

**PERIVENTRICULAR LESIONS IN MULTIPLE SCLEROSIS:
THEIR PERIVENOUS ORIGIN AND RELATIONSHIP TO
GRANULAR EPENDYMITIS**

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**Periventricular lesions in multiple sclerosis: their perivenous origin
and relationship to granular ependymitis**

The periventricular region was studied in the brains of 129 cases of multiple sclerosis, with the purpose of establishing the mechanism and order of events in the development of the periventricular plaque, and deciding whether there is any relationship between granular ependymitis and such plaques. Periventricular plaques were found in 82·2% of cases. Observation and computerized morphology showed that the early stage of the periventricular plaque is the formation of a lesion around a subependymal vein and that adjacent lesions later coalesce. These plaques do not appear to arise from the ependyma, which is against any role for the CSF in their initial development. Chronic or burnt-out periventricular lesions often show overlying granular ependymitis (10·9% of cases) and subependymal gliosis (17·8%), presumably as a result of the long-continued low-grade inflammatory process. This process, which is not specific for multiple sclerosis, is sometimes associated with transfer of IgG and C3, as shown with peroxidase methods, across the subependymal vein wall and the ependymal epithelium. Increased permeability of the inflamed ependyma constitutes a possible abnormal entry route from plaque to CSF or, in reverse, from CSF to brain.

Introduction

Periventricular lesions have been recognized as characteristic and particularly frequent lesions in multiple sclerosis (Dawson, 1916; Fog, 1965; Lumsden, 1970). In his study by serial section of two cases, Fog followed the course of perivenular plaques, extending as Dawson's 'fingers' from the

periventricular region out into the central cerebral white matter. However, Fog could shed no light on the origin of the periventricular lesion, in that the parent vein could not be identified in the confluent plaque (see Lumsden, 1970). In Lumsden's own survey, about 90% of cases showed periventricular plaques: such plaques usually appeared to be long-standing and, hence, to have developed early in the course of the disease (Adams, 1983). The periventricular distribution of the lesions could be taken to suggest that the causative agent for the disease had penetrated into the brain from the cerebrospinal fluid (Dawson, 1916). However, it was later shown in two gross specimens that early periventricular lesions are situated around subependymal veins causing focal perivenous demyelination (Allen, 1981; Adams, 1983). Hence, it could be that the periventricular plaque has no real relationship to the ependyma or CSF, and arises around small veins and venules, as is characteristic of most other (if not all) plaques in multiple sclerosis (Fog, 1964, 1965). If the lesions have no consistent relationship to the ependyma, then it would seem unlikely that the primary causative agent arises in the CSF and diffuses into the brain to cause periventricular plaques, as suggested by Dawson (1916).

The first purpose of this paper is to examine periventricular plaques to determine whether their topography and geometry are more consistent with a perivenular or a periventricular origin. The second purpose is to show that granular ependymitis is common in multiple sclerosis and to study its relationship to periventricular plaques. Granular ependymitis is associated with increased permeability to immunoreactive agents. Although, the perivenular distribution of immunoglobulin and complement has been described in detail by other workers (Esiri, 1980; Walsh & Tourtellotte, 1983), no previous immunocytochemical study has been made of the ependyma in multiple sclerosis in this respect.

Material and methods

Sections or blocks were available from 129 cases of multiple sclerosis. These were cases where there was adequate sampling of the ventricular system, with blocks usually including lateral, third and fourth ventricles. The 129 cases were derived from 84 cases out of 118 in the MRC brain bank at Guy's Hospital, from 34 cases out of 42 at the MRC Neuropathology Laboratory at Runwell Hospital, Wickford, Essex and from 11 cases at the Department of Morbid Anatomy, Addenbrooke's Hospital, Cambridge.

As controls for the study of granular ependymitis, 10 non-neurological and 10 neurological brains were examined (cases as in Adams *et al.*, 1985). Also examined were (a) 10 brains with ventricular dilatation from subjects aged 70 to 84 years (three with Parkinson's disease, three with cerebrovascular disease and four with senile atrophy), and (b) brains from 15 cases of meningitis, aged 25 to 70 years (three meningococcal, one pneumococcal otitic, six following head injury, five associated with carcinoma, lymphoma or leukaemia). There was, thus, a total of 40 control brains to study the frequency of granular ependymitis in non-multiple sclerosis subjects.

Sections from paraffin-embedded tissue were stained with haematoxylin and eosin, phosphotungstic acid haematoxylin, modified elastic trichrome with Martius Scarlet Blue, Luxol Fast Blue, Solochrome B and Holtzer's method.

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Selected cases were stained with the following immunohistochemical methods using peroxidase/antiperoxidase applied to formalin fixed paraffin embedded sections: anti-albumin (Dako), anti-immunoglobulin G (Dako), anti-fibrin (polyvalent), anti-complement C3 (Dako) and anti-glial fibrillary acidic protein (Dako). The peroxidase techniques used were as set out in Adams *et al.* (1985).

COMPUTERIZED MORPHOMETRY

The geometric circularity and centroid of the constituent plaques of some periventricular and periaqueductal lesions were determined with a Leitz image analyser, using a Hewlett Packard computer 86B, Hewlett Packard printer 82905B, Summagraphics 'Bit Pad' graphics tablet and the Imagan general morphometric program. For analysis, lesions were first photographed by inserting the microscope slide into a photographic enlarger; a negative print was obtained which was then subjected to image analysis.

If a plaque is centred on a vein, then the vein and plaque will be concentric. The same argument applies to a confluent periventricular plaque if it arises from the ependymal lining of the ventricular cavity. Only slides with a complete cross-section of the ventricle were examined (only 18 cases were suitable). The centroid (analogous to the centre of gravity) of confluent areas of periventricular plaques (including the ventricle) was determined. Likewise, the centroid of the ventricular cavity alone was determined. The distance between the centroids was divided by the diameter of a circle equivalent in area to the cross-sectional area of the ventricle. The magnitude of this number (*C*) is a measure of departure from co-centricity: a value of zero implies perfect co-centricity. The same technique was applied to perivenular plaques in various parts of the rest of the brain. To ensure comparability with the components of periventricular plaques, the plaques chosen were those with a diameter not more than four times that of the central vein.

The centroid is the 'centre of mass' of a closed loop. If the loop is drawn as a Cartesian graph, the centroid of the lesion occupies a position (*x',y'*) relative to the centroid of the vein or ventricle. The distance between lesion centroid and vein or ventricular centroid was determined by computerized morphometry. The basic formula is as follows: the centroid of the area under the curve of a function *f(x)* between *x*=*a* and *x*=*b* is located at *x',y'* where

$$\bar{x} = \frac{\int_a^b xf(x)dx}{\int_a^b f(x)dx} \quad \text{and} \quad \bar{y} = \frac{\frac{1}{2} \int_a^b [f(x)]^2 dx}{\int_a^b f(x)dx}$$

Results

Origin of periventricular plaques

In this series of 129 cases of multiple sclerosis, 106 (82.2%) showed periventricular plaques affecting at least one part of the ventricular system. The commonest site for lesions was around the lateral ventricles (78.3% of

Table 1. Disease activity in perivenous and confluent periventricular plaques, as judged by presence or absence of perivascular lymphocytic infiltration

	Active	Inactive	Total
Perivenous lesions	51	24	75
Confluent lesions	10	21	31
Total	61	45	106

$\chi^2 = 11.47$; $P < 0.001$.

the 106 cases), and then in descending order: fourth ventricle (13.2%), third ventricle (10.4%) and aqueduct (5.7%). The results for the aqueduct, however, are possibly low due to under-representation in the tissue samples. In 10.4% of the 106 cases, there were plaques around the lateral ventricles and at one other ventricular site. No plaque was seen in the extension of the third ventricle towards the optic chiasm. Some 83.6% of active lesions in the periventricular region, distinguished by lymphocytic infiltration and a shelving (or irregular) edge (see Adams, 1980), showed clear-cut subependymal perivenular demyelination (Figure 1), but only 53.3% of chronic or non-active lesions showed discrete perivenular rather than confluent periventricular lesions (Table 1).

Image analysis confirmed that there was a predominant perivenular element to the lesion (Table 2), and showed that the 18 lesions studied in the periventricular region were not centred on the ventricle. Some 69% of 52 separate small (early) non-confluent plaques in the vicinity of a ventricle showed a circular profile (Figure 1) and were presumed to have arisen from the vein that they encompassed, whereas 31% were flattened or half-circular in profile and could have arisen from either a subependymal vein or the ependymal epithelium itself. Further support for the view that periventricular plaques arise around the subependymal veins is shown by examination of the whole brain, stained in the gross by Nile blue sulphate

Table 2. Values for centroid concentricity for components of periventricular plaques in relation to the ventricular centroid and perivenous plaques elsewhere in relation to vein centroid

Plaque type	No. of observations	Mean C value (range)
Periventricular	18	0.592 (0.03-3.77)
Perivenous elsewhere	21	0.0852 (0.06-0.14)

Low values indicate co-centricity, whereas higher values indicate a separate relationship between the compared structures (for explanation see Methods).

$t = 2.7362$; $DF = 37$; $P = 0.0095$.

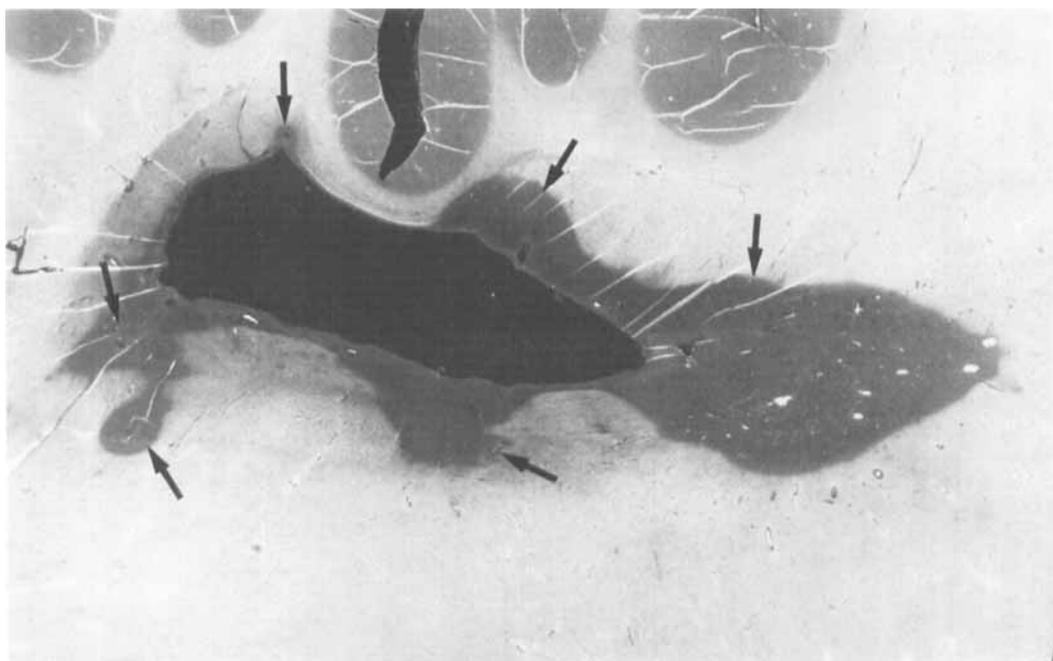


Figure 1. Negative print of periventricular region in active multiple sclerosis. Direct enlargement of the tissue section obtained by placing the stained slide in the stage of the enlarger. Grey matter and plaques appear grey, the lateral ventricle is black, while white matter is white. Arrows point to periventricular plaques (with circular or more than half-circular profiles), which were shown histologically to be centred around veins.

(Figure 2). Here it can be seen that the pale demyelinated lesions around the ventricle closely follow the course of subependymal veins.

Granular ependymitis

Some 10.9% of periventricular lesions showed evidence of active granular ependymitis (Table 3), characterized by raised pyramidal granulations with focal gliosis in the subependymal region; the overlying ependyma was atrophic, eroded or absent (Figure 3). A further 17.8% of all periventricular lesions showed extensive subependymal gliosis when stained with phosphotungstic acid-haematoxylin (Figure 4), Holtzer's stain or with the GFAP-peroxidase method (Figure 5). These regions of subependymal gliosis and of granular ependymitis were less common in active cases. Only 46.8% of cases with ependymitis or subependymal gliosis were active in the sense that they contained perivenular lymphocytic infiltrates, whereas 66.1% of cases without these ependymal changes showed lymphocytic infiltration (Table 1). This difference indicates that ependymitis and subependymal gliosis are associated more with chronic than active lesions ($P < 0.05$). Dilatation of the lateral and third ventricles was obviously present in only four cases in the

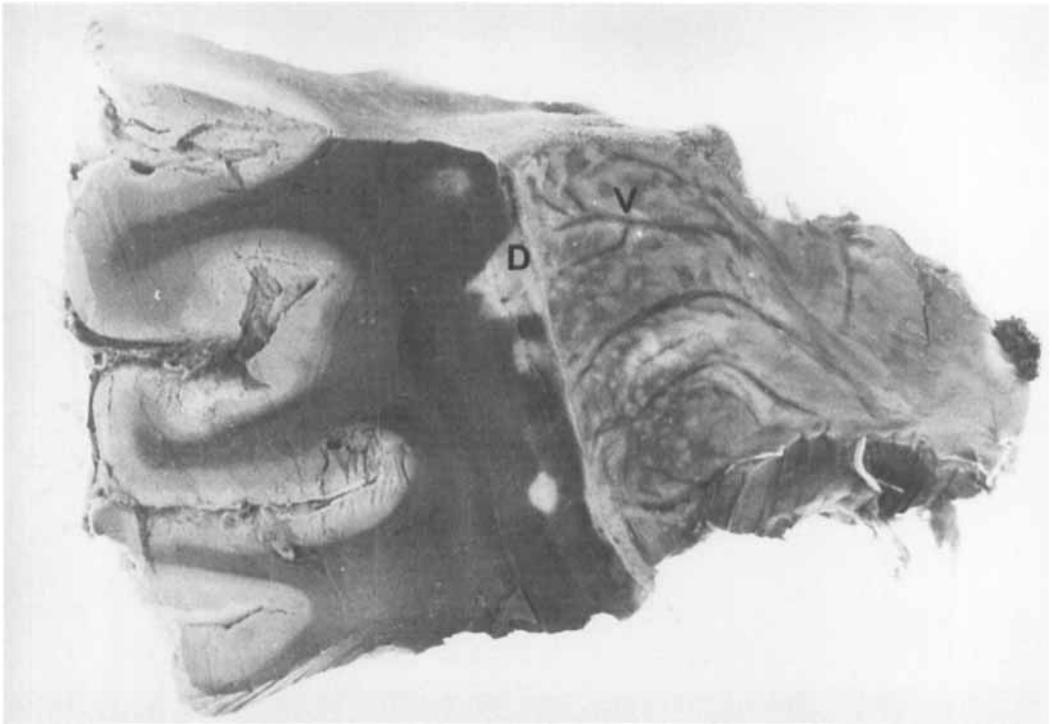


Figure 2. Slice of brain including the ependymal lining of the third ventricle and central white matter in active multiple sclerosis, stained in the gross with Nile blue sulphate. Note pallor around the subependymal veins (V), which appears as a rim of demyelination (D) in the central white matter cut at right angles to the ventricular lining.

multiple sclerosis series; these four brains showed granular ependymitis in the lateral ventricles.

The 10 non-neurological control brains showed no ependymal granulations or gliotic changes in the subependymal region. The 10 neurological control brains without ventricular dilatation only showed

Table 3. Disease activity of plaques with granular ependymitis, subependymal gliosis and with no gliosis, as adjudged by the presence or absence of perivascular lymphocytic infiltration

	Active	Inactive	Total
Granular ependymitis	5	9	14
Subependymal gliosis	17	16	33
No gliosis	39	20	59
Total	61	45	106

$\chi^2 = 3.841$; $P < 0.05$.

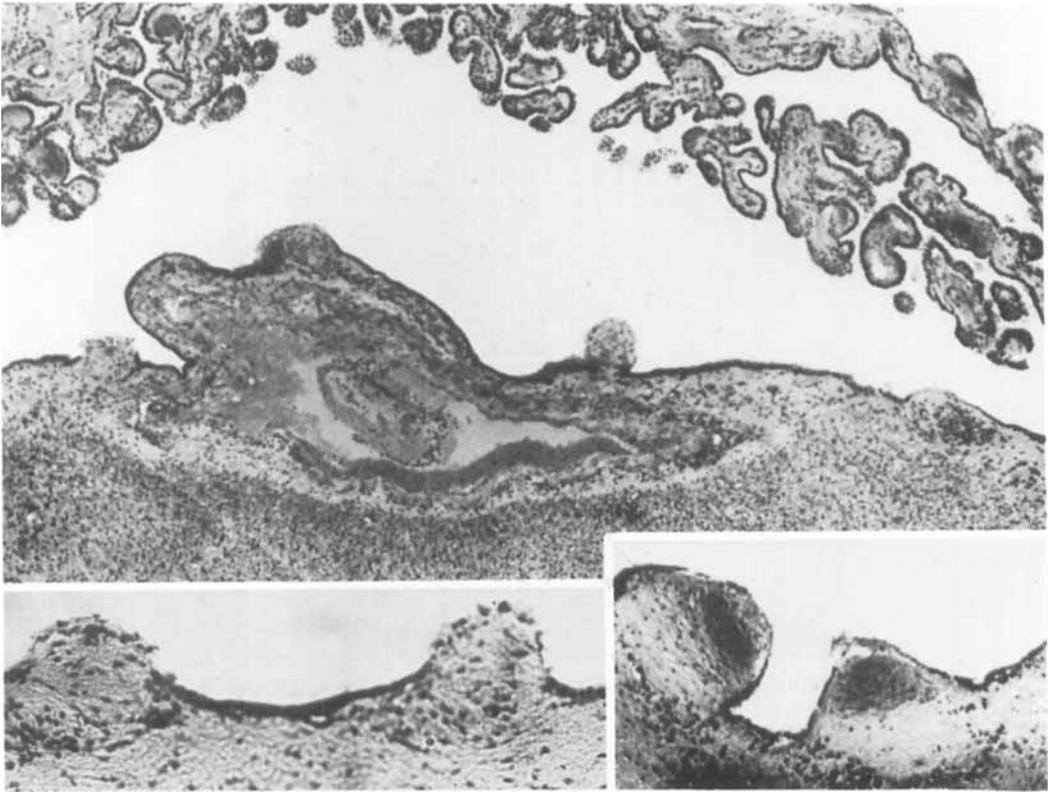


Figure 3. Three chronic cases of multiple sclerosis to show granular ependymitis (arrows) in the third ventricle, $\times 25$. Insets: granular ependymitis of (left) septum pellucidum, (right) aqueduct. Van Gieson. $\times 60$.

granular ependymitis in a case of astrocytoma with meningitis. In the ten cases from older patients with ventricular dilatation, there were seven instances of granular ependymitis or subependymal gliosis (2/3 Parkinson's disease, 2/3 vascular disease, 3/4 senile atrophy). Likewise, 10 out of 15 cases of meningitis showed granular ependymitis or subependymal gliosis (3/4 meningococcal or pneumococcal, 4/6 head injury, 3/5 neoplasia). These findings indicate that granular ependymitis is, in general, associated with ventricular dilatation or meningitis.

Immunohistochemical methods showed evidence of gross non-specific leakage of albumin into the brain substance, presumably representing post-mortem diffusion of a molecule of relatively low molecular weight. By contrast, fibrinogen showed only focal leakage around subependymal perivenous lesions, as reported previously for other perivenous lesions (Adams *et al.*, 1985). Accumulation of IgG and C3 were seen around subependymal venules (Figure 6) and focally in the ependyma and subependyma in areas of chronic granular ependymitis and subependymal gliosis (Figure 7).

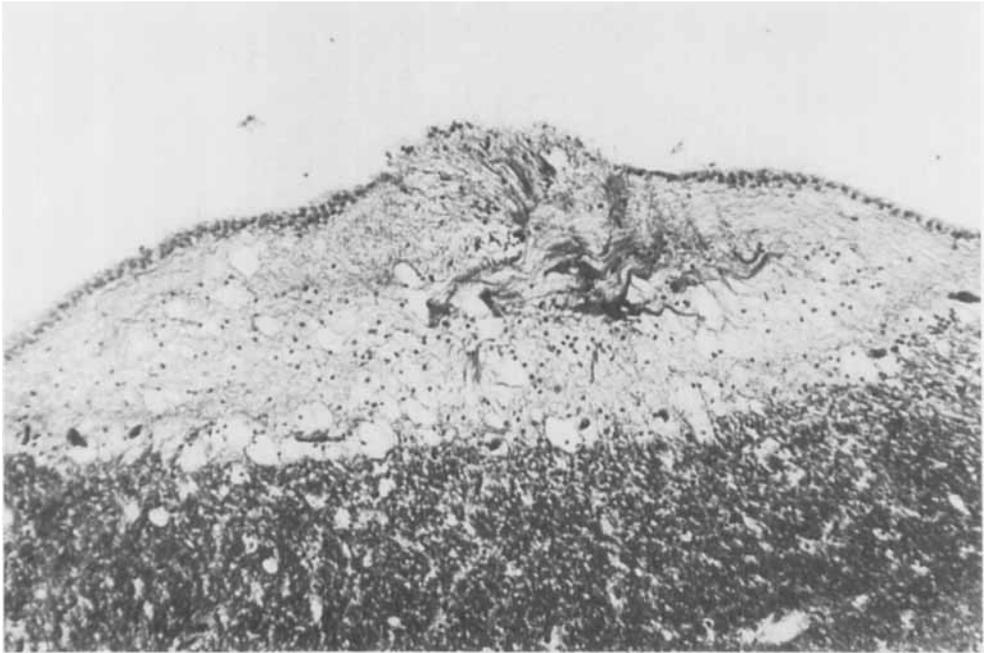


Figure 4. Chronic multiple sclerosis with granular ependymitis and subependymal gliosis. PTAH. $\times 60$.

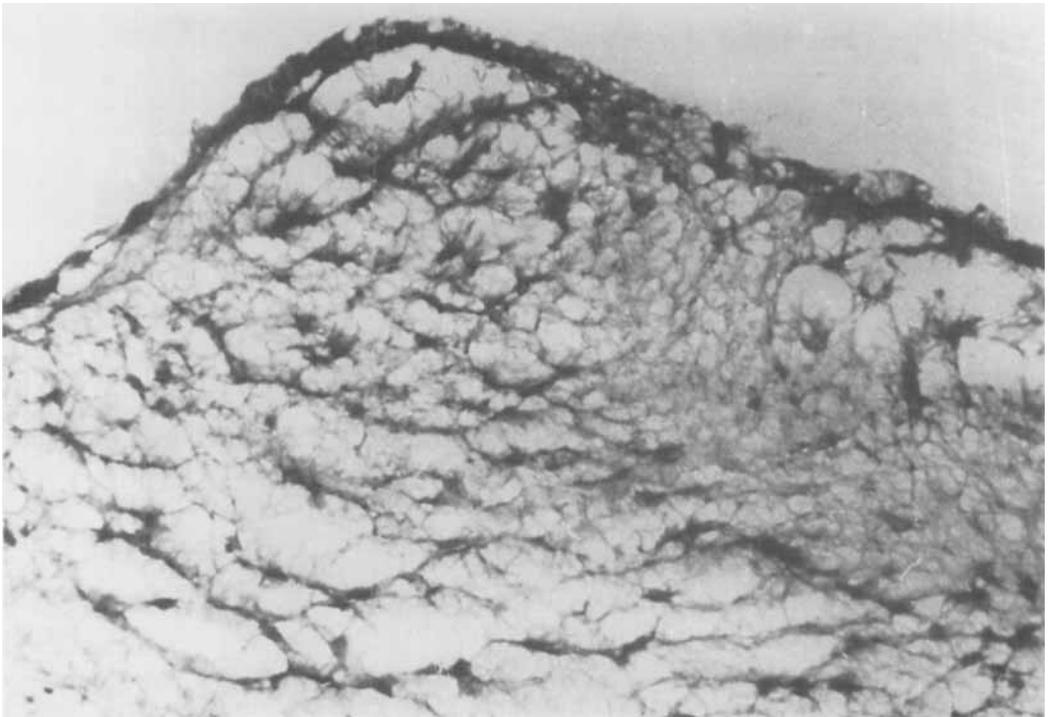


Figure 5. As for Figure 4, but stained to show glial fibrillary acidic protein in the ependyma and fibrillary astrocytes in the area of subependymal gliosis. GFAP-oxidase. $\times 120$.

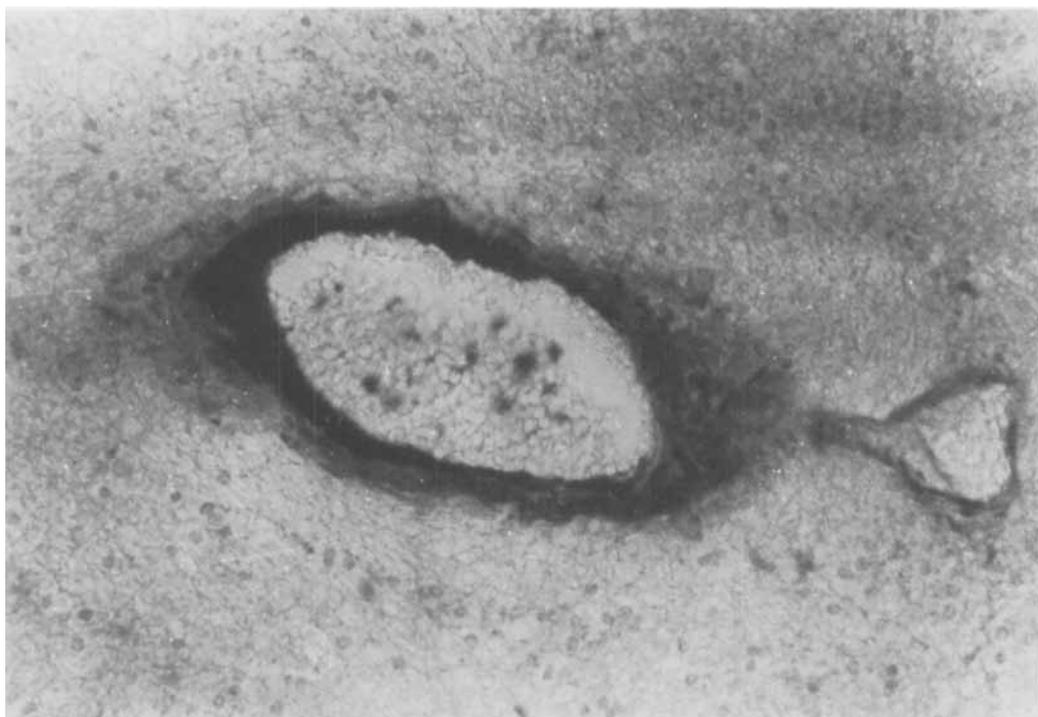


Figure 6. Complement C5 in and around wall of subependymal vein in subacute multiple sclerosis. C5-peroxidase. $\times 180$.

Discussion

Origin of periventricular plaques

These observations show that there is a predominantly perivenous demyelination in non-confluent periventricular lesions. These plaques are often accompanied by lymphocytic infiltration and show an irregular edge as evidence of active disease. Non-inflamed, sharply demarcated, supposedly older quiescent lesions are often confluent around at least part of the ventricle. These observations suggest that early lesions start around the subependymal vein wall, and subsequently coalesce with neighbouring lesions to form the characteristic old confluent periventricular or periaqueductal plaque. Computerized morphometry showed that the confluent periventricular plaque could not be explained by the supposition that it arises diffusely from the ependymal lining of the ventricle; its geometry was more precisely explained by the supposition that it arises around subependymal veins. The circular profile of most (69%) individual non-confluent components of plaques argues in favour of their perivenous origin, and against a point origin from the ependymal epithelium. Even flattened or half-circular plaques did not necessarily arise from the

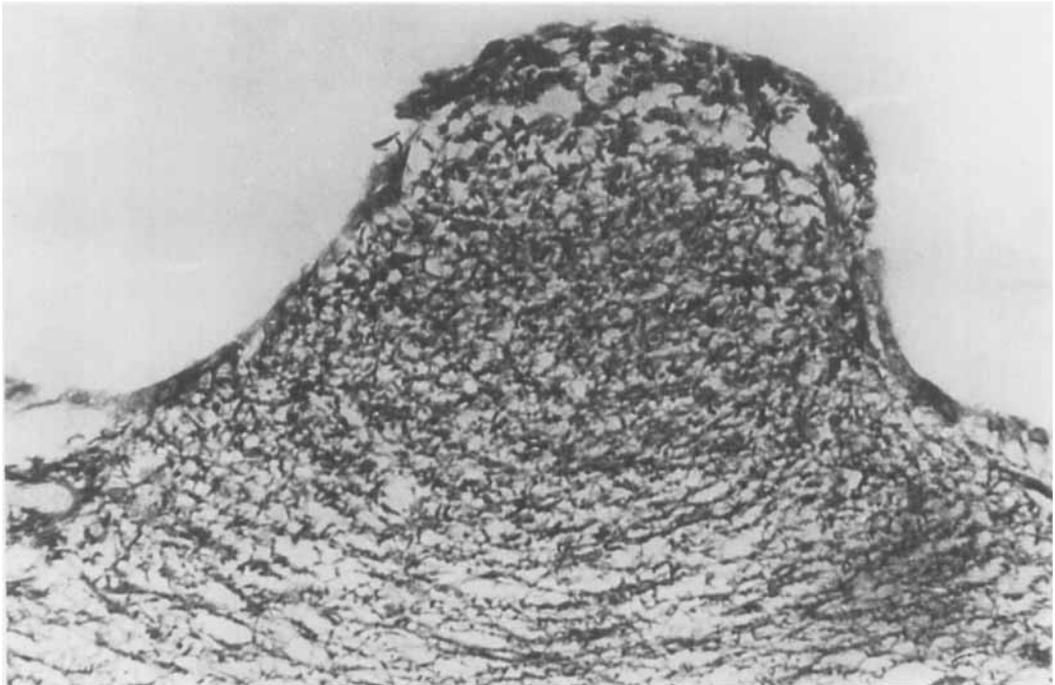


Figure 7. Immunoglobulin G in nodule of granular ependymitis and gliosis. IgG-peroxidase. $\times 120$.

epithelium and might have originated around subependymal veins immediately beneath the ependyma. A perivenous origin for the periventricular plaque was also indicated by gross observations on the brain stained with Nile blue sulphate (Figure 2). The initial perivenular demyelination along these parallel rows of subependymal veins and their subsequent coalescence to form a large confluent periventricular plaque illustrates the mechanism for plaque formation suggested previously by Adams (1975, 1977).

Granular ependymitis

Apart from the characteristic periventricular plaques of multiple sclerosis described above, many chronic or subacute cases show granular ependymitis and subependymal gliosis, which represent gliotic repair in response to loss of ependymal epithelium, presumably in this instance as a result of inflammatory changes in the vicinity of the ependyma and because the ependyma has only a limited capacity for repair (Weller *et al.*, 1971).

Granular ependymitis is distinct from the plaque, both in site (i.e. sometimes distant from plaques), and also in time of onset (i.e. less associated with lymphocytic infiltration and the early non-confluent perivenous lesion). Granular ependymitis is not an early feature of the disease and is not specific

for multiple sclerosis; for example, it is seen in association with ventricular dilatation or meningitis (see Results). Nevertheless, it does seem to provide a possible route in multiple sclerosis for movement of inflammatory agents (e.g. IgG and C3) between CNS lesion and CSF, or vice versa. This could explain the 23% incidence of raised albumin in the CSF in multiple sclerosis (Walsh & Tourtellotte, 1983), as well as the increase therein of myelin constituents, such as basic myelin protein (Cuzner *et al.*, 1978).

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