extends from Potts Creek Valley at Waiteville, Monroe County, West Virginia, at the northeast, and Big Stony Creek Valley at Kimballton, Giles County, Virginia, at the northwest, to upper Craigs Creek Valley north of Blacksburg, Montgomery County, Virginia, at the southeast, and Spruce Run Valley at Goodwins Ferry, Giles County, Virginia, at the southwest. In late August 1965, material was collected at "Baltimore" in four woods-bordered fields about evenly spaced along a 6 km stretch of Jones Falls in Green Spring Valley, elevation 90 m, Baltimore County, Maryland. Every effort was made to catch all *P. glaucus* females encountered, and, particularly at Baltimore, very few were missed. Females were not so numerous that the collector was simultaneously presented with a dark and a light one and forced to decide which morph to sample.

12. I was assisted in gathering the Mt. Lake sample of *P. glaucus* by S. N. Burns, M. P. Levin, and the members of my animal speciation class: C. Beirne, S. C. Cornick, E. S. Fouché, D. A. Graham, M. J. Hodge, A. K. Mester, W. H. Rittenhouse, E. Scvortzoff, J. T. Sigros, and D. H. Zimmerman. I collected the Baltimore sample and dissected all specimens of *P. glaucus* from both localities; most of the sample of *B. philenor* was collected by D. A. Graham (from 30 July to 3 August 1965) and dissected by him. I preserved the distal part of the abdomen of 35 Mt. Lake females in Kahle's fluid, stored crude abdominal dissections of all Baltimore females in 80 percent ethanol, and dissected out the spermatophores a few weeks later; both preservatives were satisfactory.
13. J. M. Burns, unpublished.
14. H. M. Graham, P. A. Glick, M. T. Ouye, D. F. Martin, *Ann. Entomol. Soc. Amer.* **58**, 595 (1965); M. T. Ouye, R. S. Garcia, H. M. Graham, D. F. Martin, *ibid.* **58**, 880 (1965).
15. V. B. Wigglesworth, *The Principles of Insect Physiology* (Methuen, London, 1953), pp. 469–475.
16. Although the data support this conjecture, their nature precludes rigorous statistical testing by the χ^2 test. Use of an independent 2×3 contingency table for each geographic sample of *P. glaucus* necessitates lumping the data from Mt. Lake in the three right-hand columns of the frequency distribution in Table 2, which results in the loss of some of the information critical in establishing the mating preference. For the data from Baltimore, the contingency table is in the form seen in Table 2. In each contingency table no cell has an expected frequency less than 1, but 33 percent of the cells (rather than the permissible maximum of 20 percent) have expected frequencies less than 5. For Mt. Lake, $\chi^2 = 4.61$, d.f. $= 2$, $P = .10$; for Baltimore, $\chi^2 = 4.84$, d.f. $= 2$, $.10 > P > .05$. Further testing, by adding the values from both samples, yields $\chi^2 = 9.45$, d.f. $= 4$, $.10 > P > .05$ (but with P very close to .05). A final analysis, involving calculation of χ values, their summation, and division by the square root of d.f., yields a standardized normal deviate of 2.18 corresponding to $P = .03$.
17. Supported in part by Mountain Lake Biological Station of the University of Virginia and NSF grant GB 3439. R. K. Selander critically read the manuscript.

22 April 1966

Glial Responses to Degenerating Cerebellar Cortico-nuclear Pathways in the Cat

Abstract. *Astrocytic changes in regions of long-term, cerebellar corticonuclear degeneration are characterized by large increases in cytoplasmic volume, as well as by formation of vacuoles and fibrils. Lipids are demonstrable in the vacuoles with oil red-O staining.*

Glial reactions to degenerative processes in the central nervous system have been described in a number of recent investigations, which have focused some attention on changes occurring in astrocytes in response to the degeneration of neural elements (*1–5*). We have observed reactive astrocytes in areas of the cerebellar nuclei of the cat in which large-scale degeneration of cerebellar cortico-nuclear fibers occurred after extensive destruction of the overlying cerebellar cortex.

Unilateral cortical lesions were made under aseptic conditions on the cerebellums of six adult cats by scratching the cortical surface with a needle. Subcortical white matter was largely spared. Each lesion, involving major portions of the cerebellar cortex on one side, resulted in degeneration of fibers to all three homolateral cerebellar nuclei (*6*).

All experimental animals were killed 3½ months after operation. Four were perfused with 5 percent glutaraldehyde made up in phosphate buffer (*7*). Small blocks of cerebellar nuclei (fastigius, interpositus, dentate) and adjacent white matter were removed from operated and control sides, treated with osmic acid, dehydrated, and embedded in capsules of Maraglas. Sections were cut on an LKB Ultratome, mounted on unsupported 200-mesh copper grids, stained with lead citrate (*8*), and viewed with an RCA EMU-3 electron microscope. Two animals were perfused with 10 percent formol-saline (0.9 percent NaCl); their cerebellums were cut into 20-μ frozen sections, and the sections were stained either with oil red-O (*9*), for the demonstration of lipid, or with the Nauta-Laidlaw silver method (*10, 11*), for the demonstration of degenerated fibers.

The morphology of normal macroglia found in areas of the cerebellar nuclei and adjacent white matter corresponded well with previous descriptions of these cells in other parts of the central nervous system (*4, 5, 12*). Oligodendrocytes were readily identifiable by the dense chromatin of their nuclei and by the relatively greater density of their cytoplasm. Normal astrocytes had large, round nuclei with dispersed chromatin and a cytoplasm less rich in ribosomes. Fibrous astrocytic processes were seen only rarely in normal cerebellar nuclear regions. Cells identifiable as microglia were never observed.

The responses of glial elements to long-term degeneration of cerebellar cortico-nuclear fibers was confined to cells which could be, in most cases, identified as astrocytes. There was no evidence of non-glial cellular infiltration which characterizes other degenerating systems (*2, 13*), at least not at this stage of degeneration, nor were microglia ever seen. This was a surprising observation since it is commonly held that the microglia participate in phagocytosis of degenerating neural elements.

The cytoplasmic volume of reactive astrocytes increased greatly, and numerous vacuoles and fibrils were formed. Increases in volume and vacuolation were particularly marked in perivascular astrocytic processes (Fig. 1) which are normally so attenuated that they are not easily identifiable. The membrane-bound vacuoles occupy most of the cytoplasm of the reactive cell, especially in perinuclear regions (Fig. 2). Most of them appear empty in electron micrographs, although dense material is often found in the cytoplasm in regions containing many vacuoles (Fig. 2). The remaining cytoplasm contains mitochondria and elements of granular and agranular endoplasmic reticulum (Fig. 2).

Fibrils, although not a common feature of normal astrocytes in the neuropil of deep cerebellar regions, are a distinct characteristic of the reactive astrocyte in these specimens and are easily discernible with both the light and electron microscopes (Figs. 1–3). The presence of fibrillar material, vacuoles, and glycogen (*5*) in the same cell (Fig. 3) indicates that the reactive glial cells in our specimens are astrocytes.

Frozen sections of degenerated regions, stained with oil red-O, reveal the presence of much reactive lipid; none is found in normal nuclear areas of the cerebellum. The stained lipid occupies the enlarged perivascular processes, as well as the cytoplasm of other reactive astrocytes, and its distribution corresponds well with the distribution of cytoplasmic vacuoles, indicating that most, if not all, of the lipid available for oil red-O reaction resides at these sites. Adjacent frozen sections prepared

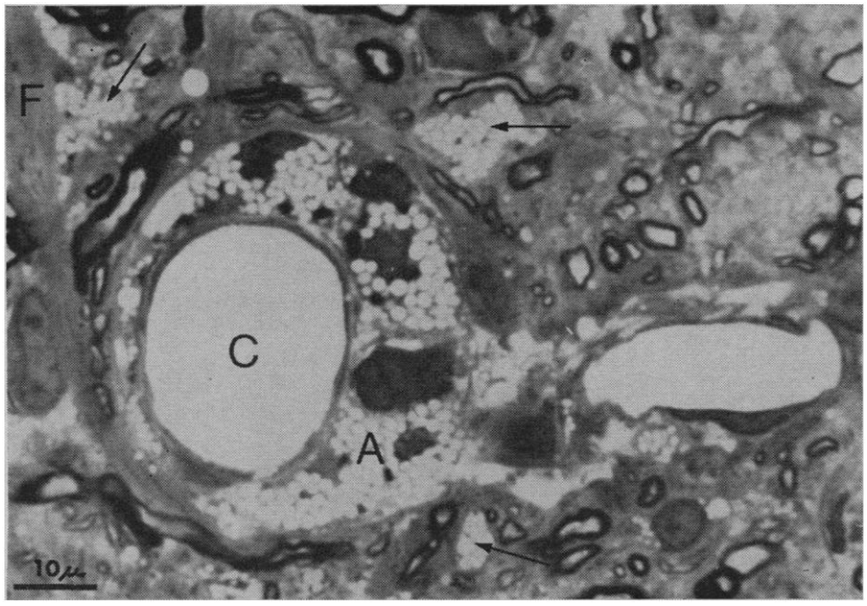

Fig. 1 (top). Extensive increase in size and vacuolation of perivascular astrocytic cytosplasm (*A*). The capillary (*C*) is distended from the perfusion pressure. Other patches of vacuolated astrocytic cytoplasm (arrows) as well as a large fibrous astrocytic process (*F*) are discernible. One-micron Maraglas section stained with toluidine blue.

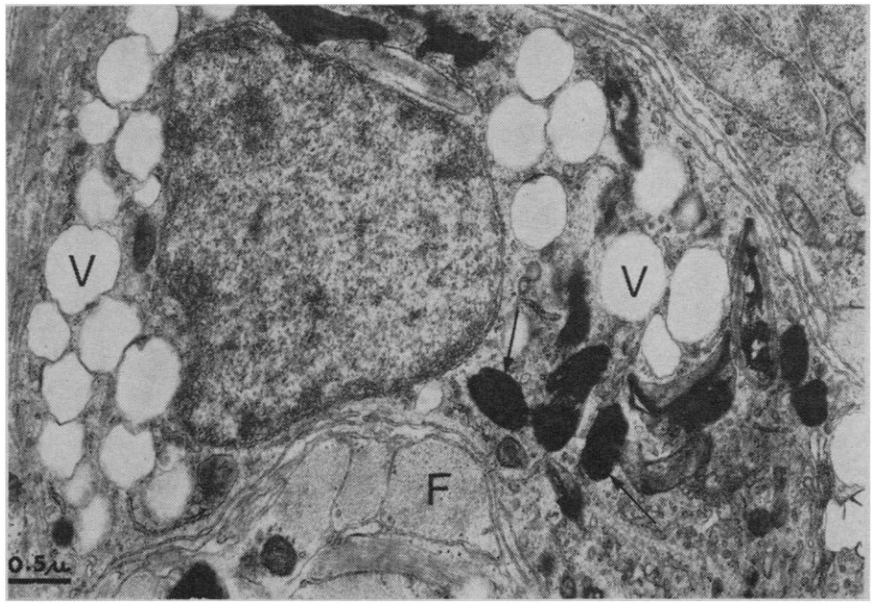

Fig. 2 (middle). Electron micrograph of a reactive astrocyte with typical membrane-bound vacuoles (*V*), and dense material (arrow) in the cytoplasm. Several fibrous processes (*F*) lie adjacent to the cell.

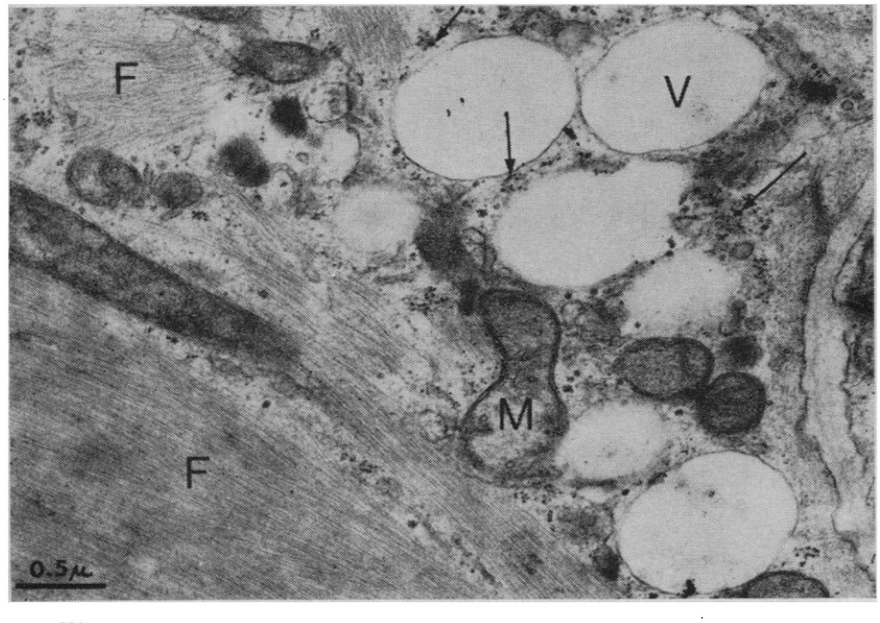

Fig. 3 (bottom). Electron micrograph of a portion of reactive astrocytic cytoplasm containing vacuoles (*V*), dense bands of fibrils (*F*), and glycogen granules (arrows). Several mitochondria (*M*) are also present in the field.

by the Nauta procedure have numbers of degenerated fibers, even at this relatively late postoperative stage. A comparison of the distribution of lipid material in Nauta-stained sections counterstained with oil red-O, shows that the two methods demonstrate different products, or the same product in two different forms. The Nauta-Laidlaw procedure, which is thought to demonstrate unsaturated lipid (*11*), stained material which was not distributed in the vacuoles of the reactive astrocyte.

The changes seen in astrocytes in our study may be, at least in part, a manifestation of some phagocytic activity. Although such a role for astrocytes has been proposed by several investigators (*3, 14*), there is no direct evidence, either from this or other studies, that the astrocytes function in such a capacity. Examination of earlier stages of the degenerative process in this system should determine whether other cellular elements, glial or non-glial, respond in similar fashion.

ROBERT P. EAGER
PATRICIA R. EAGER
Department of Anatomy,
Yale University School of Medicine,
New Haven, Connecticut

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Separation of Gases by Zeolites

In "Electrostatic aspects of physical adsorption: Implications for molecular sieves and gaseous anesthesia," (*1*) Benson and King correctly state that the adsorptive properties of zeolites can be modified by exchanging the mobile cations for different ones. However, exception must be taken to their conclusion that the separation of gases by zeolites is due primarily to adsorption on the external surface of the crystal, rather than to adsorption in the internal cavities of the zeolite.

When a zeolite is outgassed at elevated temperature under vacuum and then cooled to a lower temperature, the zeolite crystals will occlude in their cavities, molecules of any gas to which they are exposed (subject to molecular-size restrictions). Barrer and Gibbons (*2*) have shown that the saturation sorption capacity of synthetic faujasite for CO_2 corresponds quite well to the volume of the large cages in this zeolite. They have also shown that the amount of NH_3 occluded by synthetic faujasite corresponds quite well to that predicted by assuming that the large cages and sodalite cages in this zeolite are filled (*3*). Rees and Williams have similarly shown that the saturation sorption capacity of synthetic faujasite for krypton is equal to that calculated by assuming the large cages are filled with krypton atoms (*4*). These studies have shown how the sorptive properties of the zeolite depend on the kind of exchangeable cation which is present in the zeolite.

In the investigation of NH_3 sorption (*3*) it was shown that the temperature dependence of the saturation sorption value was quite similar to the temperature dependence of the density of liquid NH_3. In a previous study of the occlusion of hydrocarbons by synthetic faujasite (*5*), Barrer and co-workers showed that, near saturation of the crystals, the thermal expansivity of the occluded hydrocarbons was quite similar to the thermal expansivities of the liquid hydrocarbons. The occluded sorbate was likened to a "capillary condensate." Quoting the authors further: "Unlike usual micropore systems, however, we are now considering a regular intersecting channel system, with uniform alternations between a widest diameter of only ~12A and a narrowest diameter of only ~9A. Because of this, capillary condensation and occlusion merge, and no hystereris is observed. No part of any channel is wide enough to escape the field of sorption potential due to the surrounding lattice." It should be pointed out that the zeolite faujasite, which Barrer was studying, is one of the most open zeolite structures. It is still more certain that in other zeolites the occluded gas molecules are under the influence of a sorption potential.

Since the crystal structure of many zeolites is known, Barrer and co-workers have made some efforts to calculate the sorptive potentials. They have considered guest molecule interactions with both the mobile cations and the anionic lattice (2–4,6). Thus, it is quite certain that the gas molecules adsorbed by a zeolite are occluded in the cavities and that it is the internal surface that is responsible for this sorption. Certainly the external surface area could not be responsible for the large sorption capacities that have been observed.

The positions of the exchangeable cations are known for many zeolites (*1*). They are certainly within the interior of the crystal and not on the surface. Thus exchanging cations should modify the sorption potential at the exterior of the crystal very little. Sorption of gas molecules at the crystal faces most probably depends primarily on their interaction with hydroxy groups on the crystal faces (*8*). The weakly acidic protons in these groups should not exchange for other cations at the *p*H used to exchange the exchangeable cations that are in the zeolite. Adsorption on the exterior surface of the crystal should be a relatively constant factor. The action of a sorption potential in the interior of the zeolite will also explain why Benson and King find a linear correlation between retention volumes for nonpolar molecules and atoms and their polarizability, when it is realized that all the molecules and atoms used could penetrate the Linde 5-A (Ca-A) sieve used. No investigator of zeolites today believes that the adsorption and separation of gas molecules on zeolites is determined primarily by the relative size of the adsorbate molecules and the channels in the zeolite. Benson and King cite Barrer in 1949 (*9*) as believing this. What Barrer said was, "The molecular-sieve property of zeolites would suggest that clear-cut separations could be obtained in molecular mixtures of which one constituent was freely occluded by the zeolite, while the other constituent, having the wrong molecular dimensions, was not occluded." Barrer was only pointing out that the most striking separations that can be made on zeolites do depend on the relative sizes of the gas molecules and the channels in the zeolite. For instance, the synthetic zeolite Linde 4-A (sodium form of Linde A) will not sorb normal paraffins, isoparaffins, and N_2 (*10*). If about one-third of the sodium ions are replaced with calcium ions, by ion exchange, normal paraffins and N_2 are sorbed in large quantities, but no isoparaffins are sorbed. This phenomenon does, indeed, depend on the relative minimum cross-sections of the gas molecules for diffusion and the size of the zeolitic channel. Many other examples can be found in the literature.

Ion exchange can change the sorptive properties of zeolites for two reasons. In the first case, ion exchange changes the sorption potential in the interior of the zeolite cavities causing the kinds of subtle changes in selectivity that are normally seen in sorption on conventional adsorbents. Once it is realized that gas molecules must diffuse through narrow openings to gain access to the cavities of a zeolite, it then becomes reasonable that, if cations are located in the channels of a zeolite, there is some molecule which, although it is small enough to diffuse through the empty channel, is excluded from the partially blocked channels. If the blocking cations are removed by ion exchange, this molecule can now freely diffuse through the channel. This is what happens in the case of calcium exchange of Linde 4-A. Two sodium cations are replaced by one calcium cation and the channels become unblocked, giving normal paraffin and nitrogen molecules access to the cages in the interior of the crystal.

In summary, it can be safely concluded that occlusion of guest molecules in the cavities is responsible for the sorptive properties of zeolites. Because the internal cavities or pores are so small, all the guest molecules are under