this change preceded the entry of inflammatory cells into the brain and cord. Injection of serum from rabbits with EAE into normal guinea-pigs had no measurable effect on the cerebral vessels.

These findings are consistent with the view that EAE is a cell-mediated lesion and provide no support for the suggestion that the initial damage is due to circulating antibody.

INTRODUCTION

Experimental allergic encephalomyelitis (EAE) is an autoimmune disease in which an animal reacts against a basic protein component of its own myelin (Field, 1968). Both circulating antibody and delayed hypersensitivity to the antigen appear during the course of the disease, although the latter is generally considered to be responsible for the development of the lesion (Paterson, 1966; Adams and Leibowitz, 1969). However, there is some evidence suggesting that circulating antibody may play a role in the pathogenesis of the disease. The most interesting recent finding was that of Oldstone and Dixon (1968) who (using an immunofluorescent method) observed a leakage of albumin, γ and β_1C globulin from the cerebral vessels of rats developing EAE several days before the accumulation of inflammatory cells in the CNS. This would imply that the lesion was initiated by a circulating serum factor—such as antibody—and that the entry of mononuclear cells into the brain was a secondary phenomenon.

We have investigated this problem in the guinea-pig with a quantitative double radioisotope method which allowed changes in permeability to be assessed independently of alterations in cerebral blood volume. Vascular permeability was measured in normal guinea-pigs and in a series of animals developing EAE. The experiments were designed to test the sensitivity of the method; and to establish the relationship between the onset of cerebral oedema, the inflammatory lesion in the CNS and the development of neurological signs. The main aim was to determine whether, using a sensitive quantitative technique, it was possible to find evidence of a general increase in cerebral vascular permeability preceding the entry of inflammatory cells into the brain and cord.

MATERIALS AND METHODS

Production of EAE

Female albino guinea-pigs of the Hartley strain (250–450 g) were sensitized with a single intradermal injection of guinea-pig whole brain emulsified with an equal volume of Freund's complete adjuvant (Difco). Each animal received 1 mg of guinea-pig brain (dry weight) and 0·2 mg of *Myco. butyricum.*

The animals were weighed and examined daily for evidence of neurological disease—weakness, paralysis, loss of righting reflexes, incontinence or torticollis. They were killed with ether and the brain and spinal cord fixed in formol saline.

Measurement of vascular permeability

The cerebral vessels are relatively impermeable to protein and changes in permeability can be detected by measuring the rate of accumulation of such protein in the extravascular compartment. If a circulating serum protein is labelled with a suitable isotope, the site and severity of the leak can be recognized by the rise in radioactivity that is caused by the extravasation of the labelled compound. A refinement of this technique—used in the present investigation—is to make allowance for the amount of intravascular isotope by measuring the cerebral blood volume. This is done by injecting protein, labelled with a second isotope, immediately before the animal is killed.

Female albino guinea-pigs were used in these experiments. Under light ether anaesthesia, 2 μCi ^{131}I-labelled human serum albumin (Radiochemical Centre, Amersham) contained in 0·1–0·4 ml saline was injected into a leg vein. Twenty-four hours later 10 μCi of ^{125}I-labelled human serum albumin were injected and the animal killed by exsanguination 5 minutes later. The ^{125}I-labelled protein was a 3 per cent solution of human serum albumin containing 50 μCi/ml. Shortly before death, 2–5 minutes after the second injection 0·02 ml of blood was withdrawn from an ear vein. The ^{125}I-labelled albumin was prepared by Dr Norman Veall of the Radiophysics Department, Guy's Hospital, by the iodine monochloride method, using purified human serum albumin obtained from the Lister Institute.

The brain was removed, washed free of blood clot, and fixed for 24 hours in 10 per cent formol saline. It was separated into three portions for counting: (a) cerebral hemispheres (right cerebral hemisphere counted); (b) midbrain and medulla; (c) cerebellum. The spinal cord was exposed and fixed *in situ* for 24 hours before removal. It was then divided into three parts of approximately equal length—referred to as cervical, thoracic and lumbar segments. Each portion of the CNS was weighed and placed in a plastic container containing formol saline for counting and histology.

Determination of radioactivity

Specimens were counted in a well-type scintillation counter. The instrument was calibrated by determining the spectra for ^{131}I and ^{125}I. The channels used were those giving maximum sensitivity for each isotope and minimum 'breakthrough' between them.

Each specimen was counted in both the ^{131}I and ^{125}I channels. Since ^{125}I will not register on the ^{131}I channel, the only correction applied was for ^{131}I penetrating the ^{125}I channel; this correction was effected by counting a specimen of ^{131}I in both channels and determining the efficiency of the ^{125}I channel for ^{131}I. Thus the true ^{125}I count of the specimen=

$$\text{Channel} \times \frac{\text{Count of specimen in } {}^{125}\text{I channel—Count of specimen in } {}^{131}\text{I}}{\text{Count of pure } {}^{131}\text{I in } {}^{125}\text{I channel}}$$

$$\text{Count of pure } {}^{131}\text{I in } {}^{131}\text{I channel}$$

(see Veall and Vetter, 1955).

'Blood equivalents'

The results were expressed in the form of arbitrary units—'blood equivalents'. The reason for this is that, although radioactivity in different parts of the CNS can be legitimately compared in the same animal, comparison *between* animals is only meaningful if account is taken of the differing levels of blood radioactivity. Dividing the cpm/g for each specimen by the cpm/ml blood for the respective animal provided the blood equivalent index(BE). Hence allowance was made for variation in blood radioactivity in the different animals.

$$\text{The 'blood equivalent'} = \frac{\text{Cpm/g}}{\text{Cpm/ml blood}} \times 100.$$

Under the conditions of the experiment the ^{125}I blood equivalent (^{125}I BE) represents the blood volume of the specimen. On the other hand, the ^{131}I blood equivalent (^{131}I BE) reflects both intravascular and extravascular ^{131}I-labelled protein. The difference between the two (^{131}I BE–^{125}I BE) is therefore a measure of extravascular leakage over a peroid of 24 hours. This is referred to as the 'extravascular blood equivalent' (EVBE).

Histology

The specimens were embedded in paraffin blocks after counting. Sections taken from the mid- and hind-brain and the cervical cord were examined in all animals. A further four blocks were also sectioned in each animal killed after the 8th day. In addition, semi-serial sections of the mid- and hind-brain (1 section/100 μ) were examined in the group killed immediately before the onset of the clinical signs (Day 10).

Experimental design

EAE was produced by the method outlined above. Vascular permeability was measured in batches of animals killed 3, 6, 8, 10, 12, 13, 14, and 15 days after sensitization. Similar measurements were made in non-sensitized controls. A total of sixty-five animals were studied in four separate experiments.

Effect of EAE (rabbit) serum on permeability in normal guinea-pigs

Two lop-eared albino New Zealand rabbits were sensitized by intradermal injection of 0·4 ml of a 1:2 emulsion of rat spinal cord and Freund's complete adjuvant+3 mg/ml *M. tuberculosis*; 0·1 ml was injected into each footpad. Both animals developed paralysis of the hind limbs 12 days afterwards. They were bled before sensitization and on Days 7, 11, 13 and 18.

The serum was filtered, tested for sterility and stored at −20°. Normal female guinea-pigs (200–400 g) were injected intraperitoneally with 5 ml of serum and cerebral vascular permeability was measured over the succeeding 24 hours.

RESULTS

CEREBRAL VASCULAR PERMEABILITY IN NORMAL GUINEA-PIGS

If the cerebral vessels were completely impermeable to protein, both the ^{131}I and the ^{125}I-labelled albumin would remain in the intravascular compartment and the EVBE in normal animals would approximate to 0. However, some protein does escape and the

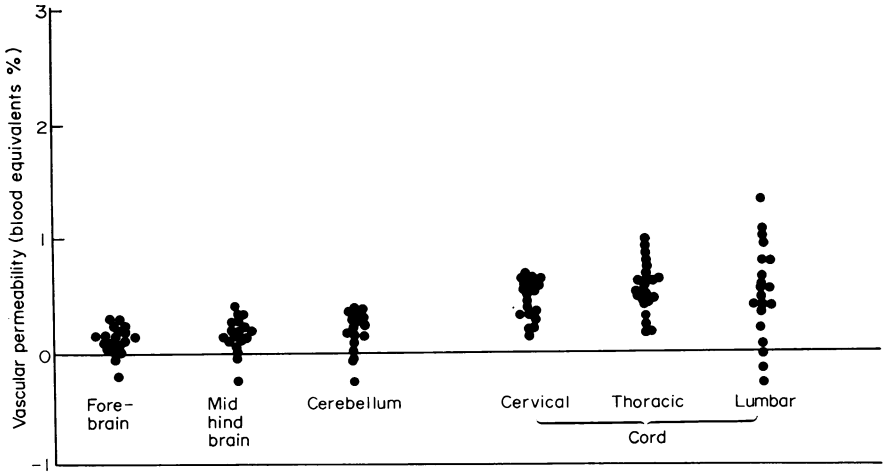

Fig. 1. Vascular permeability in the central nervous system measured by the double radioisotope method. Range of values in normal guinea-pigs.

TABLE 1

VASCULAR PERMEABILITY IN THE BRAIN AND CORD OF NORMAL GUINEA-PIGS

	Double isotope method EVBE		Single isotope method ^{131}I BE	
	Mean	SD	Mean	SD
Forebrain	0·12	0·11	1·64	0·24
Midbrain & Medulla	0·13	0·12	1·91	0·30
Cerebellum	0·18	0·15	2·17	1·39
Cervical C.	0·43	0·26	2·19	0·35
Thoracic C.	0·55	0·23	2·00	0·47
Lumbar C.	0·49	0·40	2·28	0·57

Comparison between single (I^{131} BE) and double (I^{131}–^{125}I = EVBE) radioisotope methods. The values for ^{131}I BE show a wider degree of scatter.

mean figure varies between 0·12 and 0·55 (Fig. 1). The lowest values were in the forebrain and the mid-brain and medulla (0·12 and 0·13). This small but definite leakage of protein into the extravascular compartment is probably due to two main factors. One is the passage of labelled albumin into the cerebrospinal fluid and its subsequent diffusion into the brain parenchyma from the subarachnoid and ventricular surfaces. The second is the accumulation of protein in the extravascular space of the meningeal connective tissue.

These effects are likely to be more pronounced in the cord than they are in the brain, accounting for the higher EVBE at the former site. The cord also shows a wider scatter in individual EVBE values. This increased margin of error is to be attributed to the smaller size of the cord specimen and the greater difficulty encountered in its dissection. For anatomical reasons these difficulties were most marked in the lumbo-sacral region.

Sensitivity of the method

The increased sensitivity resulting from the use of the second isotope is illustrated in Table 1. The range of values obtained in normal animals when vascular permeability was

TABLE 2

COMPARISON OF SINGLE AND DOUBLE RADIOISOTOPE METHODS FOR MEASURING VASCULAR PERMEABILITY

	No. specimens showing increased permeability	
	Double isotope method	Single isotope method
Forebrain	12	10
Midbrain & Medulla	12	9
Cerebellum	7	4
Cervical C.	7	6
Thoracic C.	19	7
Lumbar C.	11	10
Total	68	46

Table showing the increased sensitivity of the double isotope technique. Vascular permeability measured in guinea-pigs with EAE. Increased permeability = ^{131}I BE or EVBE > mean + 3 SD of the normal value.

TABLE 3

VASCULAR PERMEABILITY IN EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS IN THE GUINEA-PIG

	Mean EVBE	
	Allergic encephalomyelitis (11–15 days after sensitization)	Normal controls
Forebrain	2·16	0·12 ± 0·02
Midbrain & Medulla	2·54	0·13 ± 0·03
Cerebellum	1·52	0·18 ± 0·03
Cervical C.	1·52	0·43 ± 0·06
Thoracic C.	6·13	0·55 ± 0·05
Lumbar C.	4·54	0·49 ± 0·09

EVBE—Extravascular blood equivalent. Mean ± SE.

calculated on the basis of the ^{131}I figures only (^{131}I BE) is twice that obtained when allowance is made for the intravascular blood volume (EVBE). In an inflammatory lesion such as EAE, where the blood volume might be expected to fluctuate widely, the error introduced by failing to take these changes into account would be even greater.

This increased sensitivity is confirmed by directly comparing the results obtained with the two methods in animals with EAE. The ^{131}I BE and the EVBE were both calculated from the same block of tissue. Vascular leakage was detected in sixty-eight of seventy-eight specimens by the double isotope technique compared with forty-six using ^{131}I alone—an

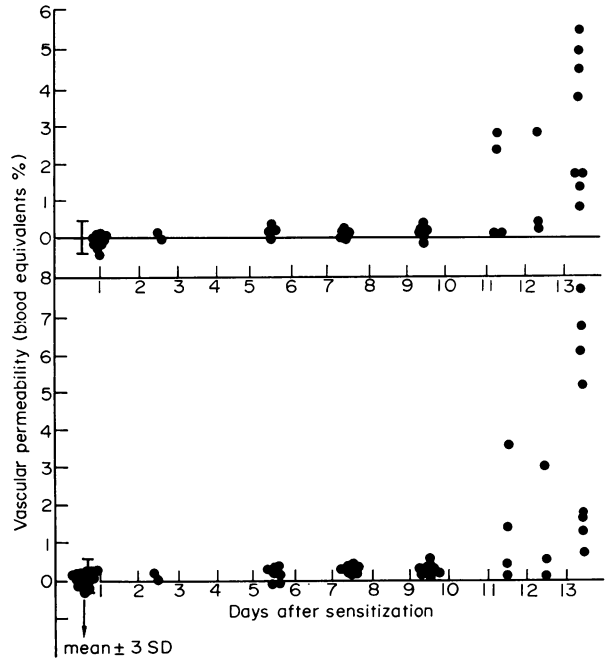

Fig. 2. Vascular permeability in the (above) forebrain and (below) midbrain and medulla of guinea-pigs developing experimental allergic encephalomyelitis. First appearance of vascular leakage 11–12 days after sensitization.

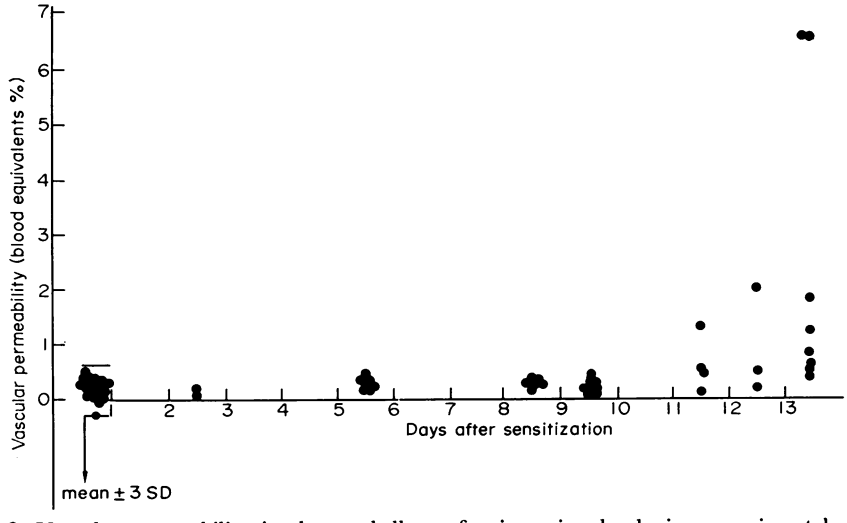

Fig. 3. Vascular permeability in the cerebellum of guinea-pigs developing experimental allergic encephalomyelitis.

increase in sensitivity of about a third (Table 2). In these experiments vascular permeability was considered to be increased when the EVBE>mean+3 SD of the normal value (P 0·015).

TABLE 4

ONSET OFOEDEMA IN RELATION TO CELLULAR INFILTRATION AND NEUROLOGICAL SIGNS

	Number of animals					
	Days after sensitization					
	Controls	2–8	9–10	11–12	12–13	13–15
Total No. animals	21	18	9	4	3	9
> Vascular permeability	0	0	0	3	1	9
Cellular infiltration	0	0	2	3	3	9
Neurological signs	0	0	0	3	1	7

EAE in the guinea-pig. Sensitization with 1 mg dry weight guinea-pig brain with Freund's complete adjuvant, 0·1 ml i.d.

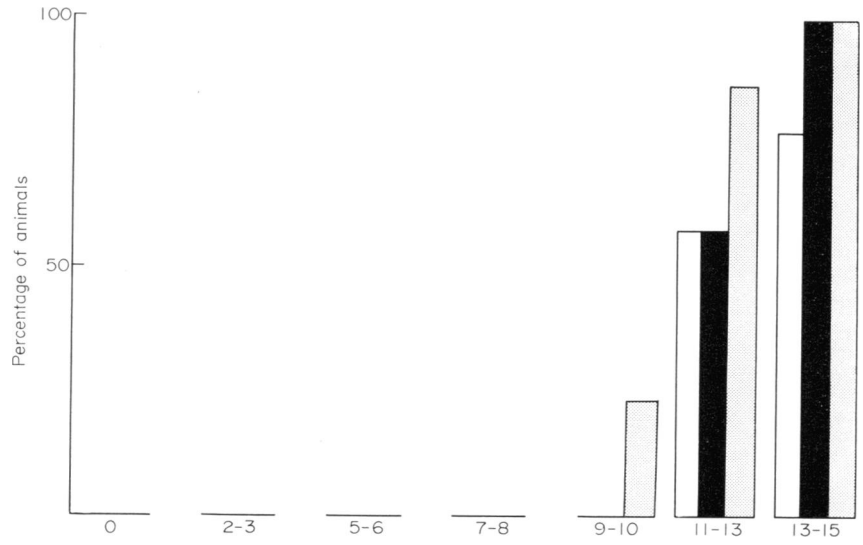

Fig. 4. Onset of oedema (solid columns), cellular infiltration (stippled columns) and neurological signs (open columns) in guinea-pigs developing experimental allergic encephalomyelitis.

Vascular permeability in EAE

Cerebral vascular permeability was markedly increased in animals with allergic encephalomyelitis (Table 3). For example, in the mid-brain and medulla the mean extravascular blood equivalent (EVBE) rose from 0·13 to 2·54 a nineteen-fold increase. The highest individual figure (8·26) represented a twenty-eight-fold increase over the highest control figure. All parts of the brain and cord were affected.

The vascular leak first appeared 11–12 days after sensitization (Figs 2 and 3). The EVBE was within normal limits in all animals tested on Days 2–3, 5–6, 7–8 and 9–10.

Vascular permeability and cellular infiltration

The pathology of EAE is that of a meningo-encephalitis. Perivascular accumulations of mononuclear cells are seen in the meninges, the choroid plexus, the subependymal

TABLE 5

RELATION BETWEEN CELLULAR INFILTRATION AND VASCULAR PERMEABILITY IN INDIVIDUAL SPECIMENS

Evidence of EAE		
Histology	> Permeability	No. Blocks
+	+	50
−	−	195
+	−	11
−	+	2

Specimens taken from the brain and cord. Experimental allergic encephalomyelitis in the guinea-pig.

TABLE 6

EFFECT OF EAE (RABBIT) SERUM ON CEREBRAL VASCULAR PERMEABILITY IN THE GIUNEA-PIG

Rabbit serum (5 ml i.p.)		Vascular permeability (EVBE)					
	Day after sensitization	Forebrain	Midbrain and Medulla	Cerebellum	Cervical C.	Thoracic C.	Lumbar C.
21 normal controls. No serum (mean ± S.D.)		0·12 ± 0·11	0·13 ± 0·13	0·18 ± 0·15	0·43 ± 0·21	0·55 ± 0·23	0·49 ± 0·40
Normal rabbit serum		0·20	0·22	0·12	0·40	0·81	0·36
Normal rabbit serum		0·05	0·33	0·43	0·60	0·64	0·82
EAE serum	11	0·27	0·21	0·26	0·59	0·65	0·76
EAE serum	7	0·38	0·28	0·25	0·29	0·52	0·44
EAE serum	11	0·16	0·18	0·22	0·42	0·36	0·78
EAE serum	14	0·16	0·19	0·41	0·24	0·23	0·36

Normal guinea-pigs injected intraperitoneally with 5 ml of serum from rabbits with EAE. Vascular permeability measured over the succeeding 24 hours by the double radioisotope method. Permeability not increased i.e. < mean + 3 SD of normal value.

region and scattered throughout the brain and cord. In the early stages the cells are predominantly small and medium-sized lymphocytes. Later the proportion of monocytes increases and the perivascular cuff appears as a tightly packed mass of histiocytic cells. Monocytic cells—some of them activated microglia—extend out into the parenchyma of the brain forming a paravascular extension of the perivascular lesion. Polymorphonuclear leucocytes may be prominent in the early stages, particularly when the lesion is acute.

The first definite evidence of cellular infiltration in the CNS in these experiments was found in two animals on the 10th day (Table 4). Although several less impressive lesions were found earlier, vascular permeability in these animals was normal. However, from the 11–12th day virtually all had well-marked histological lesions accompanied by vascular leakage. In these experiments therefore, minimal cellular infiltration was occasionally found in the absence if any change in vascular permeability, while measurable oedema was found only in the presence of histological lesions. Certainly no evidence was found of any general increase in vascular permeability in the CNS preceding the entry of inflammatory cells (Fig. 4).

This conclusion is reinforced by an examination of the results in individual blocks. Of the 258 blocks examined histologically 50 showed both cellular infiltration and vascular leakage while 195 showed neither (Table 5). There were only two with oedema and normal histology. A slight discrepancy of this order is inherent in the method. It may be explained by the fact that a proportion of the lesions will be outside the plane of the sections examined or by diffusion of extravasated protein from adjacent inflammatory areas.

Cellular infiltration with normal EVBE was found more frequently. If this does not merely reflect the limits of sensitivity of the method—it must mean that perivascular cellular infiltration can be present without measurable leakage of protein.

DISCUSSION

The cellular lesion of experimental allergic encephalomyelitis is accompanied by a striking increase in cerebral vascular permeability. This may be inferred from the electron microscopic appearances (Lampert and Carpenter, 1965; Hirano *et al.*, 1970) and can be demonstrated in tissue sections using trypan blue (Barlow, 1956) and radiolabelled serum proteins as markers (Vulpe, Hawkins and Rozdilsky, 1960; Leibowitz, 1969).

There is general agreement that in animals with established disease, these two elements —cellular infiltration and vascular leakage—can be dissociated. Vessels may show evidence of increased permeability in the absence of a cuff of inflammatory cells; and conversely, there may be perivascular cuffing without observable protein loss (Cutler, Lorenzo and Barlow, 1967). A similar dissociation has been observed in electron microscopic studies of the tuberculin reaction in the skin (Wiener, Lattes and Spiro, 1967).

Autoradiographic evidence of vascular leakage unaccompanied by cells may be explained in a number of ways. For example, proteins leaving a vessel at the site of a cellular lesion might diffuse along it in the perivascular space. Sections taken through adjacent areas would then show the vessel surrounded by labelled protein in the absence of a cuff of inflammatory cells. Alternatively this dissociation may reflect a more fundamental aspect of the cell-mediated lesion—namely the action of 'lymphokines'. The addition of specific antigen to sensitized lymphocytes in culture results in the release of a number of factors, at least one of which increases vascular permeability when injected into the skin (Dumonde *et al.*, 1969). If such factors were actually responsible for producing the delayed lesion *in vivo* this might well explain the anomalous autoradiographic findings. Sensitized lymphocytes in contact with antigen may release pharmacologically active substances capable of inducing vascular leakage at some distance from the initial accumulation of inflammatory cells. It is clear therefore, that some degree of dissociation between the cellular and vascular events is to be expected in delayed hypersensitivity and, perhaps, in all inflammatory lesions.

However, widespread permeability changes preceding the entry of inflammatory cells by several days would imply a dissociation of quite a different order and could not be explained on a basis of delayed hypersensitivity alone. It would mean that the lesion in the central nervous system was initiated by a circulating serum factor and that the appearance of the cells in the brain was secondary. The myelinolytic antibody found in the serum of animals with EAE (Bornstein and Appel, 1965) or antibody to the encephalitogenic protein itself (Lisak *et al.*, 1970) must be considered as possible candidates for such a role.

The present series of experiments were designed to detect the earliest vascular changes and to relate them to the entry of inflammatory cells into the central nervous system. The technique employed—measurement of radiolabelled protein in large blocks of tissue—would appear to be the method of choice for detecting minute but generalized vascular changes developing slowly over a period of days. The increased sensitivity resulting from the use of a second isotope is apparent from an analysis of the results.

One possible criticism however, is that 24 hours may not represent the optimal pulse time for ^{131}I-albumin and that some degree of sensitivity was lost on this account. At the end of 24 hours the ^{131}I in the blood falls to about a third of its original level due to equilibration with the extravascular space, while the clearance-exchange of accumulated protein presumably continues relatively unchanged. The question of the optimal pulse-time was not explored in these experiments and attention to this point might well enhance the precision and sensitivity of the method. Any improvement would depend upon a number of factors, including the rate at which labelled protein was passing into (and out of) the lesions in the CNS and the rate at which it was disappearing from the circulating blood.

The onset of EAE in these experiments was accompanied by a marked increase in cerebral vascular permeability. The relation between the intensity of this oedema and the clinical signs was sufficiently close for it to be considered as a major factor in the development of the paralysis. However, no evidence was found that changes in vascular permeability preceded the entry of inflammatory cells into the CNS. A similar pattern emerged from the autoradiographic studies of Cutler *et al.* (1967) in which vascular and cellular changes were demonstrated on the first day of the clinical illness. Oldstone and Dixon (1968) however, reached the opposite conclusion in an investigation of EAE in Lewis rats. Using a fluorescent-antibody method they observed a leakage of serum fibrinogen, β_1C globulin and IgG from the cerebral vessels several days before the appearance of inflammatory cells in the central nervous system. The escape of fibrinogen, deposited as fibrin in the immediate vicinity of blood vessels was very convincingly illustrated in the accompanying photomicrographs.

The discrepancy may be due to a species difference. If not, the question resolves itself into a comparison between the relative sensitivities of the methods used. The present investigation has clearly failed to confirm, by a quantitative radioisotope method, results obtained using immunofluorescence in tissue sections.

These findings, together with the failure of serum from rabbits with EAE to influence vascular permeability in normal guinea-pigs, argue against the notion that a circulating cytotoxic antibody is involved in the pathogenesis of allergic encephalomyelitis. The relation between vascular leakage and cellular infiltration in these experiments (and the pattern of the vascular change observed autoradiographically) are quite compatible with the view that the EAE lesion in the guinea-pig is due to delayed hypersensitivity—and initiated by cells alone.

ACKNOWLEDGMENT

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REFERENCES

ADAMS, C. W. M. and LEIBOWITZ, S. (1969). 'The general pathology of demyelinating diseases.' *The Structure and Function of Nervous Tissues* (Ed. by G. H. Bourne), Vol. 3. Academic Press, New York.

BARLOW, C. F. (1956), 'A study of abnormal blood-brain permeability in experimental allergic encephalomyelitis.' *J. Neuropath. exp. Neurol.*, **15**, 196.

BORNSTEIN, M. B. and APPEL, S. H. (1965). 'Tissue culture studies of demyelination.' *Ann. N.Y. Acad. Sci.*, **122**, 280.

CUTLER, R. W., LORENZO, A. V. and BARLOW, C. F. (1967). 'Brain vascular permeability to 1251 gamma globulin and leucocytes in allergic encephalomyelitis.' *J. Neuropath. exp. Neurol.*, **26**, 558.

DUMONDE, D. C., WOLSTENCROFT, R. A., PANAYI, G. S., MATTHEW, M., MORLEY, J. and HOWSON, W. T. (1969), 'Lymphokines: Non-antibody mediators of cellular immunity generated by lymphocyte activation.' *Nature (Lond.)*, **224**, 38.

FIELD, E. J. (1968). 'The brain and nervous system in allergic disease.' *Clinical Aspects of Immunology* (Ed. by P. G. H. Gell and R. R. A. Coombs). Blackwell, Oxford.

HIRANO, A., DEMBITZER, H. M., BECKER, N. H., LEVINE, S. and ZIMMERMAN, M. H. (1970). 'Fine structural alterations of the blood-brain barrier in experimental allergic encephalomyelitis.' *J. Neuropath. exp. Neurol.*, **29**, 432.

LAMPERT, P. and CARPENTER, S. (1965). 'Electron microscopic studies on the vascular permeability and mechanisms of demyelination in experimental allergic encephalomyelitis.' *J. Neuropath. exp. Neurol.*, **24**, 11.

LEIBOWITZ, S. (1969). 'Cerebral vascular permeability in allergic encephalomyelitis.' *Neuropat. Pol.*, **7**, 303.

LISAK, R. P., FALK. G. A., HEINZE, R. G., KIES, M. W. and ALVORD, E. C. (1970). 'Dissociation of antibody production from disease suppression in the inhibition of allergic encephalomyelitis by myelin basic protein.' *J. Immunol.*, **104**, 1435.

OLDSTONE, M. B. A. and DIXON, F. J. (1968). 'Immunohistochemical study of allergic encephalomyelitis.' *Amer. J. Path.*, **52**, 251.

PATERSON, P. Y. (1966). 'Experimental allergic encephalomyelitis and autoimmune disease.' *Advanc. Immun.* **5**, 131.

VEALL, N. and VETTER, H. (1958). '*Radioisotope Techniques in Clinical Research and Diagnosis.*' Butterworth, London, pp. 103–121.

VULPE, M., HAWKINS, A. and ROZDILSKY, B. (1960). 'Permeability of cerebral blood vessels in EAE studied by radioactive estimated bovine albumin.' *Neurology*, **10**, 171.

WIENER, J., LATTES, R. G. and SPIRO, D. (1967). 'An electron microscopic study of leucocyte emigration and vascular permeability in tuberculin sensitivity.' *Amer. J. Path.*, **50**, 485.