ULTRASTRUCTURE OF THE CAROTID BODY

T. J. BISCOE and W. E. STEHBENS

From the Departments of Physiology and Experimental Pathology, The John Curtin School of Medical Research, Canberra, Australia. Dr. Biscoe's present address is The Cardiovascular Research Institute, San Francisco Medical Center, San Francisco, California

ABSTRACT

An electron microscope investigation was made of the carotid body in the cat and the rabbit. In thin-walled blood vessels the endothelium was fenestrated. Larger vessels were surrounded by a layer of smooth muscle fibers. Among the numerous blood vessels lay groups of cells of two types covered by basement membranes. Aggregates of Type I cells were invested by Type II cells, though occasionally cytoplasmic extensions were covered by basement membrane only. Type I cells contained many electron-opaque cored vesicles (350 to 1900 A in diameter) resembling those in endocrine secretory cells. Type II cells covered nerve endings terminating on Type I cells and enclosed nerve fibers in much the same manner as Schwann cells. The nerve endings contained numerous microvesicles ($\sim$ 500 A in diameter), mitochondria, glycogen granules, and a few electron-opaque cored vesicles. Junctions between nerve endings and Type I cells were associated with regions of increased density in both intercellular spaces and the adjoining cytoplasm. Cilia of the 9 + 0 fibril pattern were observed in Type I and Type II cells and pericytes. Nonmyelinated nerve fibers, often containing microvesicles, mitochondria, and a few electron-opaque cored vesicles (650 to 1000 A in diameter) were present in Schwann cells, many of which were situated close to blood vessels Ganglion cells near the periphery of the gland, fibrocytes, and segments of unidentified cells were also seen. It was concluded that, according to present concepts of the structure of nerve endings, those endings related to Type I cells could be efferent or afferent.

The carotid body is a chemoreceptor sensitive to changes in concentrations of blood oxygen and carbon dioxide. It is supplied by two nerves. One of these is the sinus nerve, which contains the chemoreceptor afferent fibers (9, 40). The other nerve is a postganglionic branch of the superior cervical ganglion (36), excitation of which increases the activity recorded in the chemoreceptor afferents (4, 28, 34) and decreases the blood flow through the carotid body (10).

De Castro (11–13) suggested that certain parenchymal cells within the carotid body functioned as chemoreceptors, now a generally accepted view. If De Castro is correct, one might expect specialization at the nerve ending–receptor cell junction, but this has not been demonstrated. Therefore, the fine structure of the carotid body was reexamined, with special reference to the nerve endings, and this paper presents additional morphological observations pertaining to the physiological role of the carotid body. A brief preliminary account of a few of the early results has been published (5).

METHODS

One carotid body was removed from each of 10 cats and 8 rabbits, in the following manner. The animals were anesthetized with intraperitoneal sodium pentobarbitone, 30 mg/kg. The trachea was cannulated, and the larynx and pharynx were reflected in the

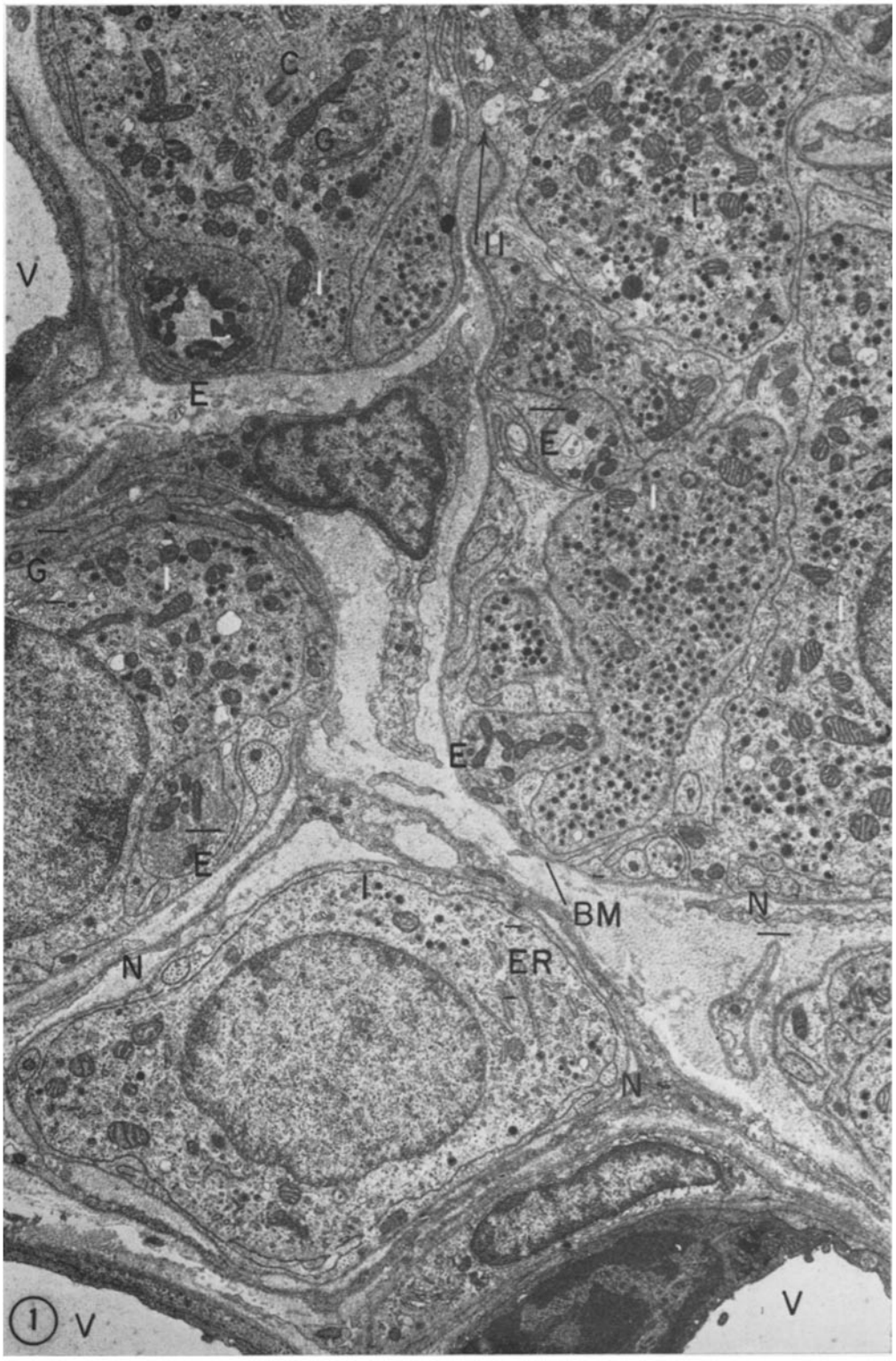

FIGURE 1 Carotid body tissue showing blood vessels (*V*), Type I cells (*I*) containing numerous electron-opaque cored vesicles, Type II cells (II), nerve endings (*E*), endoplasmic reticulum (*ER*), centriole (*C*), nerve fibers (*N*), and Golgi apparatus (*G*). In one Type II cell there are large vacuoles of the Golgi apparatus (arrow). Note the basement membrane (*BM*) around the cell groups. × 10,000.

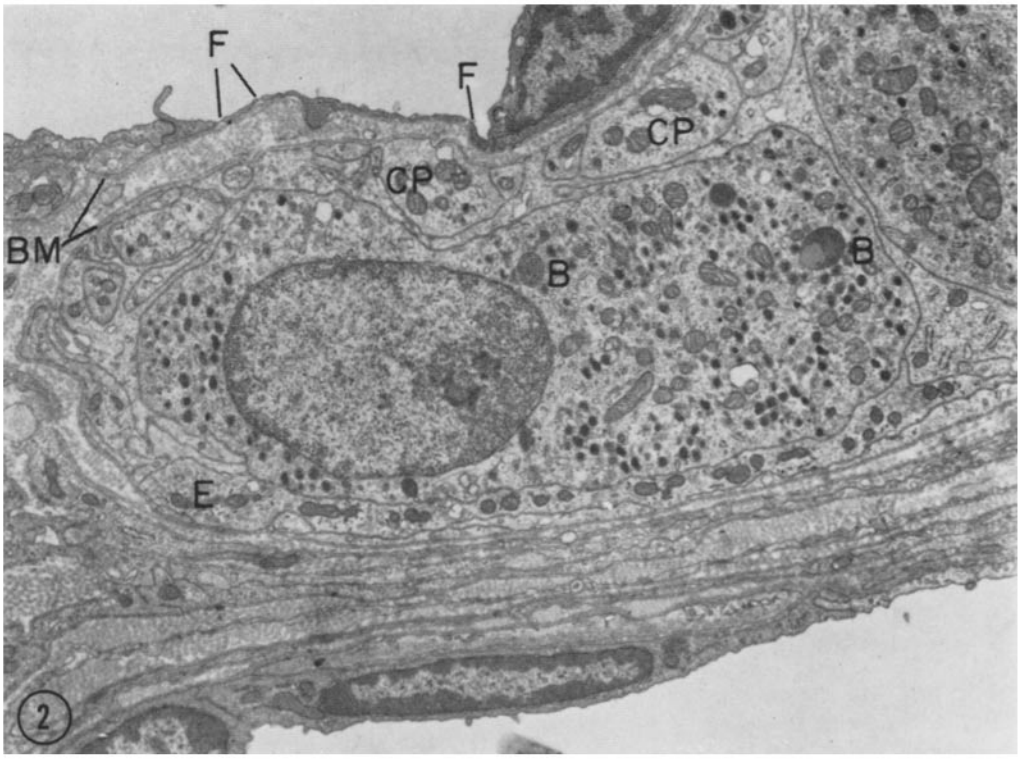

FIGURE 2 Type I cell containing electron-opaque cored vesicles, several rounded bodies (*B*) (probably lysosomes), and mitochondria. It is surrounded by a Type II cell and basement membrane (*BM*). A nerve ending (*E*) is in contact with the Type I cell over a length of 6 μ. Fenestrae (*F*) are present in the endothelium of one blood vessel which is separated by basement membrane (*BM*) and collagen from parenchymal cells. The other vessel (bottom) is separated from these cells by unidentified cell processes alternating with layers of collagen. Within the Type II cell are cell processes (*CP*), some containing electron-opaque vesicles, which are probably extensions of Type I cells. Some of these processes are covered on one side only by basement membrane. × 12,500.

midline to expose the medial aspect of the common carotid bifurcation. The external carotid artery was ligated and a Polythene cannula inserted into the common carotid artery. Prior to commencing perfusion, flow through this artery was arrested for no more than 30 sec during which time there was invariably a reflux of blood into the cannula from anastomotic vessels in the sinus region. The sinus and the carotid body were perfused, via the cannula, with 5 to 10 ml of either 5% glutaraldehyde in Sorensen's phosphate buffer at pH 7.3–7.4 or 1% buffered osmium tetroxide at pH 7.3–7.4 (78). Perfusion pressure was sufficient to overcome the reflux of blood into the carotid body vein as viewed with the dissecting microscope. The carotid body was then removed, cut into small blocks, and placed in fresh fixative. After perfusion with glutaraldehyde, fixation was continued in fresh glutaraldehyde for 1 to 3 hr. The blocks were washed in phosphate buffer for 1 to 2 hr, postfixed in 1% osmium tetroxide for 16 hr, dehydrated in ethanol, and embedded in Araldite. After perfusion with osmium tetroxide, fixation was continued for 16 to 18 hr, and the blocks were then dehydrated in ethanol and embedded in Araldite. Thin sections were cut on an LKB microtome, stained with lead (51) and uranyl acetate, and examined in a Siemens Elmiskop I.

The illustrations are of cat tissue fixed in glutaraldehyde, unless otherwise stated.

RESULTS

The basic morphology of the carotid body was similar in the cat and the rabbit.

The parenchymal cells, found in groups among blood vessels (Figs. 1 and 2), were of two main

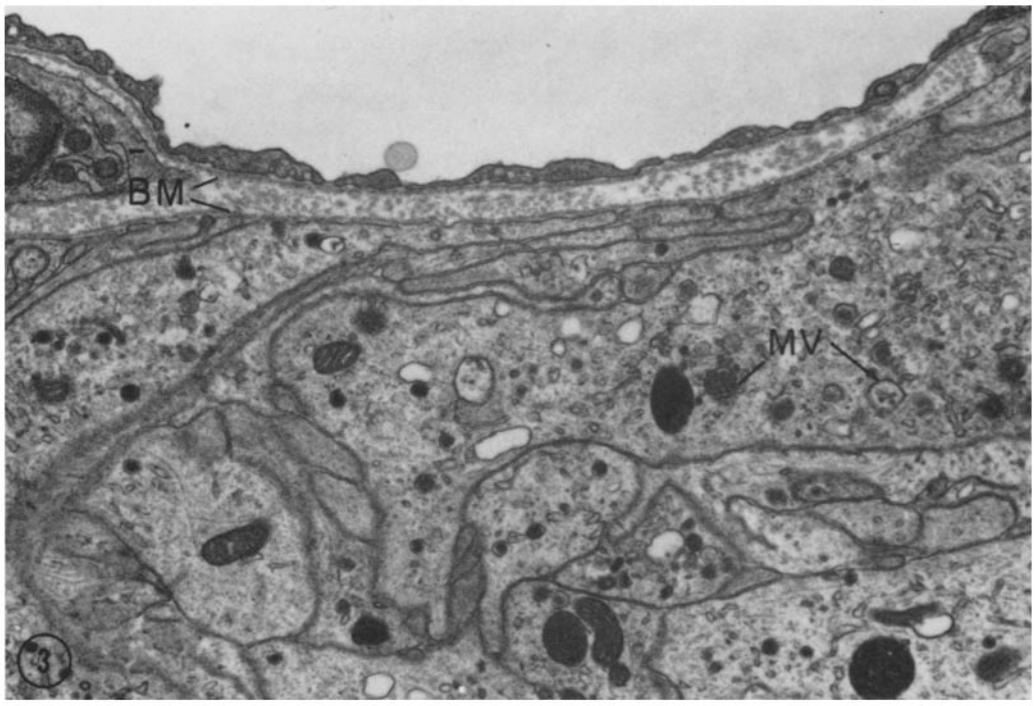

FIGURE 3 Two processes of Type I cells covered on one side only by basement membrane (*BM*) separated by collagen from the basement membrane of fenestrated endothelium. Multivesicular bodies (*MV*) are present in one cell. × 15,000.

types. One cell type, which contains vesicles having an osmiophilic, electron-opaque core, has been variously referred to as the glomus cell (49), chemoreceptor cell (69), or Type I cell (14, 15) and probably corresponds to De Castro's epithelioid cell. The second cell type has no osmiophilic granules. It has been variously called pericyte (49), sustentacular cell (69), or Type II cell (14, 15). The terms Type I cell and Type II cell (14) will be used because no specific function is thus implied.

Blood Vessels

Blood vessels were numerous and were more than 7 μ in diameter. In cross-section they were lined by three or more endothelial cells. Some vessels were partly invested by pericytes, while larger vessels were surrounded by a layer of smooth muscle fibers. There was no elastic tissue in their walls. The layer of flattened endothelium was continuous and of variable thickness. The endothelial cells contained the usual cytoplasmic constituents (32, 56, 75), and fenestrae (500 to 700 A in diameter) were found in attenuated segments of thin-walled vessels (Figs. 2 and 3). Cilia were observed in pericytes; they projected from the endothelial aspect of the cells, but their fibril pattern was not determined.

Type I Cells

These cells were complex in shape. They occurred in groups and were invested by Type II cells (Figs. 1, 2, and 4). Occasionally, exposed cytoplasmic extensions were covered by only the basement membrane surrounding the cell groups (Figs. 3 and 4). Sometimes these exposed segments of Type I cells were in close proximity to the basal surface of vascular endothelium and its associated basement membrane (Fig. 3).

The distinguishing feature of Type I cells was the presence of electron-opaque cored vesicles (49, 69), 350 to 1900 A in diameter, widely distributed throughout the cytoplasm, though their concentration varied from cell to cell. Each vesicle was bounded by a single trilaminar membrane and contained material which varied from dense to

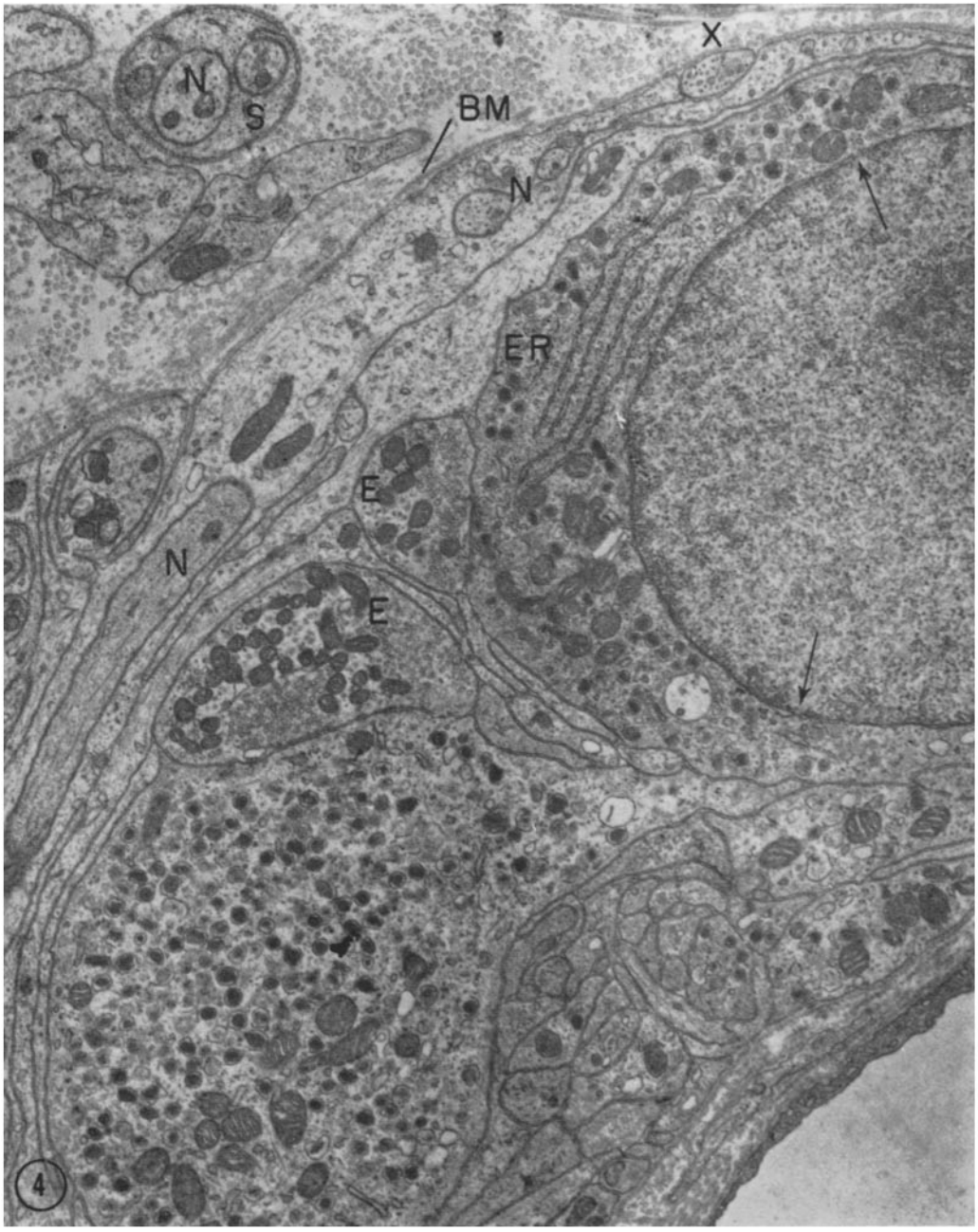

FIGURE 4 Type I cells with nerve endings (*E*), showing the complexity of the cell outlines within the basement membrane (*BM*) of the Type I/Type II cell complex. Nuclear pores (arrows), endoplasmic reticulum (*ER*). Nerve fibers (*N*) are surrounded by Type II cells and a Schwann cell (*S*). Nerve fiber at *X* is covered by basement membrane on one side. × 15,000.

lightly granular (Figs. 2 to 4) and rarely filled the vesicles. At times, this electron-opaque material appeared in the form of a ring with a less dense center. These vesicles were plentiful in glutaraldehyde-fixed tissue, whereas in OsO_4-fixed tissue they showed depletion of their con-

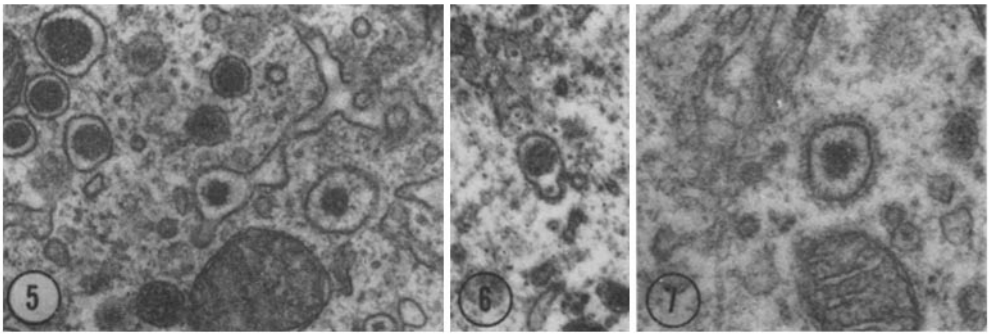

FIGURE 5 Electron-opaque cored vesicle is fused with part of the Golgi apparatus. × 45,000.

FIGURE 6 Electron-opaque cored vesicle is apparently fused with a second vesicle having a fibrillary outer coat. × 30,000.

FIGURE 7 Electron-opaque cored vesicle with fibrillary outer coat. × 60,000.

tents and rupture of the limiting membrane. Occasionally, they were intimately related to other cytoplasmic organelles. They fused with, or budded from sacs of the Golgi apparatus (47, 49, 69) (Fig. 5) but rarely fused with dense-walled (75) or coated vesicles (70) (Fig. 6). An occasional osmiophilic granule had an external fibrillary coating similar to that of the coated vesicles (Fig. 7). Several coated vesicles (400 to 1200 A in diameter) were scattered throughout the cytoplasm, and some opened on the cell surface (Fig. 8). Flattened, elongated sacs of granular endoplasmic reticulum were frequently found in parallel arrangements (Fig. 4), and dense granules (160 to 190 A in diameter) akin to those associated with the endoplasmic reticulum were dispersed either freely or in groups in the cytoplasm. Mitochondria up to 1.3 μ in length and 0.2 to 0.35 μ in width were plentiful, and branched forms were not uncommon. Also present were rounded or oval trilaminar membrane-bounded structures (0.3 to 0.7 μ in diameter) whose contents were electron opaque and granular or homogeneous (Figs. 2, 3, and 10). Some of these structures were multilobed and often contained a rounded, sharply demarcated lighter zone of homogeneous material up to

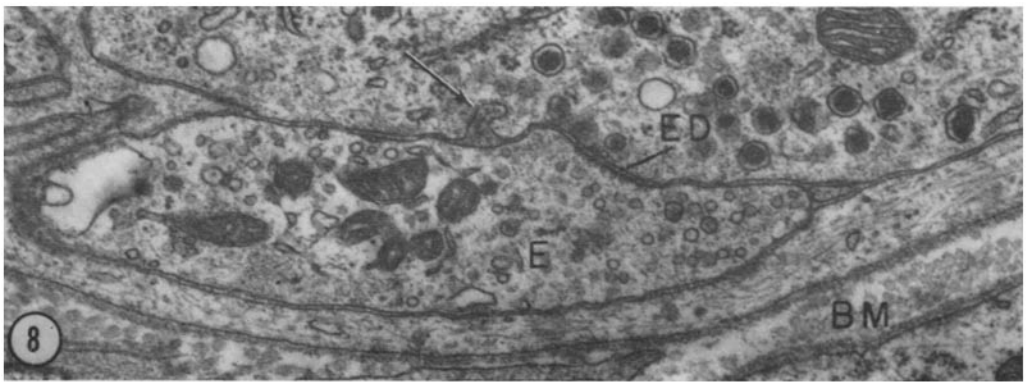

FIGURE 8 Nerve ending (*E*), Type I cell and Type II cell containing fibrils and bounded by a basement membrane (*BM*). The nerve ending contains microvesicles and mitochondria and shows electron-opaque cytoplasm (*ED*) at one point on the junctional region with the Type I cell. There is an invagination (arrow) of the Type I cell membrane which shows a fibrillary coating on the cytoplasmic surface and moderate density of the contents. × 30,000.

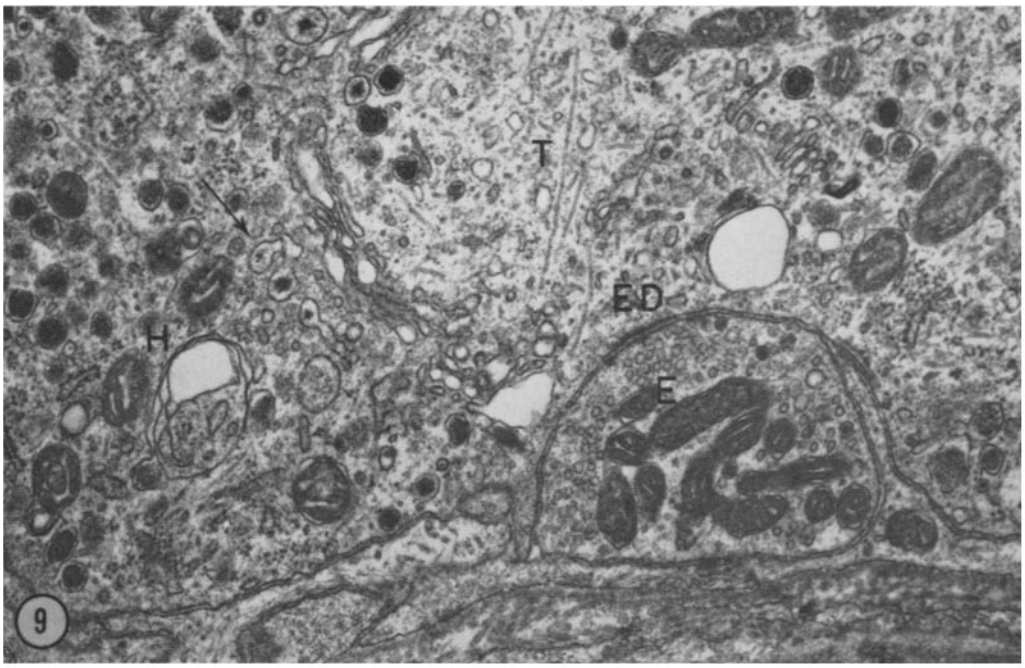

FIGURE 9 A nerve ending (*E*) containing mitochondria, microvesicles and a few small, dense cored vesicles. There is an increase in the electron opacity (*ED*) of the subjacent cell cytoplasm at one point in the junctional region. In the Type I cell are the Golgi apparatus with associated electron-opaque cored vesicles (arrow) and microtubules (*T*) and fragments of the endoplasmic reticulum. × 30,000.

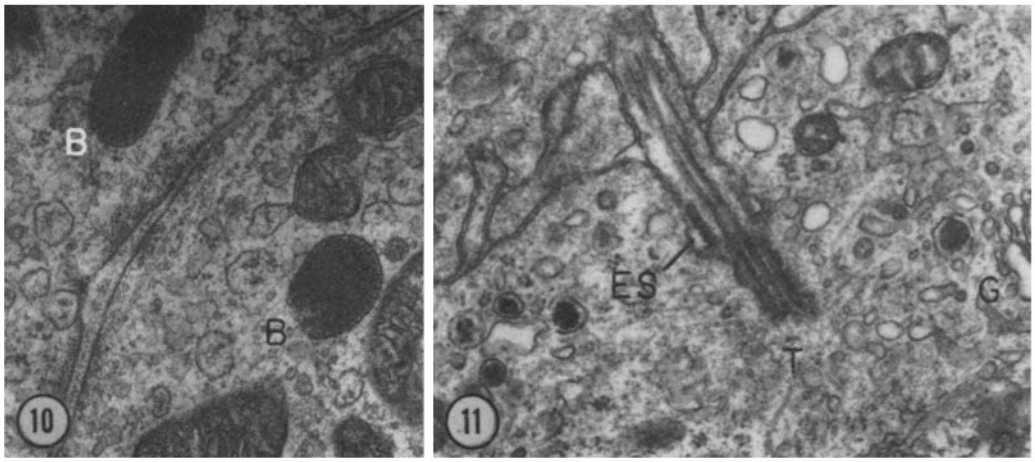

FIGURE 10 A junctional region between two Type I cells from OsO_4-fixed tissue, showing a complex similar to the *zonula adhaerens*. Two opaque granular bodies (*B*), probably lysosomes, can be seen. X 40,000.

FIGURE 11 A cilium arising from a basal body in a Type I cell and showing the extracellular space (*ES*). Part of the Golgi apparatus (*G*) and microtubules (*T*) are also shown. × 13,000.

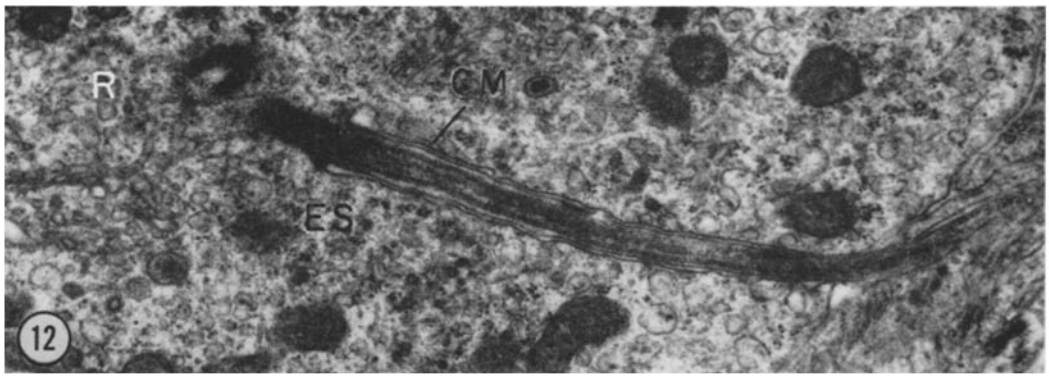

FIGURE 12 Cilium arising from a basal body, one of a pair of centrioles, within a Type I cell. Cell membrane (*CM*), and extracellular space (*ES*). The electron-opaque cored vesicles are depleted (OsO_4) fixation). The structure marked *R* may be a rootlet or pericentriolar body. × 30,000.

0.3 μ in diameter (Fig. 2). These were probably lysosomes, though hydrolytic enzyme studies were not performed to identify them.

Microtubules, 170 to 250 A in diameter, were prevalent (Fig. 9) particularly in the centrosomal region. Paired centrioles with axes at right angles to each other occurred in close association with the Golgi apparatus. Some centrioles were continuous with a cilium, over the surface of which the cell membrane was reflected down to the attachment to the centriole in which there was an extracellular sac (Figs. 11 and 12). The cilia had an over-all diameter of about 2200 A and contained circumferentially arranged fibrils each 250 A wide. In transverse sections they were seen to have the 9+0, or in some cases the 8+1, filamentous structure. Several cilia extended beyond the normal cell margin and lay parallel to the surface of the Type I cell where they were covered by Type II cells.

Occasional junctional complexes occurred between adjacent Type I cells. One variety (Fig. 10), similar to the *zonula adhaerens* (7, 30), displayed slight separation of opposing cell membranes, with a linear density in the intercellular cleft. The related cytoplasmic densities were as much as 800 A wide. A few junctions were seen in which the intercellular cleft was narrowed considerably. The outer laminae of the opposed membranes did not fuse, and the adjacent cytoplasmic density was never more than 100 A wide.

Type II Cells

Attenuated cytoplasmic extensions of these cells invested and separated Type I cells, nerve fibers, and nerve endings. The fingerlike extensions of Type I cells were not always completely enveloped (Fig. 3), segments being in direct apposition with the basement membrane (350 to 650 A wide) surrounding the cell complexes. Nerve fibers were related to Type II cells in much the same manner as they are to Schwann cells (Figs. 1, 2, and 4).

The nucleus, at times multilobed, was situated in the broadest segment of the cell to one side of groups of Type I cells. Cytoplasmic inclusions, not so numerous as in Type I cells, were mostly concentrated in the nuclear region. There were cilia of the 9+0 fibril pattern, exhibiting the same relationship to the centrioles as in the Type I cells. Vesicles of the Golgi apparatus were as much as 0.8 μ in diameter (Fig. 1), probably corresponding to the "vacuolated area" described by De Kock and Dunn (15). The attenuated cytoplasmic extensions contained primarily microtubules (150 A in diameter) and fine fibrils (50 to 100 A in diameter). Densities up to 0.1 μ long and 300 A wide were found along the plasma membrane adjoining the basement membrane.

Nerve Fibers

Nonmyelinated nerves within Schwann cells were common between blood vessels and parenchymal cells. Single nonmyelinated fibers occurred frequently within Type II cells (Figs. 1 and 4), often abutting on Type I cells and, in a few instances, lying between adjacent Type I cells. Within the nerves and also the Schwann cells there were two types of filament, viz. fine fibrils,

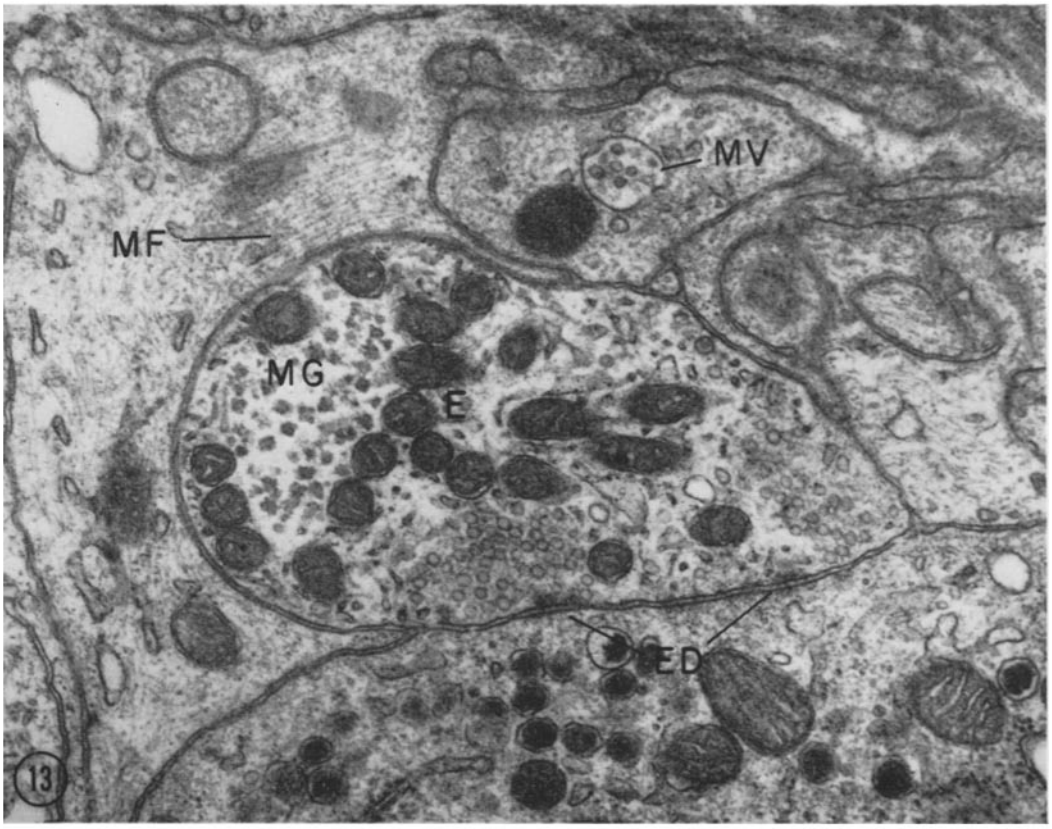

FIGURE 13 A nerve ending (*E*) containing microvesicles, small mitochondria, and mulberry-shaped granules (*MG*). There is an increased electron opacity (*ED*) of the cytoplasm of the nerve ending and the Type I cell at the junctional region. Microfibrils (*MF*) are present in the cytoplasm of the Type II cell. A multivesicular body (*MV*) is shown in an unidentified cell process. × 50,000.

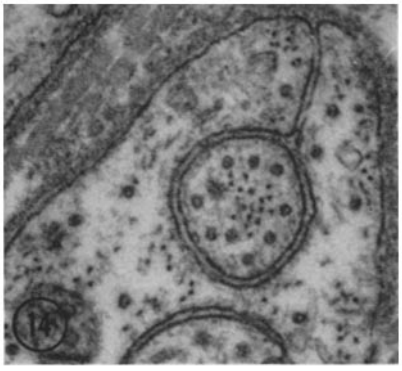

FIGURE 14 Nonmyelinated nerve fiber within a Schwann cell, both showing small and large fibrils. × 60,000.

50 to 100 A in diameter, and microtubular forms (*see* Elfvin, 22, 23), 250 to 300 A in diameter. (Fig. 14).

Myelinated nerves were less numerous than nonmyelinated fibers. They were most common near the periphery of the carotid body, but their presence elsewhere indicated that they were not restricted to this area as has been suggested previously (69).

Nerve Endings and Type I Cells

Nerve endings were recognized and distinguished from nerve fibers passing through the Type II cells by the presence of accumulations of mitochondria and small vesicles (49). The mitochondria in nerve endings were smaller than

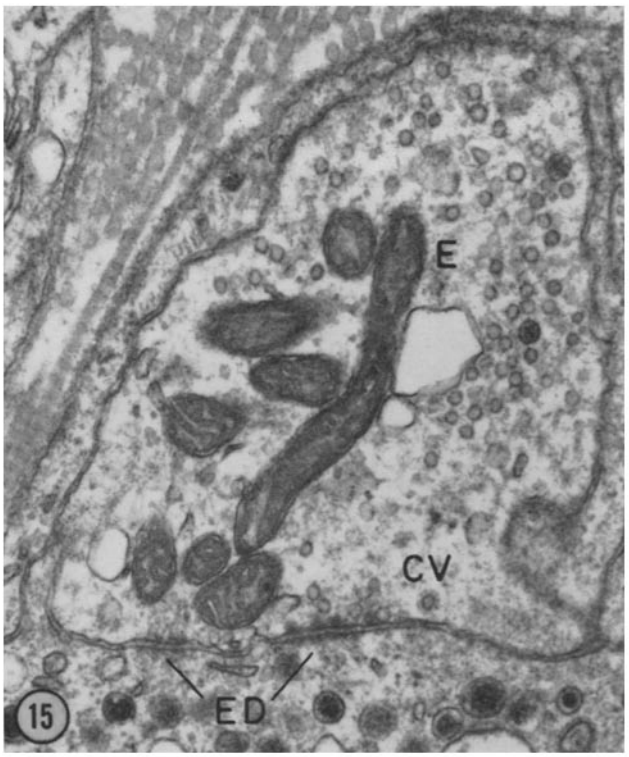

FIGURE 15 A nerve ending (*E*) containing microvesicles, small mitochondria, two small vesicles with electron-opaque cores, and a vesicle similar to the complex vesicle (37) (*CV*). There are regions of increased cytoplasmic density (*ED*), one of which has a serrated appearance. × 45,000.

those in Type I cells and were 0.10 to 0.18 μ wide and less than 0.6 μ long. The small vesicles (up to 500 A in diameter) in nerve endings were often present in large numbers. They were similar to the synaptic vesicles described at synapses in the nervous system (38). They were sometimes concentrated where the nerve endings were in apposition with Type I cells (Figs. 4, 9, and 13), but never were they within the cytoplasm of the adjoining Type I cells. Other less numerous vesicles, 500 to 1000 A in diameter, had electron-opaque cores, 400 to 850 A wide (Figs. 9, 16, and 17). These were larger, less dense, and fewer in number than those in sympathetic nerve endings of the pineal gland (6, 65, 77), vas deferens (63), iris (64), and pancreatic blood vessels (48).

The nerve endings had additional constituents, including glycogen granules (62), about 100 A in diameter, mostly in mulberry-shaped aggregates up to 500 A in diameter. These were either interspersed with the vesicles and mitochondria or occurred in areas of axoplasm of diminished density sometimes encircled by mitochondria (Fig. 13). Occasionally, they were surrounded by concentrically arranged membranes. Coated or dense-walled vesicles sometimes opened on the surface of the nerve endings, but showed no special predilection for the nerve ending–Type I cell junction. A few fibrils, 100 A in diameter, were sometimes arranged parallel to the plasma membrane (Fig. 16). Microtubules, 250 A in diameter, and collapsed irregularly shaped membranous sacs were also seen.

The diameter of the nerve endings (0.5 to 2.0 μ) was greater than that of the nerve fibers (0.2 to 0.5 μ) enclosed by Type II cells. Some endings were completely enveloped by Type II cells, whereas others were closely applied to the surface of Type I cells. According to the plane of section, some lay in a shallow groove in the Type I cell, and others extended around the periphery of Type I cells (Fig. 2) for as much as 9 μ. Usually, only one nerve ending was found associated with or enclosed by the cytoplasm of a Type I cell. Sometimes 2 or 3 endings were seen, though these could have been from the same nerve fiber coursing over the cell surface.

The plasma membranes of the nerve endings and the Type I cells were 100 to 200 A apart. The striking feature of these opposed cell surfaces

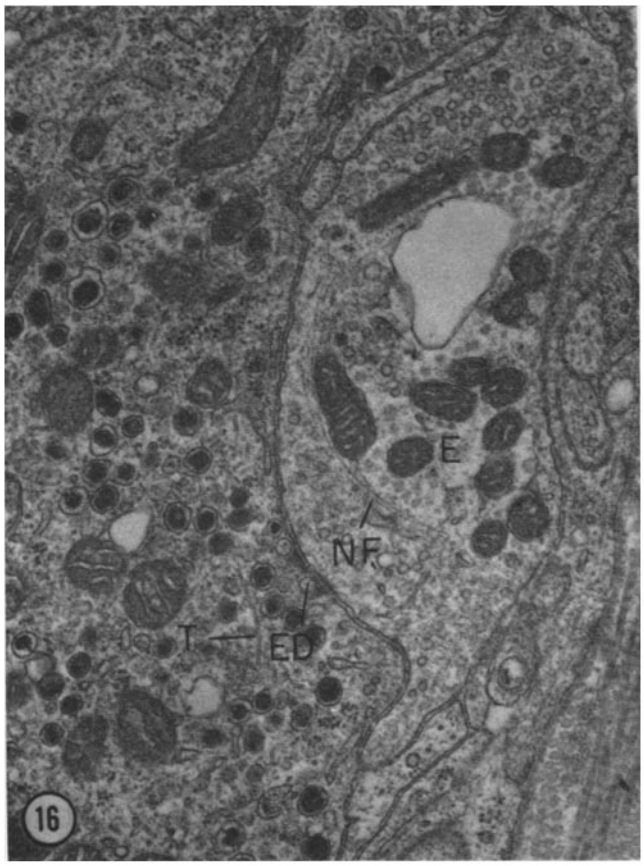

FIGURE 16 Nerve ending (*E*) containing microvesicles, small mitochondria, two small vesicles with electron-opaque cores and fibrils (*NF*). There is a small increase in cytoplasmic density at *ED*. In the Type I cell a microtubule (*T*) is shown. × 30,000.

was the presence of one or more specialized electron-opaque zones 0.07 to 0.5 μ in length (Figs. 8, 9, 13, 15, and 16), not unlike the *zonula adhaerens* (7, 30). Here the plasma membranes were sometimes separated by an additional 50 A. The intercellular cleft was of slightly increased density, and occasionally a central linear density was observed. The adjacent cytoplasms of the nerve ending and Type I cell were of increased density for a distance of up to 600 A from the cell membranes. Usually the cytoplasmic densities were wider in Type I cells than in nerve endings. A few of these densities were serrated (Fig. 15) on one or the other side of the junction, rather than on one side only as in the Type I synaptic thickening of Gray (38). From one to eight of these specialized sites occurred along the area of apposition. However, no such specialization was present elsewhere on the nerve endings.

Nerve Endings Related to Blood Vessels

Schwann cells containing several small nerve fibers, at times only 0.1 μ in diameter, were prevalent. Frequently, they were close to the endothelial basement membrane of blood vessels. Often the fibers were not completely invested by Schwann cells, being covered on one side only by the surrounding basement membrane (Fig. 17). Nerve endings in these Schwann cells were recognized by their content of microvesicles (about 500 A in diameter), mitochondria (0.1 to 0.13 μ diameter), and vesicles (650 to 1000 A diameter) with electron-opaque cores (Fig. 17). On several occasions, nerve endings containing mitochondria and microvesicles were separated from the muscle cells about blood vessels by basement membrane only.

Other Cells

Ganglion cells, morphologically similar to those in the superior cervical ganglion, were occasionally observed near the periphery of the carotid body. Numerous portions of unidentified cells closely applied to one another were associated with clumps of Type I and Type II cells. The extraordinarily

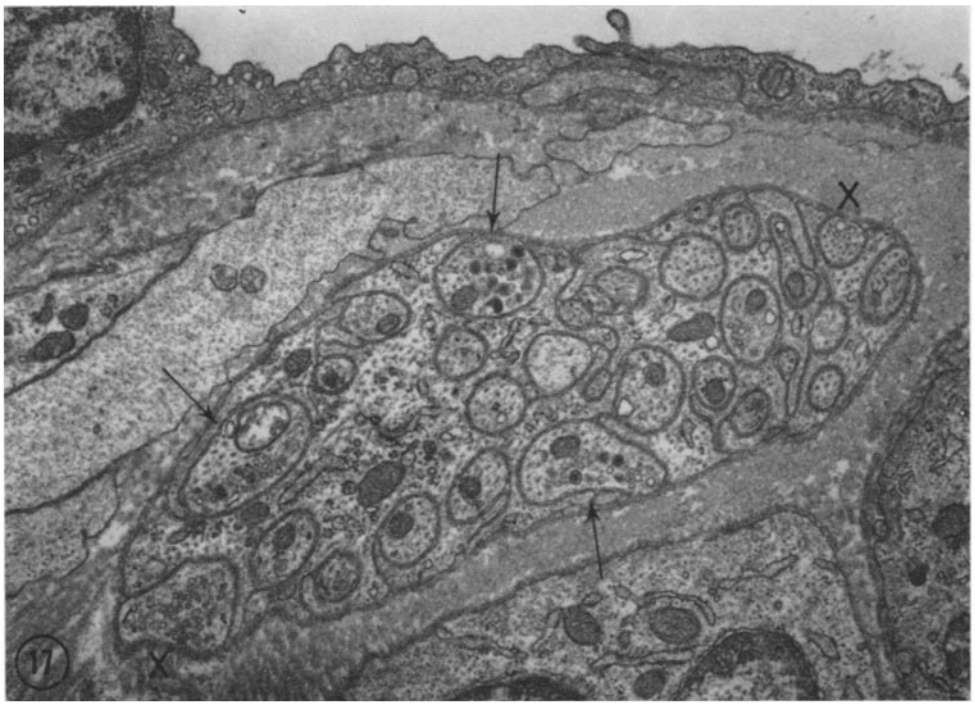

FIGURE 17 Beneath a blood vessel lies a Schwann cell with many nonmyelinated nerve fibers, some of which (arrows) contain electron-opaque cored vesicles and microvesicles. Nerve fiber at *X* is covered by basement membrane only on one side. × 20,000.

complex pattern sometimes produced could be explained by the interlocking of numerous cytoplasmic extensions of both Type I and Type II cells.

DISCUSSION

This investigation has revealed several new features relevant to the functional role of the carotid body. With regard to the general morphological organization of the body, there are important differences between our findings and those of previous workers (15, 25, 35, 41, 49, 69).

Blood Vessels

In the previous electron microscope studies (15, 25, 35, 41, 49, 69), fenestrated endothelium was not observed. Fenestration is known to occur in attenuated vascular endothelium in the stomach, intestines, endocrine glands, kindey, and eye (32, 42) and in unusual sites in the frog (75). In general, fenestration is said to indicate an intermediate degree of vascular permeability (32).

Type I and Type II Cells

The vesicles which have an electron-opaque core are remarkably similar in appearance to the secretory granules found in parenchymal cells of endocrine glands (8, 24, 31, 46, 54, 71), argentaffin cells of the bronchial glands (3) and stomach (61), specialized cells (Merkel cells) of the epidermis (52) and also in sweat glands (53). This distribution has led to the general belief that the vesicles are storage sites for physiologically active substances. It is pertinent, therefore, that they occur in considerable numbers in Type I cells, at which site the nature of such a stored substance remains in doubt, though Lever et al. (49) reported that reserpine treatment depleted the vesicles of their electron-opaque contents. However, in the present investigation, depletion of these vesicles resulted from osmium tetroxide fixation alone. Since Lever et al. fixed their material in osmium tetroxide, the possibility of a fixation artifact must be taken into account in an evaluation of the effect of reserpine administration. Nevertheless, the deduction, from this observation, that a catecholamine is found in the electron-opaque, cored vesicles is supported by the finding that noradrenaline is present in sites in which Type I cells are most prevalent (55, 60).

The results of Priimak (58) and Rodríguez-Pérez (66) support this contention, though the evidence is by no means conclusive. Tests for other pharmacologically active substances were not made by these authors. The relationship of Type I cells to Type II cells is morphologically clear, though it remains functionally obscure. Like Schwann cells, Type II cells may be supporting or sustentacular cells (69) for the many nerve fibers coursing through them.

The infrequent intimate connection between dense cored vesicles and coated or dense-walled vesicles is similar to that reported in yolk formation in mosquito oocytes (70). These specialized vesicles, believed to be concerned with the selective uptake of protein, were observed in all cell types and appear to be ubiquitous.

Cilia similar to those seen in this investigation have been described by Rogers (67) in cells of the amphibian carotid labyrinth which are comparable to Type I cells. The cilia in the carotid body and in the labyrinth have a diplosomal basal structure. Their associated 9+0 fibril pattern has been demonstrated in endocrine cells (2, 18), various sensory receptors especially those sensitive to light (16, 20), the nervous system (19, 39, 76), and in many other diverse sites (1, 18, 73). Thus, this type of cilium appears to be widely distributed in the animal kingdom, and to draw any general conclusion regarding its function does not seem possible. It is thought to be nonmotile; and the notion that the cilium is a receptor (2, 47) is without experimental foundation. The function of the cilia within the carotid body is undetermined.

Fenestrations in vascular endothelium and cells containing dense-cored vesicles and cilia are obvious structural similarities between the carotid body and endocrine glands. Type I cells differ from endocrine cells in that, for the most part, they are invested by Type II cells.

Ganglion Cells

Smith (72) and Rogers (68) have described sympathetic ganglion cells participating in the embryological development of the carotid body. The occurrence of small numbers of ganglion cells in the present study is consistent with the findings of these and previous workers (11, 12, 45, 59).

Nerve Endings

The junction of nerve endings and Type I cells exhibits many features of synapses seen elsewhere in the central nervous system (38). If the Type I cell is the chemoreceptor element of the carotid body, then the information it detects should be transmitted to the chemoreceptor afferent nerve endings. For some years, it has been held that this transmission is chemical in nature (26, 27, 29, 50). However, one of the most interesting features of the junction is the presence of vesicles, morphologically synaptic in type (17, 33, 57), within the nerve ending (5, 49), but not in the adjoining cytoplasm of the Type I cell. One might expect that chemical transmission (21) would then be from the nerve ending to the Type I cell and that the nerve ending would be efferent (presynaptic) to the carotid body. However, synaptic vesicles *have* been found in afferent nerve endings (38). This being so, nerve endings related to Type I cells in the present investigation could be afferent and Type I cells could be chemoreceptors, thus supporting the hypothesis of De Castro (11, 12).

The possibility also exists that the Type I cells are not receptors and that the nerve endings are efferent to them; in this case, it would be necessary to look elsewhere for the chemoreceptor nerve endings. Alternatively, if the Type I cells are receptors the nerve *fibers* lying adjacent to the Type I cells could be the chemoreceptor endings, the efferent fibers modulating the activity of the receptor as in the cochlea (43, 44, 74).

In the present state of knowledge, the nerve endings on the Type I cells could be efferent or afferent, and the logical sequel is to investigate the chronic effects of cutting the nerves to the carotid body. Lever et al. (49) found no alterations following sympathectomy in the rabbit 1 wk previously, but this may be insufficient time for degenerative changes to appear (74).

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