ELECTRON MICROSCOPIC DEMONSTRATION OF CONNECTIONS BETWEEN GLIA AND MYELIN SHEATHS IN THE DEVELOPING MAMMALIAN CENTRAL NERVOUS SYSTEM

MARY BARTLETT BUNGE, RICHARD P. BUNGE, and GEORGE D. PAPPAS. From the Department of Anatomy and Laboratory for Cell Physiology, Columbia University College of Physicians and Surgeons, New York City

Evidence is accumulating that central myelinogenesis in mammals and amphibians is accomplished by spiral wrapping of glial cell processes (1–5). As the glial process spirals around an axon, the contained cytoplasm is obliterated and the spiraled plasma membrane comes together to form compact myelin. This mechanism of spiral wrapping is basically similar to the way in which myelin is formed in the peripheral nervous system (6–8). The sheath-glial or sheath–Schwann cell relationships that result from this mechanism are similar in some ways, dissimilar in others. It has long been thought by Penfield, among others, that the alignment of oligodendrocytes into rows and the envelopment of nerve fibers by their processes resemble the relationship of the sheath of Schwann cells to peripheral fibers (9). Two differences are that a central sheath is not completely encircled by cell cytoplasm and that every central sheath is not conspicuously associated with a cell perikaryon.

It is claimed by electron microscopists that cytoplasm on the central sheath exterior is confined to the paramesaxonal area or to its modified counterpart, the loop (1–5). And, yet, whether the substance in these areas is truly cytoplasm or derived therefrom could be questioned, because in the adult the usual organelles are lacking. From our studies of younger myelinating central nervous tissue, however, we have found that cytoplasmic organelles are indeed present in such areas. This is as would be expected if the central sheath is formed by a mechanism similar to that for peripheral myelinogenesis. It has been postulated, therefore, despite lack of observed connecting processes, that (*a*) a central sheath is connected

to a cell as in peripheral nerve but that (*b*) the central sheath and its glial perikaryon may be separated by some distance (1, 3, 5). It is the purpose of this paper to demonstrate these two points in developing central nervous tissue.

MATERIALS AND METHODS

Kitten spinal cord was harvested at varying intervals by the following technique. All the cerebrospinal fluid available at cisternal puncture was removed and fixative was injected into the subarachnoid space to replace it. The fixing solution consisted of 2 per cent OsO_4 and 0.01 per cent $CaCl_2$ in pH 7.4 veronal-acetate buffer. A cut through the meninges was made in the lumbar region and, as more fixative was injected into the cisterna, excess fluid dripped out below. When such a cut is made just after the initial injection of fixative, the peripheral cord is already blackened. The subarachnoid space flush was continued for 20 minutes at which time a segment of cervical cord was extirpated. The cord was then immersed in fresh fixative, cooled to 4°C, and kept this way so that fixation time from the first injection totaled 2 hours. The cord segment was dehydrated in 30, 50, and 70 per cent ethanol. At this time, small wedges were cut from the cord periphery. Dehydration was continued in ethanol and propylene oxide preparatory to embedding in Epon 812 (10). Sections, cut with glass knives on a Servall Porter-Blum microtome, were placed on formvar-coated grids and stained in 7.5 per cent uranyl acetate for 2½ to 4 hours at room temperature. They were examined in an RCA EMU-3C electron microscope and photographed on Ilford N.60 plates. Initial magnifications range from 2,000 to 13,500.

OBSERVATIONS AND DISCUSSION

In transversely sectioned myelinating spinal cord, outer loops vary greatly in size. While the small loops contain little more than characteristic particles, the larger ones contain recognizable cell cytoplasm. Mitochondria, particles presumed to be ribosomes which are free and in association with vesicles and cisternae of endoplasmic reticulum, and vesicles of agranular membrane are contained within them. Where outer mesaxons replace loops, such patches of cytoplasm are located on one side of the mesaxon. An outer loop results from fusion of one of the two mesaxonal membranes with compact myelin (1, 2).

A number of the outer loops or paramesaxonal enlargements are elongated, the tip farthest from the sheath often appearing obliquely sectioned. If such a loop or paramesaxonal enlargement is examined in neighboring sections, it is seen to lengthen and become continuous with a nearby glial perikaryon. Thus it was discovered in myelinating cord that cytoplasm in the outer loop is continuous with a glial cell. To establish that such a connection exists, the membrane emerging from compact myelin must be seen to be continuous with the membrane bounding the glial cell (Figs. 1 and 2).

That a typical connection between a sheath and glial cell may be long and slender is suggested by the finding that, in a series of sections, it appears and disappears. Table I shows that connections vary in length. The sheath may indent the perikaryon or be as far as 12.3 μ from the nucleus. Since the point of departure of the connecting process from the perikaryon is sometimes difficult to ascertain, the distance from the sheath to the nearest portion of nuclear membrane was chosen arbitrarily to express this variation. Most of the connections observed are at the level of the nucleus. The thickness of the connecting process also varies (Table I). The longest process found, 12.3 μ from nucleus to sheath, was only 0.1 μ thick in one area. The same connection was even thinner at a nearby level; the bounding plasma membrane was closely apposed, with all cytoplasm obliterated, for a distance of 0.7 μ. Two connections (Figs. 3 to 6) but no more than two were seen to emerge from one perikaryon at a given level. The orientation of the attachment of the process to the sheath and the inner mesaxon is consistent with the spiral wrapping theory. We have no conclusive evidence as yet to support our idea that there is but one connection per internode.

Contained within a typical connection is cytoplasm like that in the perikaryon. Mitochondria, Golgi complex, free ribosomes, and ribosomes associated with cisternae of endoplasmic reticulum are present. The cisternae, often distended and containing flocculent material, are sometimes oriented. Frequently there are oriented cisternae near the attachment to the sheath (Figs. 1 and 3). Of the two distinct glial types in adult peripheral spinal cord, oligodendrocytes contain oriented cisternae but fibrous astrocytes never do (5). Identification is further aided by the lack of astrocytic compact fibril bundles in the cells dispatching connecting processes. Thus, it appears that the sheaths are connected to oligodendrocytes 1 to 2 weeks after birth, the intervals studied most thoroughly thus far. Whether the myelin-forming

cells display similar structural characteristics at an earlier stage is currently being investigated.

Except where the glial process connects to the sheath, we believe that the glial cytoplasm is confined to a ridge along the internode. Such a ridge is seen in cross-section as a loop. If this ridge and the glial connection were stainable and visible in the light microscope—and we know that some of the loops and connections are certainly large enough in young tissue—one would expect to obtain a picture like that presented by Ramón-Moliner (Fig. 1, reference 11). He shows an oligodendrocyte from kitten brain in which fine, long processes emerge and run parallel to fibers. Penfield also considers that expansions from an oligodendrocyte pass upward and downward on a fiber, and he illustrates this point with a picture taken from del Rio Hortega (Fig. 13, p. 441, reference 9). The additional processes that he finds encircling the fiber could conceivably represent areas in which cytoplasm is retained around the circumference of, as well as along, the sheath. These points are offered in support of the concept of central myelin-glial relationships we have summarized in Fig. 18 in reference 5.

Whether the connections between glia and sheaths persist throughout adulthood is another question. Whereas they have not yet been observed in the adult, it seems likely that they are indeed maintained because cytoplasm, admittedly modified, persists in the inner and outer paramesaxonal areas, in the outer loops, and in the sheath endings delineating nodes. Connecting processes may not be seen so readily in mature cord because the sheaths are packed together so much more closely. This question is under investigation.

TABLE I

Representative Connections between Glia and Myelin Sheaths Observed Electron Microscopically

No.	Shortest distance* between sheath and glial nucleus via connection	Narrowest thickness* of connecting process
	μ	μ
1.	0.2	Sheath indents perikaryon
2.	0.3	1.0
3.‡	1.0	0.5
4.	1.2	0.06
5.	4.3	1.5
6.	4.4	0.8
7.	7.3	0.6
8.§	(*a*) 6.0	1.6
	(*b*) 11.2	0.6
9.	12.3	0.1

Diameters* of axons within sheaths connected to these glia range between 0.9 and 1.8 μ; diameters* of nuclei in glia displaying these connections are between 3.6 and 5.8 μ.

* All measurements were obtained from random micrographs of thin sections. Hence, they do not represent necessarily the largest or smallest dimension that would be found by examining all other levels.

‡ Fig. 1.

§ Fig. 3.

SUMMARY

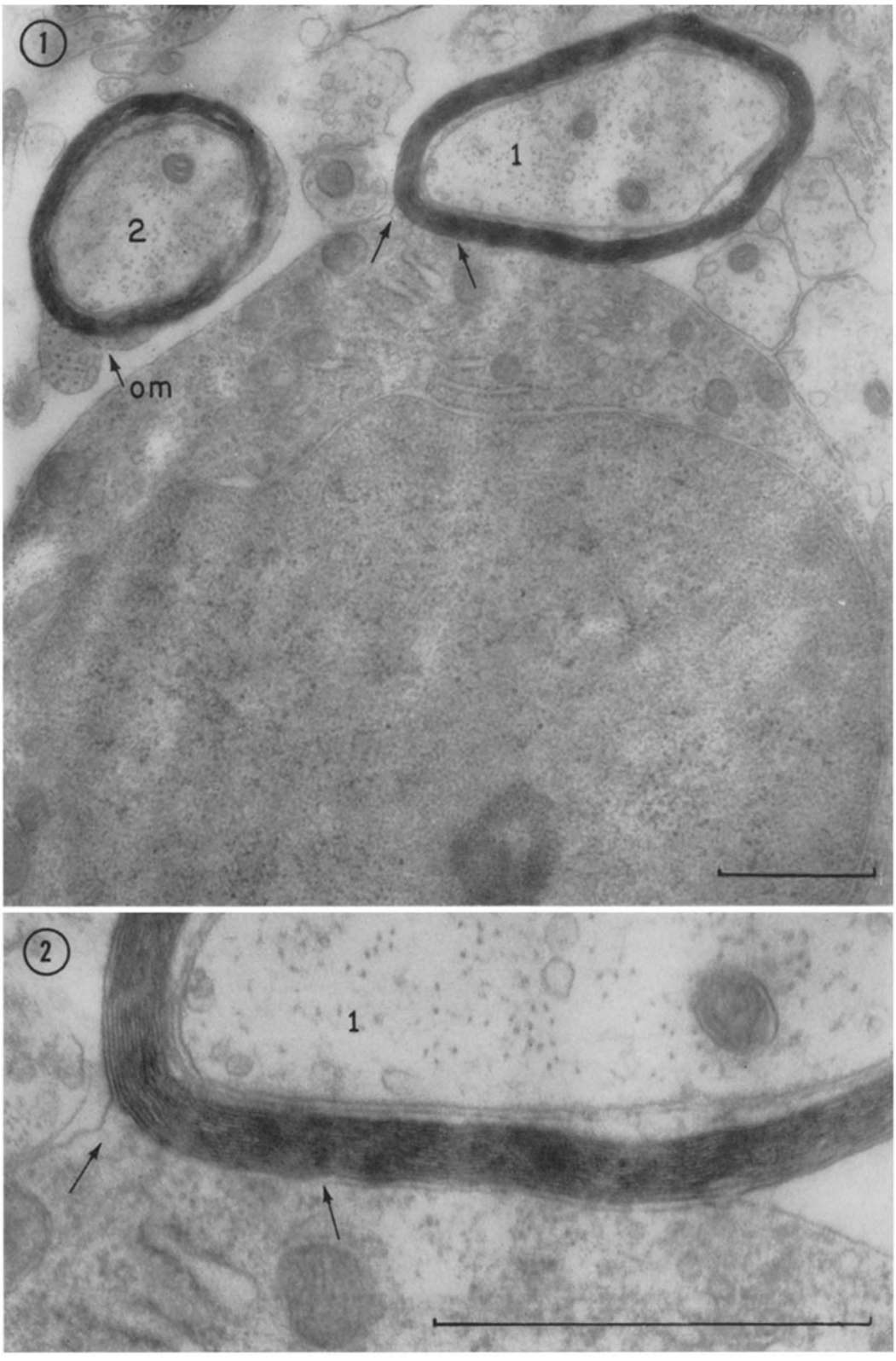
This paper demonstrates for the first time with the electron microscope that connections between myelin sheaths and glia exist in developing mam-

Figure Legends

The bar equals 1 micron.

FIGURES 1 AND 2

These electron micrographs demonstrate continuity of the glial cell plasma membrane with the outermost major dense line of the myelin sheath surrounding axon *1*. The distance delineated by the arrows is the glial-myelin sheath connection. The myelin investing axon *2* is a more typical example of transversely sectioned sheaths. Only a little cytoplasm is seen on the sheath exterior, an outer mesaxon (*om*) is visible, and there is no suggestion of a cell connection. 12 days after birth. Fig. 1, × 24,000; Fig. 2, × 68,000.



malian central nervous tissue. A perikaryon and its sheath may be separated by some distance. The connecting processes are described. Central sheath-glial relationships are discussed briefly and compared to possible correlates observed in the light microscope.

Dr. Mary Bunge holds a Postdoctoral Fellowship from the National Institute of Neurological Diseases and Blindness, United States Public Health Service.
Dr. Richard Bunge is a Postdoctoral Fellow of the National Multiple Sclerosis Society.
This study was supported in part by a research grant (No. B-2314 C) from the National Institute of Neurological Diseases and Blindness, United States Public Health Service.

Received for publication, August 21, 1961.

LITERATURE CITED

1. MATURANA, H. R., *J. Biophysic. and Biochem. Cytol.*, 1960, **7**, 107.
2. PETERS, A., *J. Biophysic. and Biochem. Cytol.*, 1960, **7**, 121.
3. PETERS, A., *J. Biophysic. and Biochem. Cytol.*, 1960, **8**, 431.
4. METUZALS, J., European Regional Conference on Electron Microscopy, 1960, Delft, Nederlandse Vereniging Voor Electronenmicroscopie, 1961.
5. BUNGE, M. B., BUNGE, R. P., and RIS, H., *J. Biophysic. and Biochem. Cytol.*, 1961, **10**, 67.
6. GEREN, B. B., *Exp. Cell Research*, 1954, **7**, 558.
7. ROBERTSON, J. D., *J. Biophysic. and Biochem. Cytol.*, 1955, **1**, 271.
8. ROBERTSON, J. D., *J. Biophysic. and Biochem. Cytol.*, 1958, **4**, 349.
9. PENFIELD, W., *in* Cytology and Cellular Pathology of the Nervous System, Section IX, (W. Penfield, editor), **2**, New York, Paul B. Hoeber, Inc., 1932.
10. LUFT, J. H., *J. Biophysic. and Biochem. Cytol.*, 1961, **9**, 409.
11. RAMÓN-MOLINER, E., *J. Comp. Neurol.*, 1958, **110**, 157.

FIGURES 3 TO 6

The oligodendrocyte (*ol*) in Fig. 3 displays two processes connected to myelin sheaths. The continuity between glial plasma membrane and myelin of axon *1* is shown in Fig. 4. This enlargement was obtained from another level. The second connecting process is shown at different levels in Figs. 3 and 6. In Fig. 3, the process appears to be connected to both the sheath surrounding axon *2* and a sheath (star) adjacent to it. The starred sheath, however, is but an outpocketing of the sheath around axon *2*; it has disappeared at the level shown in Fig. 6. In Fig. 6, the connection to the sheath investing axon *2* is more clear. Fig. 5 is an enlargement obtained from this level. The glial plasma membrane can be followed all around the sheath because some glial cytoplasm (*gc*) has been retained on the sheath exterior. Both the outer mesaxon (*om*) and inner mesaxon (*im*) are visible. Inner and outer mesaxons of other sheaths can also be seen in Fig. 5. 12 days after birth. Fig. 3, × 10,000; Fig. 4, × 40,000; Fig. 5, × 33,000; Fig. 6, × 9,000.