Electron Microscopic Observations of the Carotid Body of the Cat*

By LEONARD L. ROSS, PH.D.

(From the Department of Anatomy, Cornell University Medical College, New York)

PLATES 134 TO 139

(Received for publication, March 13, 1959)

ABSTRACT

Carotid bodies were removed from cats, fixed in buffered 1 per cent osmic acid, embedded in deaerated, nitrogenated methacrylate, and cut into thin sections for electron microscopic study. The carotid body is seen to be composed of islands of chemoreceptor and sustentacular cells surrounded by wide irregular sinusoids. These cells are separated from the sinusoids by relatively broad interstitial spaces which are filled with collagen, fibroblasts, and many unmyelinated nerve fibers with their Schwann cell sheaths. The chemoreceptor cells are surrounded by the flattened, multiprocessed sustentacular cells which serve to convey the axons from an interstitial to a pericellular location. These sustentacular cells are assumed to be lemmoblastic in origin. Relatively few axons are seen to abut on the chemoreceptor cells.

The cytoplasm of the chemoreceptor cell is characterized by numerous small mitochondria, units of granular endoplasmic reticulum, a small Golgi complex, and a variety of vesicles. There are many small vesicles diffusely scattered throughout the cytoplasm. In addition, there is a small number of dark-cored vesicles of the type which has been previously described in the adrenal medulla. These are usually associated with the Golgi complex. These findings are discussed in relation to the concepts of the origin of the chemoreceptor cell and the nature of the synapse.

INTRODUCTION

The general structure, vascular pattern, and nerve supply of the mammalian carotid body have been subjects of investigations for more than one hundred years. This work has been extensively reviewed by Adams (2). More recently, morphological studies of this organ have been directed toward elucidation of the cytology of the chemoreceptor cells (18, 19, 24, 46) and their relationship to the neural elements (29, 1, 8, 20). Knowledge of the structure of the chemoreceptor cells has kept pace with our improved understanding of the physiological role of the carotid body in the respiratory reflex (47, 31, 16).

Recent electron microscope studies have, to a great extent, confirmed the light microscopic observations on the carotid body and have clarified many details of structure previously obscure (25, 12, 17, 13). Osmiophilic bodies have been described in the cytoplasm of the glomus cells which were not observed with the light microscope (25), and two distinct cell types have been distinguished on the basis of variations in cytoplasmic density (25, 17, 13).

The present study was undertaken with the purpose of defining in greater detail the mode of nerve termination on the chemoreceptor cells of the carotid body. The observations substantiate many of the earlier light and electron microscopic investigations and provide additional information on the finer cytology of the chemoreceptor cells, the nature of the nerve endings and the relation of the neurolemmal elements to these endings. These observations are correlated with the previous histochemical investigations (46) and their physiological implications are discussed.

* Supported by a grant from the United States Public Health Service (B-1523).

Materials and Methods

The observations are based on a study of the carotid body of the cat. This species was chosen because of the large size and compact, encapsulated nature of its carotid body. The organ was removed as previously described (46), observing the necessary precautions as to anesthesia, oxygen intake, and maintenance of blood supply during exposure. The carotid body, free of its surrounding connective tissue, was immediately immersed in 1 per cent osmium tetroxide buffered to pH 7.4 to 7.6 with veronal acetate buffer (32). Thin (1 mm.) blocks of tissue were cut from the immersed organ and fixation was continued for 1 hour. After a brief wash in physiological saline the blocks were dehydrated in a graded series of alcohols and infiltrated for 3 hours in three changes of a mixture of *n*-butyl and methyl methacrylate (8:1). The tissues were then placed in gelatin capsules which contained the same mixture of methacrylates to which 1 per cent benzoyl peroxide had been added as a catalyst. This embedding mixture had been previously deaerated and kept under pure nitrogen for 2 hours prior to use. This is a modification of the technique originally proposed by Moore and Grimley (30). The plastic was allowed to polymerize overnight in an oven at 60°C. Sections were cut on a Porter-Blum microtome (39) using glass knives, and were examined without removal of the plastic. Preliminary studies, previously reported in abstract form (45), were made on a Phillips EM 100A electron microscope. The micrographs presented here were made on an RCA electron microscope, model EMU-3B at magnifications of 2,000 to 10,000. Greater magnifications were obtained by photographic enlargement.

OBSERVATIONS

Histological Organization.—The carotid body of the cat as seen in the light microscope is composed of islands of chemoreceptor cells separated from one another by irregular blood sinusoids and surrounded by a rich plexus of nerve fibers. The whole is enclosed by a dense fibrous connective tissue capsule. Bundles of myelinated nerve fibers and solitary ganglion cells are found in the periphery of the organ. Two types of cells can be identified in the glomera. The more prevalent type is a polygonal cell with a rounded nucleus. This type is considered to be the chemoreceptor cell. Another cell type having a flat elliptical nucleus and elongated processes is found in close association with the chemoreceptor cell. Nerve fibers are intimately related to both types of cell.

A survey of the carotid body with the electron microscope confirms the over-all impression gained by light microscopy (Fig. 1). Groups of epithelioid cells are found in close association with wide irregular endothelial-lined sinusoids. There is, however, no very intimate contact between the chemoreceptor cells and the blood channels. Instead, these two elements are separated from one another by interstitial spaces that are occupied by connective tissue cells, bundles of collagen fibers, and unmyelinated axons ensheathed by Schwann cells (Figs. 2, 4, 6, 7). The fusiform cells found in the interstitial spaces appear to be attenuated fibroblasts with randomly oriented slender (100 to 150 mμ) processes coursing between the glomera (Figs. 4, 5, 7). Their cell bodies are located just beneath the sinusoidal endothelium. They are separated from the chemoreceptor cells and do not contact them even via their processes. The connective tissue fibers are found in moderately high concentration between the sinusoids and the islands of chemoreceptor cells. Although these fibers abound in the interstitial spaces, they never penetrate into the glomera. In the unmyelinated nerves, which ramify extensively around the islands of parenchymal cells, the axons are enfolded by plasma membranes of Schwann cells in such a way as to form characteristic mesaxons (Figs. 1, 2, 4 to 7) identical to those that have been described elsewhere in the peripheral nervous system (13). Of particular interest is the fact that, in this interstitial location, each Schwann cell encloses no more than one or two axons. Myelinated fibers were never seen in the interior of the carotid body, although they are found in close proximity to the more peripherally located glomera.

Within the cell islands, two types of cells are distinguished. The one which is larger and more numerous is irregularly polygonal in shape and has a round or oval nucleus (Figs. 1, 4 to 7). Processes do not appear to arise from this cell. For reasons to be discussed presently, this cell type is considered to be the *chemoreceptor cell.*

The other, less numerous cell type possesses a flat cell body with a flat, oval nucleus and exhibits long, branching processes which run between and around the chemoreceptor cells. These will be referred to as *sustentacular cells.* The observational basis for this interpretation will be considered below. The bodies of these cells are found, for the most part, in the periphery of the glomera, and their processes run to the islands, separating the chemoreceptor cells from one another (Figs. 1, 4, 6, 7). Although the nuclear region of this cell type is seen only occasionally, its ramifying proc-

esses can be identified around almost every chemoreceptor cell in any field. These processes are so numerous that they form multiple layers around the individual chemoreceptor cells (Figs. 4, 5, 7).

Chemoreceptor Cell.—The chemoreceptor cell is a rounded or polygonal cell, 6 to 10 micra in diameter (Figs. 1, 4 to 7). Its round, centrally placed nucleus (5 to 6 micra in diameter) exhibits a finely granular karyoplasm of moderate density and generally contains a single prominent nucleolus. The nuclear envelope shows the usual double membrane form (Figs. 4, 10), but there are no obvious nuclear pores.

The endoplasmic reticulum is made up of one or more discrete systems of parallel cisternae in the juxtanuclear cytoplasm (Figs. 1 to 5, 10). Where more than one such group of membrane-limited flat cavities is present, they are usually situated at opposite poles of the nucleus (Fig. 5). The width of their profiles varies from 20 to 40 mμ, and they appear to be identical with the endoplasmic reticulum found in a variety of other cell types (34, 35, 38). Associated with the membranes are small (10 to 15 mμ), dense granules apparently identical to those previously identified as ribonucleoprotein (33, 35, 38) (Figs. 2, 3, 5, 10). Very few of these granules are free in the cytoplasm. This observation is consistent with previous histochemical studies of the carotid body (46), which demonstrated that ribonucleoprotein (*i.e.*, pyroninophilia abolished by ribonuclease digestion) was localized in discrete areas within the cytoplasm of the chemoreceptor cell, usually near the nucleus.

A small Golgi complex is occasionally observed in the juxtanuclear region (Figs. 4, 5, 8). It appears as systems of narrow, flattened tubules and small (20 to 40 mμ) vesicles in close association (Fig. 8). The tubules are seen as thin membrane profiles, while the vesicles appear as membrane-limited structures with contents whose density is greater than that of the surrounding area. The high concentration of vesicles in this region and the dispersion of vesicles of similar appearance elsewhere in the cytoplasm suggests the possibility that most, if not all, such vesicles are formed in the region of the Golgi complex (5).

In addition to these 20 to 40 mμ vesicles the cytoplasm contains other membranous profiles. One is a large (60 to 120 mμ) membrane-bounded cavity of low internal density which is found throughout the cell (Figs. 2 to 4, 8 to 10). In micrographs these vesicles appear as thin walled, smooth surfaced profiles surrounding a clear area. Because of their size, smooth walls, and general disposition in the cell, these vesicles are reminiscent of the vesicular agranular reticulum of Palade (34, 35). It might be pointed out that the coexistence of both types of reticulum has been noted in other situations (35) including the neuron (38).

A third type of vesicle found in most chemoreceptor cells is present in far smaller numbers than the types just described. This structure possesses a distinct membrane which encloses a content of relatively high electron density. Its diameter varies from 60 to 80 mμ (Figs. 4, 5, 8 to 10). Often the contents of this vesicle have an extremely dense core which varies in size from a small central dot to a dense body occupying most of the vesicle (Figs. 9, 10). The dark-cored vesicles are often closely associated with the Golgi complex (Figs. 4, 8). Although the majority of chemoreceptor cells exhibit a sparse population of this type of granule, cells are occasionally found which have them in a high concentration (Fig. 9). In such cells many of the vesicles have a uniformly dense content. These dark-centered vesicles closely resemble those which de Robertis and Vaz Ferreira (43) have described in the adrenal medulla and interpretated as catechol-containing droplets. Although the adrenal medullary droplets were, on the average, larger in diameter (87 to 230 mμ) than those seen in the chemoreceptor cell (60 to 80 mμ), the general appearance of the two is strikingly similar, and it is noteworthy that de Robertis occasionally saw catechol-containing droplets as small as 40 mμ in diameter.

Large vacuoles (200 to 300 mμ) with a definite limiting membrane (Figs. 4, 5, 9, 10) are occasionally noted in the chemoreceptor cell cytoplasm. These contain small vesicles, 20 to 30 mμ in diameter, and similar vesicles often accumulate immediately outside the vacuoles as well (Figs. 4, 10). The exact nature of these structures is unknown, but Palay and Palade (38) have reported them also in the dorsal root ganglion cell where they may be related to the agranular reticulum (Golgi complex). In the chemoreceptor cell these vesicles do not seem to possess any such relation.

The mitochondria of the chemoreceptor cell are small (200 to 400 mμ), numerous, and uniformly distributed in the cell (Figs. 1 to 10). They are generally round to oval in shape, but a few

branching forms are seen (Fig. 4). The mitochondrial matrix is fairly dense and, as a rule, the cristae are of uniform size and parallel in their orientation.

Sustentacular Cell.—Closely associated with the chemoreceptor cells are "sustentacular cells," located at the periphery of the glomus and possessing processes which ramify extensively among the chemoreceptor cells.

The sustentacular cells can be seen to best advantage in a section which passes through the edge of a glomus and thus includes only one or two chemoreceptor cells (Figs. 4 to 7). These supportive elements abut directly on the chemoreceptor cells and their long processes branch out and surround one or more of them. When only one or two chemoreceptor cells are in the field, the processes of the same cell can be followed almost completely around individual glomus cells. When the section passes through a large group of parenchymal cells, however, the branchings of the sustentacular cell processes are usually too complex to follow (Figs. 1, 2). The sustentacular cell can be distinguished from the chemoreceptor cell by several characteristics. Its nucleus is generally flattened and finely granular. The cytoplasm of the sustentacular cell is less dense than that of the glomus cell and contains fewer organelles. One outstanding characteristic of the cytoplasm is the presence of large osmiophilic bodies, presumably lipide in nature (Figs. 4, 5, 7). These spherical bodies range in size from 300 to 450 mμ and are usually located in the perinuclear region. The chemoreceptor cell too may have lipide inclusions, but less frequently than the sustentacular cell.

Elongated or round membrane profiles are occasionally noted in the sustentacular cells, but well differentiated cisternae of the endoplasmic reticulum are rarely seen (Figs. 5, 7). These membranes enclose areas which are lighter than the surrounding cytoplasm and have on their outer surfaces small (10 to 15 mμ), dense granules, presumably ribonucleoprotein. Small groups of these granules, free of membrane attachment, are few in number and widely dispersed in the cytoplasm. The mitochondria are small (200 to 300 mμ), few in number, and have a dense matrix and sparse cristae. There are a few, widely dispersed, small (40 to 60 mμ), light-centered vesicles, but these do not approach the number of those found in the chemoreceptor cell.

The disposition of the sustentacular cell processes is quite intricate and requires further elaboration. The cell bodies always lie at the periphery of the glomus. Generally, their processes surround the one or two chemoreceptor cells adjacent to the cell bodies, and do not seem to extend beyond this range. It is, however, not possible to follow the processes for any great distance. No more than three or four processes arise from any one sustentacular cell (Figs. 4 to 7). The sustentacular processes may embrace whole glomus cells (Figs. 5, 6), parts of glomus cells (Figs. 4, 7), other sustentacular processes (Figs. 4, 7), or combinations of these. Usually the last condition obtains. The processes may be wide (300 to 500 mμ) (Figs. 4, 6, 7) or quite narrow (15 to 30 mμ). In their widest regions, they contain most of the formed elements found in the juxtanuclear cytoplasm. In their narrowest zones they are usually devoid of organelles and inclusions, exhibiting only an extremely fine granularity. In these attenuated regions, the processes reveal a complex, overlapping relation with one another, forming an interwoven plexus of slender processes on the surface of the chemoreceptor cell (Figs. 3, 8, 9).

Neural Elements.—Groups of myelinated nerve fibers can be seen coursing through the connective tissue surrounding the carotid body. Distributed among the myelinated fibers are smaller groups of unmyelinated axons suspended within Schwann cell sheaths. In the interglomerular connective tissue, the ratio of myelinated to unmyelinated fibers reverses and comparatively few myelinated fibers are seen amid large numbers of unmyelinated axons. At the level of the periglomerular connective tissue there is a dense plexus of nerve fibers consisting solely of unmyelinated axons and their Schwann cell sheaths (Figs. 1, 2, 4, 6). The axon, which is deeply recessed in the surface of the Schwann cell, exhibits a relatively clear axoplasm, a few small (20 to 30 mμ) vesicles, and small (50 to 100 mμ) dense mitochondria (Figs. 2, 4, 7). The axons range in diameter from 200 to 400 mμ.

In this periglomerular location, of course, the axons have no relationship with the chemoreceptor cells. This relationship is not established until the axons become enfolded by the sustentacular cell processes. As the axon passes from the periglomerular neurolemma, it becomes enveloped by a typical mesaxonic infolding of the sustentacular cell membrane (Figs. 4 to 8). It may be situated in any part of the sustentacular cell and may be only partially enveloped, remaining exposed over part

of its surface. In such areas the axolemma abuts directly on the chemoreceptor cell membrane and an intimate relationship between these two elements is established (Figs. 3 to 5). Completely free axons are never seen, nor are axons ever seen which are not related in some manner to the sustentacular cell membrane. It is also worthy of note that, in comparison to the number of axons seen in periglomerular locations, few are seen which actually touch the chemoreceptor cells.

At the point of contact between the axon and the chemoreceptor cell (Figs. 3 to 5) there is no specialization of the adjoining surfaces, nor is there an accumulation of synaptic vesicles or of mitochondria. The axon terminal may be slightly dilated, averaging 400 to 500 mμ in diameter, but no other local modifications can be seen. Yet, as far as could be determined, these points of apposition are synaptic junctions.

It will be apparent from the foregoing description why the multiprocessed cell that envelopes the chemoreceptor cell has been designated a "sustentacular cell." The principal basis of this interpretation is the relationship of this cell to the intraglomerular axon. In other special sensory receptors, the sustentacular cell is that cell which supports the receptive elements and surrounds the axon in its passage from its Schwann cell sheath to its termination on the receptor. This is true of the organ of Corti (10, 11), the taste bud (28), and the cristae ampullares (49). In all these situations, the axon has continual support by either Schwann cell or sustentacular cell until it reaches its termination on the receptor cell.

Before leaving this subject, particular note should be made of the complexity of the sustentacular cell processes in the areas between the glomus cells (Figs. 2, 3, 5, 8, 9). It is in these regions that the axons are most intimately enmeshed by the supporting cells and in which contact is made between the axon and the chemoreceptor cell. Sustentacular cell processes cross and overlap one another; ends of glomus cells are involved in this network; and, through it all, the axons course, suspended by their mesaxons. Adequate three-dimensional interpretation of the relationships between these elements would be impossible in these areas without reconstruction from serial sections, which is not technically feasible at the present time. For this reason a preliminary study of sections made through the periphery of the glomus, where only one or two glomus cells are included, helps to establish identifying morphological characteristics of the various elements involved. These criteria, once established, can then be applied to the interpretation of the more complex areas in the interior of the glomus.

DISCUSSION

Of all the features of carotid body morphology which have been studied, the one which has been the subject of the most controversy has been the nature of the nerve termination. While many of the workers in the field held the view that the specific glomus cells were either true, or modified, nerve cells (23, 29, 15, 20), the work of de Castro (6–8) and others (4, 18) would seem to have demonstrated that the glomus cells are of mesenchymal origin, non-neuronal in structure, and in synaptic relation with the afferent fibers of the glossopharyngeal nerve. The concept of carotid body synapses is further reinforced by the extensive physiological and pharmacological investigations of the chemoreceptor reflex and the actions of drugs on this reflex (16). Once this point was established, attention was directed to the nature of the contact between the nerve ending and the chemoreceptor cell.

De Castro (8) holds that the nerve fiber penetrates the chemoreceptor cell to form a menisciform ending in close contact with the nucleus. Intracytoplasmic nerve endings have also been reported by Boeke (3) and Abraham (1), among others. De Kock (20) maintains that an interstitial cell (of Cajal) is interposed between the nerve fiber and the chemoreceptor cell with its fine branches entering the cytoplasm of this cell. The electron microscopic observations have demonstrated that no element penetrates the chemoreceptor cell membrane and that the nerves end in contact with this membrane as in other synaptic relationships. Furthermore, both pharmacological (26, 16) and histochemical (21, 22, 46) studies have indicated that acetylcholine or some other choline ester may serve as the synaptotropic agent in this system.

The morphology of synapses in general has been the subject of much study with the electron microscope and its characteristic features would seem to have been well established (41, 36, 44, 37, 40). These include: (*a*) separation of the pre- and postsynaptic membranes by a definite intercellular space, with the apposing surfaces often being specialized in a manner similar to that seen

in desmosomes and terminal bars; (*b*) the presynaptic member contains a dense accumulation of small vesicles, termed "synaptic vesicles," which tend to congregate in the axoplasm near the surface membrane; and (*c*) the presynaptic member may be enlarged and contain an accumulation of small mitochondria.

Not all of these features were observed in the synapses of the carotid body. There was no particular thickening of the apposing plasma membranes, although there was a definite space separating the two. Small vesicles the size of the "synaptic vesicle" were evenly distributed throughout the entire chemoreceptor cell cytoplasm (presynaptic) yet were not especially concentrated in the areas of axon contact. The small mitochondria did not congregate in such areas of contact on the presynaptic side although they were often found in the postsynaptic axoplasm. Moreover, there is no evidence that the region of the axon which abuts on the chemoreceptor cell is terminal. It is entirely possible that the nerve fiber, in its course around the chemoreceptor cell, is contiguous with the glomus cell at more than one point; serial sections would be needed to demonstrate this.

Non-specific esterase and, to a lesser extent, acetylcholinestrase, has been demonstrated histochemically in the peripheral cytoplasm of small groups of chemoreceptor cells (21, 22). This finding would be consonant with the concept that the glomus cell constitutes the terminal sensory receptor and is thus presynaptic to the axon terminals. Thus it might be possible that the small vesicles, which are observed in the chemoreceptor cells, contain acetylcholine and are, indeed, "synaptic vesicles" as was proposed by de Robertis (40) and Palay (37) for other situations. If this should prove to be the case, then the regions of contact between the axon and the glomus cell do not appear to be particularly differentiated, anatomically, for impulse transmission. While the vesicles are in greater number in the chemoreceptor cell, their lack of concentration at the regions of contact would argue against a synaptic contact confined to a small area. Added to this is the fact that, in comparison to the large numbers of periglomerular axons, relatively few axons seem to establish contact with the chemoreceptor cell membrane. Thus, it would be more in accord with the observations reported here to suggest that the axon may be stimulated by release of a synaptotropic agent from a cell with which it is not contiguous. Actual contact and discrete morphological polarity would not seem to be necessary for axon action potential propagation in the case of the carotid body synapse. Polarity does exist, but it may involve the total cell-axon relationship and not just a circumscribed area of contact.

In line with these comments it would be of interest to compare the axon-receptor cell relationship in the carotid body with that which has been observed in other sensory receptors, particularly with respect to the three aforementioned features which have been designated as being most characteristic of a synaptic relationship. In the retina only two of these are found. In the circumscribed areas of the rod or cone cells contacted by the bipolar cell processes, there is an increased density of the apposing plasma membranes together with a concentration of small vesicles in the immediately adjacent presynaptic area (42). Accumulations of mitochondria are not observed. In the taste bud synapses, the opposite condition obtains (28). That is, an accumulation of vesicles and mitochondria in the postsynaptic (axon) side of the synapse.

In the case of the nerve endings around the hair cells in the organ of Corti several aspects of structural relationships are still in doubt. Smith and Dempsey (48) describe the axon endings on the hair cells as containing accumulations of many mitochondria and many small vesicles. These endings, in their descriptions, are the postsynaptic members. The cytoplasm of the hair cells only occasionally exhibit localized accumulations of vesicles. Engström and Wersall (11), on the other hand, described two types of nerve endings on the hair cells. One type is large and almost completely free of vesicles, while the other is small and densely packed with vesicles and mitochondria. Wersall (49) has described the same sort of apparently double innervation for the vestibular sensory epithelium. In a further analysis of this point, Engström (10) maintains that the duality of morphologically identifiable endings is a reflection of the fact that these sensory receptors receive both an afferent and an efferent innervation. The vesicle-filled ending is represented as being the efferent terminal, while the vesicle-free ending constitutes the afferent terminal. However, as this author points out, if the morphological criteria are correct, the large number of vesicle-filled endings would indicate a greater efferent

nerve supply than had been previously supposed. In any case, it is important to note that the receptor cell cytoplasm never exhibited any specialization of structure in the regions of axon contact.

Thus, in reviewing the studies of synapses in special sensory receptors, the conclusion must be drawn that there is no set pattern of morphological standards such as have been described for motor and associational synapses. Mitochondria are found inconstantly in either presynaptic or postsynaptic locations. In fact, de Robertis (40) questions the role of these organelles in synaptic function. It is this author's contention that it is more likely that the polarization of the synapse is dependent on the location of the "synaptic vesicles" in the presynaptic side of the junction. But, as we have seen, the localization of the vesicles is inconsistent, receptors may exhibit them in the presynaptic member, in the postsynaptic member, or in neither member. While there were small vesicles distributed throughout the cytoplasm of the chemoreceptor cells of the carotid body whose size range would put them in the category of synaptic vesicles, they were never particularly concentrated in the regions of axon-cell junction. Although these vesicles were on the presynaptic side of the synapse, it would be an unwarranted extrapolation to call them "synaptic vesicles."

The designation of the multiprocessed cells which surround the specific chemoreceptor elements as *sustentacular cells* may require further explanation inasmuch as this term is being applied to these cells for the first time. The use of this term is based on comparison with electron and light microscopic observations of other special sensory receptors, notably the cristae ampullares (49), the taste buds (28), and the olfactory mucosa (27). In these sites, the axon loses its Schwann cell investment as it enters the receptor organ and becomes enveloped by the sustentacular cell and maintains this relationship until it reaches its termination on the receptor cell. In the case of the olfactory mucosa, the sustentacular cell sheathes both the axon and dendrite of the primary olfactory neuron. The analogy between these situations and that obtaining in the carotid body is apparent. In this organ, the sustentacular cell serves to conduct the axon from the Schwann cell to the chemoreceptor cells.

It is tempting to speculate on the nature, origin, and functional role of these sustentacular elements. Because of their neurolemmal-type relationship to the axon, it is entirely possible that they are lemmoblastic in origin. If this be the case, it is conceivable that the sustentacular cell is analogous to the Schwann cell and may play a similar physiologic role in impulse conduction (14). Further, the topographical relationship which the sustentacular cell bears to the chemoreceptor cell is reminiscent of the satellite cell-ganglion cell association. This may have a bearing upon the question of the origin of the chemoreceptor cell. Many authors adhere to the concept of their non-neuronal origin based, in part, on the embryological studies of Boyd (4). Further investigation of this sustentacular-chemoreceptor cell association might shed much light on the problem. The foregoing discussion is, however, still speculative and the present study does little to illuminate this question.

These sustentacular cells have been previously described with the light microscope by Meyling (29), Goormaghtigh and Pannier (15), and, more recently, by de Kock (20). In the main, these authors used methylene blue or silver preparations to demonstrate these cells and called them interstitial cells. They maintained that the nerve fibers terminated on (or in) the interstitial cells whose processes, in turn, terminated on (or in) the chemoreceptor cells. With the information which has been derived from the electron microscopic observations of the sustentacular cell-axon associations it is not difficult to understand how these authors arrived at their conclusions regarding the interstitial cell plexus. In light of the current observations these concludions are no longer tenable.

The observation of a dense-cored granule similar in structure to that observed by de Robertis and Vaz Ferreira (43) in the adrenal medulla is significant in light of previous light and electron microscopic investigations of the carotid body. While early workers (23) reported a positive chromaffin reaction in the chemoreceptor cells and thus classified the carotid body with the paraganglia, most of the more recent work (6, 4, 18, 24, 46) has demonstrated only a faintly positive reaction in scattered glomus cells. This fact has troubled many investigators who have sought to account for this feeble reaction. No satisfactory explanation, short of conceding the sympathetic origin of chemoreceptor cells, was forthcoming. The present observations would seem to have demonstrated the morphological basis for the posi-

tive chromaffin reactions of the glomus cells. The striking structural similarity between the dense-cored droplets of the glomus cells and the catechol-containing droplets of the adrenal medulla suggests the presence of a compound or compounds like those found in the adrenal medullary cells, be it adrenalin, noradrenalin, or some other phenol derivative. The sparse and random distribution of these droplets would account for the faintness of the chemoreceptor cell chromaffin reaction. These granules have been seen by other investigators of the fine structure of the carotid body. Lever and Boyd (25), in a preliminary communication, describe such dark-cored granules and point out their similarity to those seen in the adrenal medulla. Garner and Duncan (13) have reported the presence of dark-cored droplets, but these authors believe such structures to be artifactual. The present observations do not favor this interpretation.

The presence in the chemoreceptor cells of droplets which may contain catechol amines when taken with the weak positive chromaffinity of these cells would indicate the need for a reconsideration of the possible origins of the chemoreceptor cells. While the present study does not pretend to settle the problem of the nature and origin of the chemoreceptor cells, it does point out the fact that the views of the possible neural origins of these cells should not be entirely discounted.

The experimental demonstration that carotid body function depends on normal carbohydrate metabolism (16) and that the chemoreceptor cells can be stimulated by the accumulation of the metabolic products of anaerobic glycolysis (31) and by high energy phosphate bonds (9) coordinates well with the morphological observations of large amounts of alkaline phosphatase and non-specific esterase in the glomus cells. These concepts of chemoreceptor function point toward a vigorously metabolizing cell. This is affirmed by the presence in the cytoplasm of the chemoreceptor cell of large numbers of small mitochondria, many vesicles, and a well developed endoplasmic reticulum.

The author wishes to express his appreciation to Dr. Don W. Fawcett for many suggestions and assistance in revising the manuscript. He wishes also to acknowledge the able technical assistance of Miss Vira Levytska.

Bibliography

1. Abraham, A., Uber das Nervensystem des Glomus caroticum beim Menschen, *Acta Univ. Szegediensis*, 1942, **1,** 1.
2. Adams, W. E., The Comparative Morphology of the Carotid Body and Carotid Sinus, Springfield, C. C. Thomas, 1958.
3. Boeke, J., Nerve endings, motor and sensory, *in* Cytology and Cellular Pathology of the Nervous System, (W. Penfield, editor), New York, Hoeber, 1932.
4. Boyd, J. D., The development of the human carotid body, *Carnegie Contrib. Embryol.*, 1937, **152,** 1.
5. van Breemen, V. L., *et al.*, An attempt to determine the origin of synaptic vesicles, *Exp. Cell Research*, 1958, suppl. 5, 153.
6. de Castro, F., Sur la structure et l'innervation de la glande intercarotidienne (glomus caroticum) de l'homme et des mammiferes, *Trav. Lab. Recherches biol. Univ. Madrid*, 1926, **24,** 365.
7. de Castro, F., Sur la structure et l'innervation du sinus carotidien de l'homme et des mammiferes, *Trav. Lab. Recherches biol. Univ. Madrid*, 1927–28, **25,** 331.
8. de Castro, F., Sur le structure de la synapse dans les chemocepteurs, *Acta Physiol. Scand.*, 1951, **22,** 14.
9. Dontas, A. S., Effects of energy donors, metabolic inhibitors and substrates on carotid chemoreceptor activity, *J. Pharmacol. and Exp. Therap.*, 1955, **115,** 46.
10. Engström, H., On the double innervation of the sensory epithelia of the inner ear, *Acta Oto-Laryngol.*, 1958, **49,** 109.
11. Engström, H. and Wersall, J., The ultrastructural organization of the organ of Corti and of the vestibular sensory epithelia, *Exp. Cell Research,* 1958, suppl. 5, 460.
12. Engström, H., *et al.*, The carotid body, *Acta Oto-Largyngol.*, 1957, **48,** 379.
13. Garner, C. M., and Duncan, D., Observations of the fine structure of the carotid body, *Anat. Rec.*, 1958, **130,** 691.
14. Gasser, H. S., Comparison of the structure, as revealed with the electron microscope, and the physiology of the unmedullated fibers in the skin nerves and in the olfactory nerves, *Exp. Cell Research*, 1958, suppl. 5, 3.
15. Goormaghtigh, N., and Pannier, R., Les paraganglions du coeur et des zones vasosensibles carotidienne et cardio-aortique chez le chat adulte, *Arch. biol.*, 1939, **50,** 455.
16. Heymans, C., Action of drugs on carotid body and sinus, *Pharmacol. Rev.*, 1955, **7,** 119.
17. Hoffman, H., and Birrell, J. H. W. The carotid

body in normal and anoxic states: an electron microscopic study, *Acta Anat.*, 1958, **32,** 297.

18. Hollinshead, W. H., A cytological study of the carotid body of the cat, *Am. J. Anat.*, 1943, **73,** 185.
19. Hollinshead, W. H., Effects of anoxia upon carotid body morphology, *Anat. Rec.*, 1945, **92,** 255.
20. de Kock, L. L., The intra-glomerular tissues of the carotid body, *Acta Anat.*, 1954, **21,** 101.
21. Koelle, G. B., The histochemical differentiation of types of cholinesterases and their localizations in tissues of the cat, *J. Pharmacol. and Exp. Therap.*, 1950, **100,** 158.
22. Koelle, G. B., The elimination of enzymatic diffusion artefacts in the histochemical localization of cholinesterases and a survey of their cellular distributions, *J. Pharmacol. and Exp. Therap.*, 1951, **103,** 153.
23. Kohn, A., Uber den Bau und die Entwicklung des sog. Carotisdruse, *Arch. mikr. Anat.*, 1900, **56,** 81.
24. Langer, Erick, Beitrage zur Orthologie und Pathologie des Glomus caroticum, *Beitr. path. Anat. u. allg. Path.*, 1952, **112,** 252.
25. Lever, J. D., and Boyd, J. D., Osmophile granules in the glomus cells of the rabbit carotid body, *Nature*, 1957, **179,** 1082.
26. Liljestrand, G., and Zotterman, Y., The effect of certain choline esters on the chemoreceptor activity in the carotid body, *Acta Physiol. Scand.*, 1954, **31,** 203.
27. de Lorenzo, A. J., Electron microscopic observations of the olfactory mucosa and olfactory nerve, *J. Biophysic. and Biochem. Cytol.*, 1957, **3,** 839.
28. de Lorenzo, A. J., Electron microscopic observations on the taste buds of the rabbit, *J. Biophysic. and Biochem. Cytol.*, 1958, **4,** 143.
29. Meyling, H. A., Bau und Innervation von Glomus caroticum und Sinus caroticus, *Acta Neerl. Morphol.*, 1938, **1,** 193.
30. Moore, D. H., and Grimley, P. M., Problems in methacrylate embedding for electron microscopy, *J. Biophysic. and Biochem. Cytol.*, 1957, **3,** 255.
31. Neil, Eric, Chemoreceptor areas and chemoreceptor circulatory reflexes, *Acta Physiol. Scand.*, 1951, **22,** 54.
32. Palade, G. E., A study of fixation for electron microscope, *J. Exp. Med.*, 1952, **95,** 285.
33. Palade, G. E., A small particulate component of the cytoplasm, *J. Biophysic. and Biochem. Cytol.*, 1955, **1,** 59.
34. Palade, G. E., Studies on the endoplasmic reticulum, *J. Biophysic. and Biochem. Cytol.*, 1955, **1,** 567.
35. Palade, G. E., The endoplasmic reticulum, *J. Biophysic. and Biochem. Cytol.*, 1956, **2,** No. 4, suppl., 85.
36. Palay, S. L., Synapses in the central nervous system, *J. Biophysic. and Biochem. Cytol.*, 1956, **2,** No. 4, suppl., 193.
37. Palay, S. L., The morphology of synapses in the central nervous system, *Exp. Cell Research*, 1958, suppl. 5, 275.
38. Palay, S. L., and Palade, G. E., The fine structure of neurons, *J. Biophysic. and Biochem. Cytol.*, 1955, **1,** 69.
39. Porter, K. R., and Blum, J., A study of microtomy for electron microscopy, *Anat. Rec.*, 1953, **117,** 685.
40. de Robertis, E., Submicroscopic morphology and function of the synapse, *Exp. Cell Research*, 1958, suppl. 5, 347.
41. de Robertis, E., and Bennett, H. S., Some features of the submicroscopic morphology of synapses in frog and earthworm, *J. Biophysic. and Biochem. Cytol.*, 1955, **1,** 47.
42. de Robertis, E., and Franchi, C. M., Electron microscope observations on synaptic vesicles in synapses of the retinal rods and cones, *J. Biophysic. and Biochem. Cytol.*, 1956, **2,** 307.
43. de Robertis, E., and Vaz Ferreira, A., Electron microscope study of the excretion of catechol-containing droplets in the adrenal medulla, *Exp. Cell Research*, 1957, **12,** 568.
44. Robertson, J. D., The ultrastructure of a reptilian myoneural junction, *J. Biophysic. and Biochem. Cytol.*, 1956, **2,** 381.
45. Ross, L., An electron microscopic study of carotid body chemoreceptors, *Anat. Rec.*, 1957, **127,** 481.
46. Ross, L., A cytological and histochemical study of the carotid body of the cat, *Anat. Rec.*, 1957, **129,** 433.
47. Schmidt, Carl F., and Comroe, J. H., Jr., Functions of the carotid and aortic bodies, *Physiol. Rev.*, 1940, **20,** 115.
48. Smith, C. A., and Dempsey, E. W., Electron microscopy of the organ of Corti, *Am. J. Anat.*, 1957, **100,** 337.
49. Wersall, J., Studies on the structure and innervation of the sensory epithelium of the cristae ampullares in the guinea pig, *Acta Oto-Laryngol.*, 1956, suppl., 126.

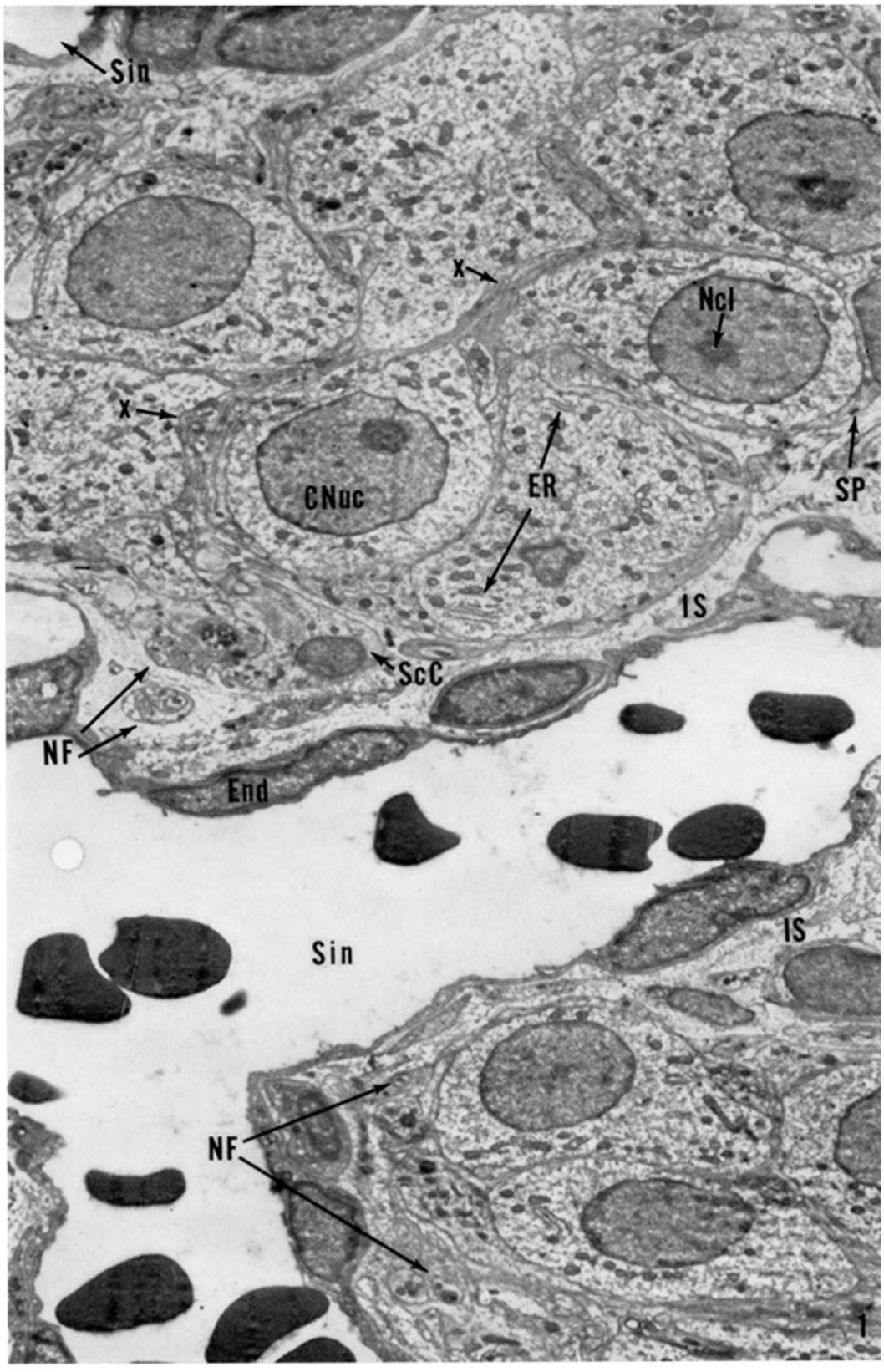
Explanation of Plates

Abbreviations Used in the Figures

CC, chemoreceptor cell.
CNuc, chemoreceptor cell nucleus.
CTP, connective tissue cell process.
DVe, dark-cored vesicle.
End, endothelium.
ER, endoplasmic reticulum.
GC, Golgi complex.
IS, interstitial space.
M, mitochondrion.
Ncl, nucleolus.
NF, nerve fiber.
Nuc, nucleus.
OB, osmiophilic body.
ScC, Schwann cell.
Sin, sinusoid.
SNuc, sustentacular cell nucleus.
SP, sustentacular cell process.
Va, vacuole.
Ve, vesicle.

Plate 134

Fig. 1. A lower power electron micrograph of the carotid body illustrating some general features of its morphology. A broad, irregular sinusoid (*Sin*), lined by endothelium (*End*) divides and almost surrounds two islands of parenchymal cells. A portion of this same sinusoid appears in the upper left corner. The nuclei of the chemoreceptor cells (*CN*uc) with their nucleoli (*N*cl) are prominent as is the endoplasmic reticulum (*ER*). Many unmyelinated nerve fibers (*NF*) are found in the interstitial spaces (*IS*). A Schwann cell (*ScC*) is seen in this space with one of its processes passing into the chemoreceptor cell island. At the periphery of the glomus (upper right of figure) a portion of a sustentacular cell nucleus can be seen with one of its processes (*SP*) coursing around a chemoreceptor cell. The complex interweavings of the sustentacular cell processes are indicated at *x*. × 5,000.

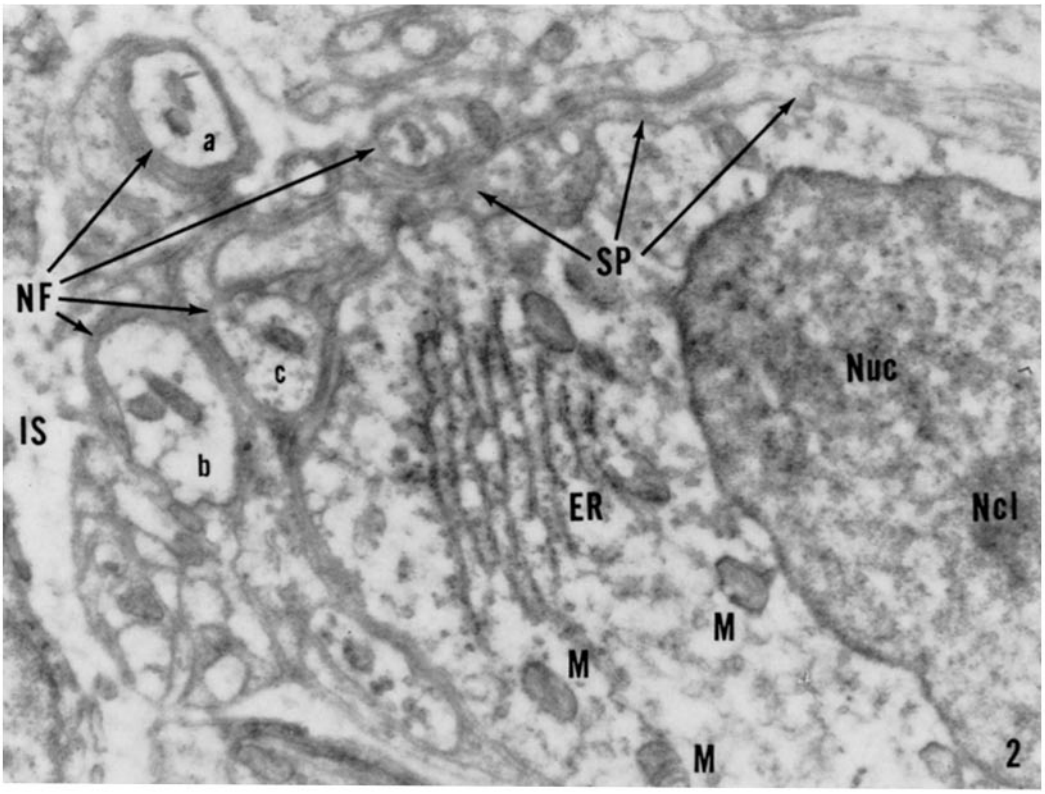


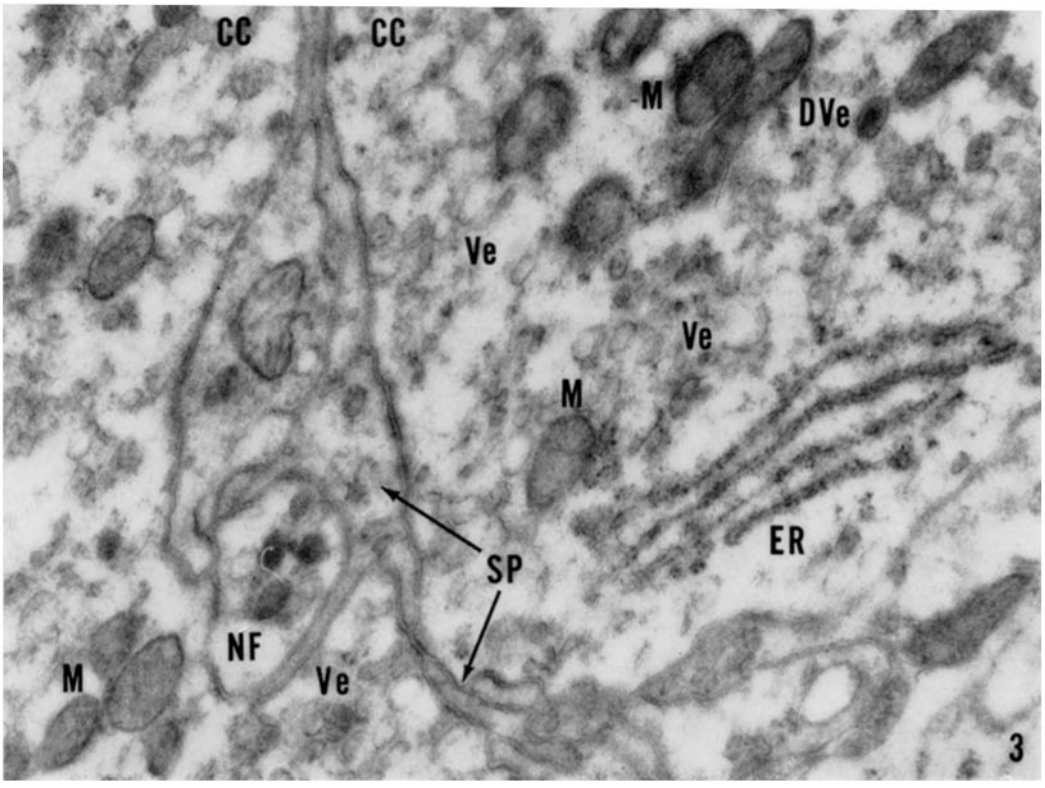
(Ross: Carotid body fine structure)

PLATE 135

FIG. 2. A portion of a chemoreceptor cell and surrounding interstitial space (*IS*). At the left of the micrograph a group of nerve fibers (*NF*) can be seen. The axon indicated *a* lies in the interstitial space and can be seen to be enfolded by a Schwann cell mesaxon. Those labelled *b* and *c* are encircled by sustentacular cell processes (*SP*) which likewise surround the chemoreceptor cell. The axons characteristically exhibit small, dense mitochondria and small vesicles. The complexity of the distribution of the sustentacular cell processes can be seen. The chemoreceptor cell has a prominent granular endoplasmic reticulum (*ER*), small mitochondria (*M*), and many small vesicles. × 19,000.

FIG. 3. An enlarged portion of two adjacent chemoreceptor cells (*CC*) with intervening sustentacular cell processes (*SP*), the complex overlapping of which can be clearly seen. The chemoreceptor cell cytoplasm exhibits granular endoplasmic reticulum (*ER*), mitochondria (*M*), dark-cored vesicles (*DVe*), and many small clear vesicles (*Ve*). At the lower left of the plate a nerve fiber (*NF*) can be seen which, in part, abuts on a chemoreceptor cell. Most of the axon, however, can be seen to be surrounded by the sustentacular cell process. The fiber contains several small dense mitochondria and a few vesicles. × 48,000.

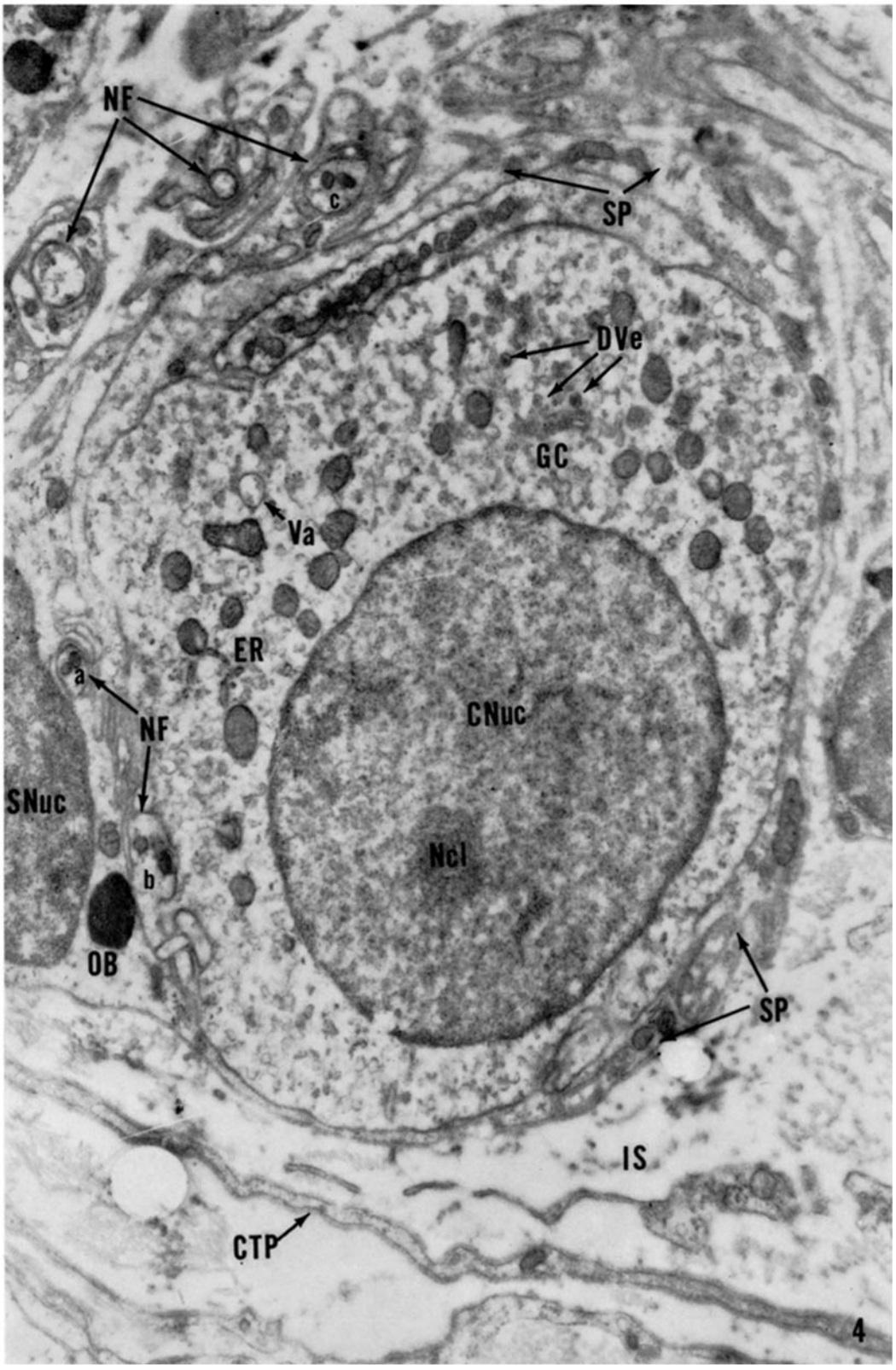




(Ross: Carotid body fine structure)

PLATE 136

FIG. 4. A micrograph showing an isolated chemoreceptor cell with a sustentacular cell, whose nucleus (*SNuc*) appears at the left of the plate. Sustentacular cell processes (*SP*), in complex array, completely surround the chemoreceptor cell. In the interstitial space (*IS*) can be found connective tissue cell processes (*CTP*), collagen fibrils, and nerve fibers (*NF*). The axon labelled *c* is in passage from an interstitial location to one where it will be enfolded by sustentacular cell processes. The axons labelled *a* are definitely encircled by the sustentacular cell, while *b* has one aspect contiguous with the chemoreceptor cell. A dense osmiophilic body (*OB*) lies in the sustentacular cell cytoplasm. In the chemoreceptor cell cytoplasm, several dark-cored vesicles (*DVe*) can be seen to be closely associated with the Golgi complex (*GC*). × 16,000.

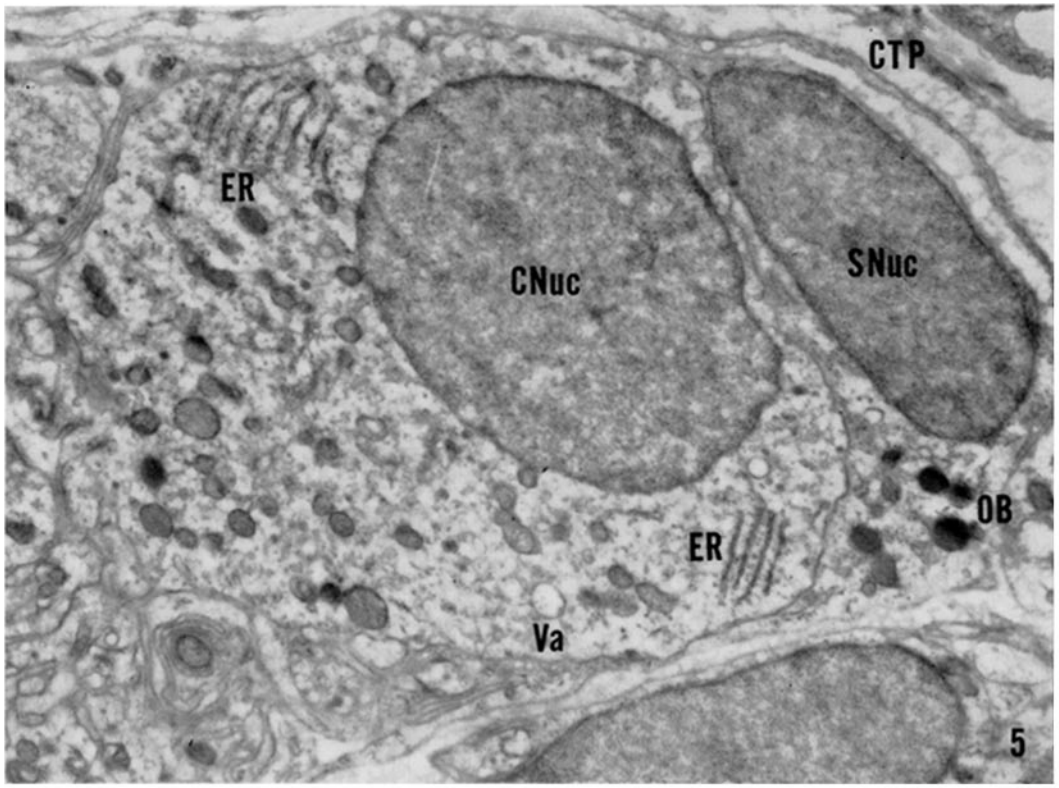


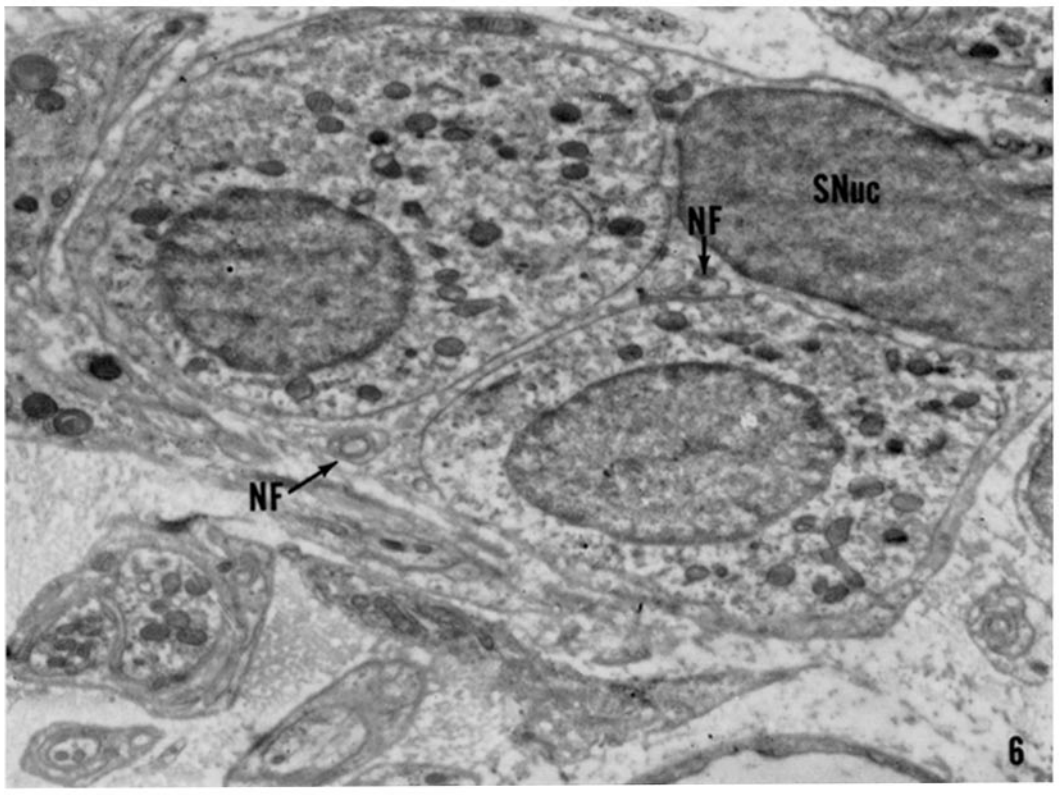
(Ross: Carotid body fine structure)

PLATE 137

FIG. 5. A chemoreceptor cell surrounded by a sustentacular cell. Except for the complex region at the left, the processes of this sustentacular cell can be traced almost completely around the chemoreceptor cell. A connective tissue cell process (*CTP*) can be seen to course around the glomus. × 13,000.

FIG. 6. A micrograph showing two chemoreceptor cells surrounded by the processes of one sustentacular cell. Nerve fibers (*NF*) can be seen in these processes. × 11,000.



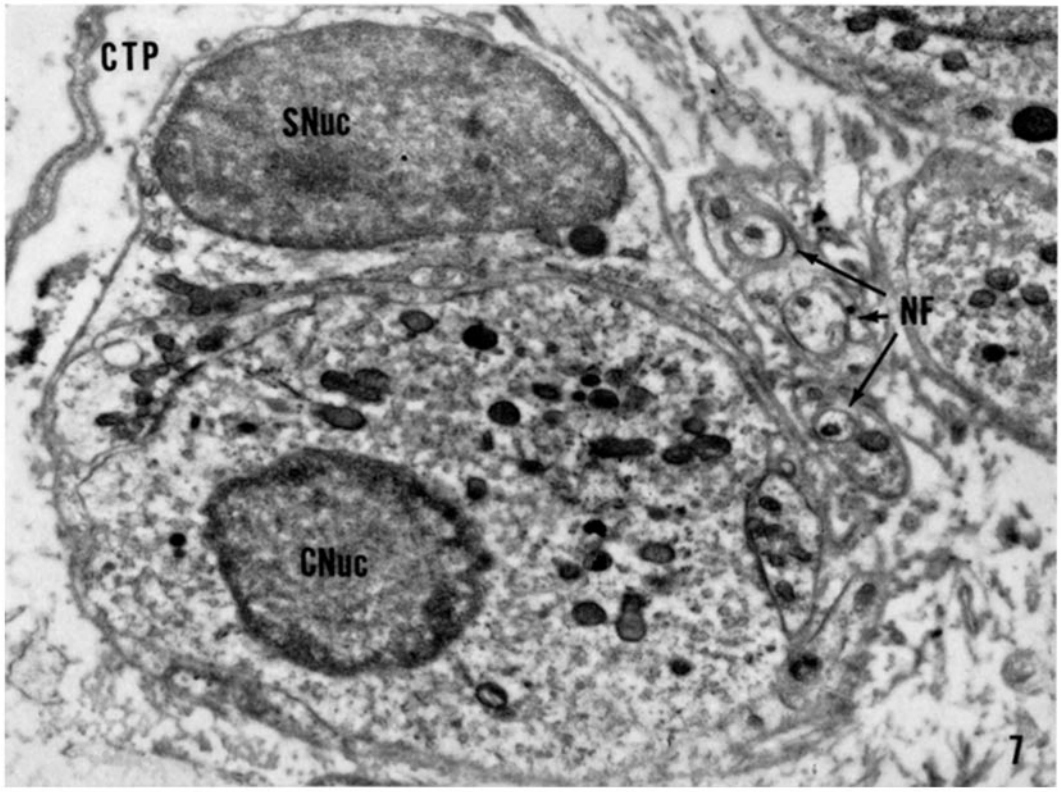


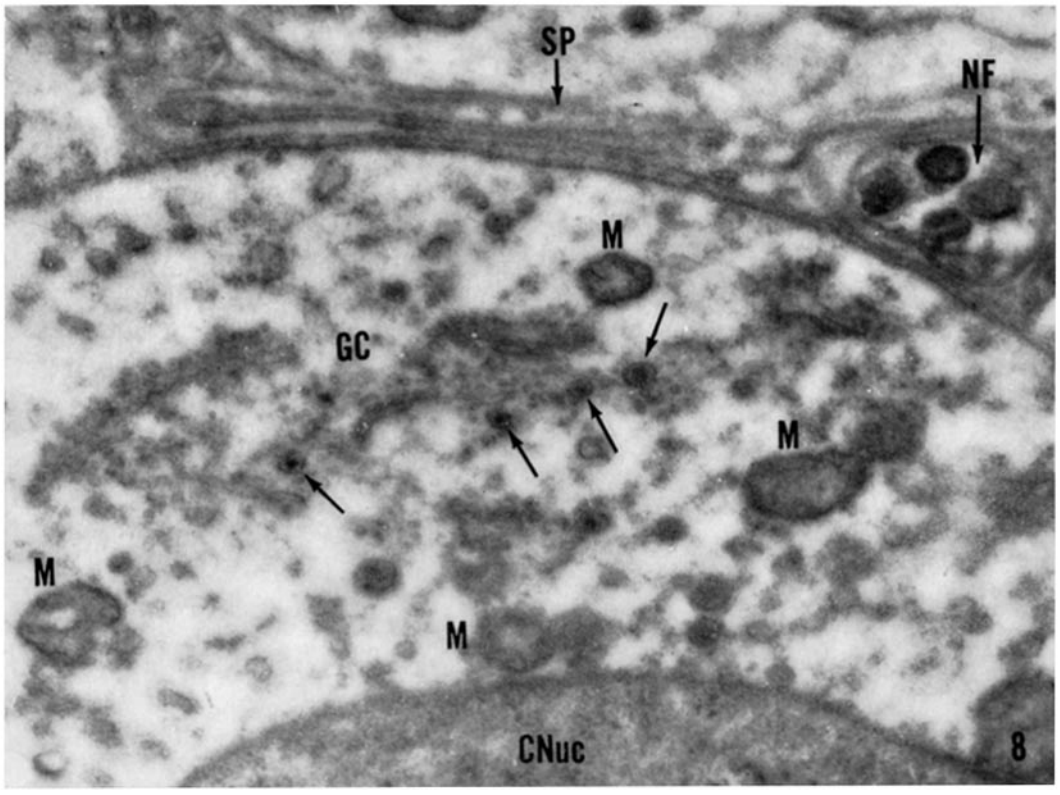
(Ross: Carotid body fine structure)

PLATE 138

FIG. 7. An isolated chemoreceptor cell with its surrounding sustentacular cell. Several unmyelinated nerve fibers (*NF*) can be seen in the interstitial space in their Schwann cell mesaxons. Portions of chemoreceptor and sustentacular cells appear in the upper right of the figure. A fibroblast process (*CTP*) lies in the interstitial space. × 12,000.

FIG. 8. An enlarged micrograph of a portion of a chemoreceptor cell with its surrounding sustentacular cell processes (*SP*). A nerve fiber (*NF*) can be seen to be enmeshed in these processes. A Golgi complex (*GC*), consisting of short tubules and vesicles, lies above the nucleus (*CN*uc). Closely associated with this complex are several dark-cored vesicles, indicated by arrows. Other smaller vesicles are scattered throughout the cytoplasm. × 40,000.



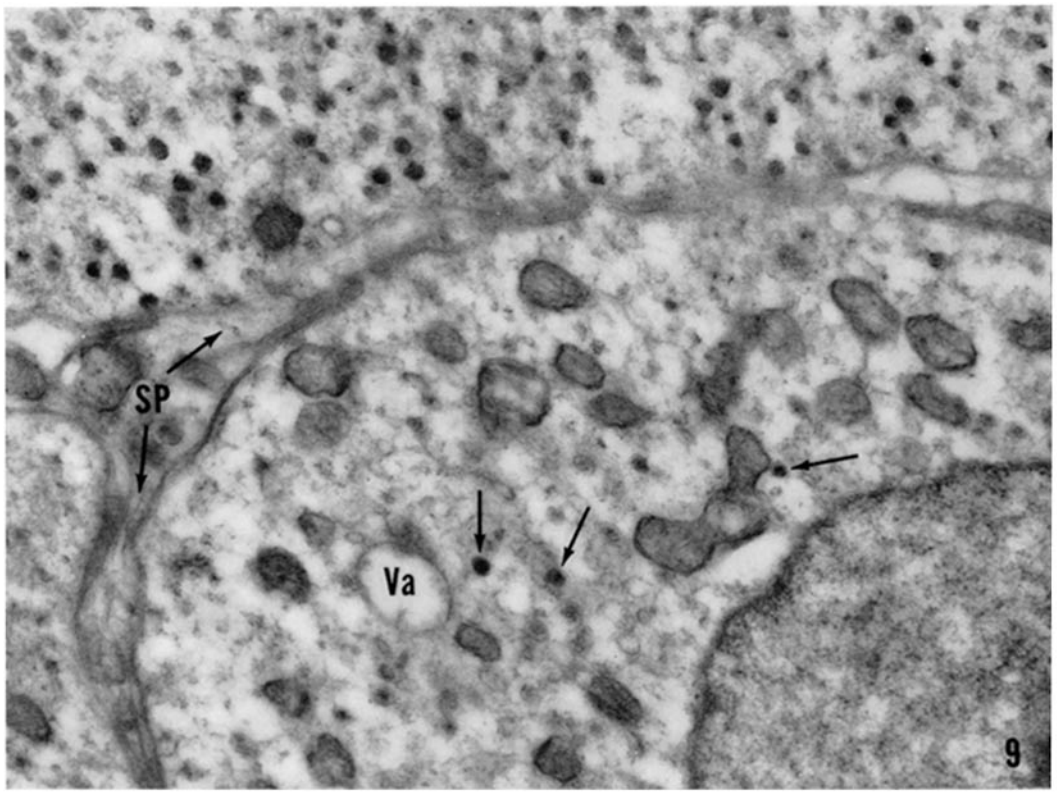


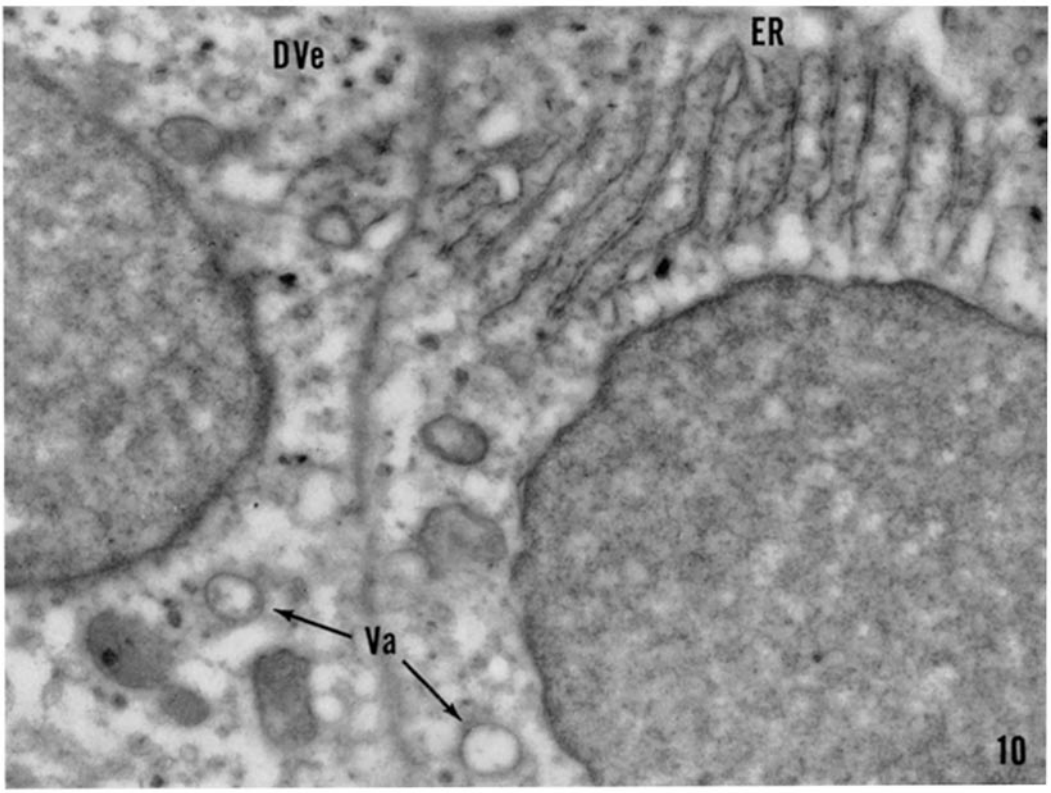
(Ross: Carotid body fine structure)

PLATE 139

FIG. 9. A micrograph showing enlarged regions of three chemoreceptor cells with the intervening sustentacular cell processes (*SP*). The cytoplasm of the cell at the top of the field exhibits large numbers of dark-cored vesicles, many of them totally dense. Similar vesicles in the other cells are indicated by arrows. In addition the cytoplasm exhibits a large vacuole (*Va*) and many small clear vesicles. × 30,000.

FIG. 10. Two adjacent chemoreceptor cells which apparently are contiguous. The cytoplasm possesses a granular endoplasmic reticulum (*ER*), dark-cored vesicles (*DVe*), and vacuoles (*Va*) which have small vesicles associated with them. × 25,000.





(Ross: Carotid body fine structure)