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The ultrastructure and connections of blood vessels supplying the rat carotid body and carotid sinus

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Summary

The morphology of blood vessels supplying the carotid body and carotid sinus was analysed in 41 rats by using a combination of light microscopic, transmission electron microscopic and scanning electron microscopic methods. We found that a large sphincter-like intimal cushion was located at the orifice of the *carotid body artery*, where the vessel arose from the external carotid or occipital artery. The sphincter contained circumferential smooth muscle and constricted the diameter of the orifice to less than half. After reaching the carotid body, the carotid body artery typically divided into three or four first-order and five or more second-order branches. Usually three or four *second-order branches* supplied the carotid body, but all other branches continued on to such structures as the superior cervical ganglion, nodose ganglion, vagus nerve and carotid sinus. *Third* and *fourth-order* branches gave rise to *terminal arterioles* that supplied the glomus tissue. Vessels resembling *precapillary sphincters* were located at the junction of terminal arterioles and capillaries. Precapillary sphincters had a wall comprised of protruding endothelial cells surrounded by smooth muscle cells or pericytes. Most terminal arterioles gave rise to two types of capillaries. *Type I capillaries* penetrated a glomus cell cluster and had an intimate association with glomus cells of that cluster had a luminal diameter ranging from about 8 to over 20 μm, but varied in calibre along their length. These vessels followed a winding course, made one or more U-shaped turns, and usually had multiple connections with venules. Type I capillaries had a thin fenestrated endothelium, an incomplete covering of pericytes, and a thin basal lamina. By contrast, *type II capillaries* did not penetrate glomus cell clusters, had a uniform diameter of about 7 μm, and had both straight and curved regions. Both types of capillaries were bypassed by *arteriovenous anastomoses* formed by terminal arterioles that joined small venules directly. *Venules* of the carotid body were interconnected with one another and joined major veins of the neck via several routes.

Arterioles derived from the carotid body artery also supplied an extensive network of vasa vasorum in the adventitia of the carotid sinus. Short capillaries and larger shunt vessels connected arterioles with the numerous venules in the sinus wall; and the venules in turn were connected to the venous plexus at the surface of the carotid body.

We conclude that the arterial branching pattern, intimal cushions and precapillary sphincters participate in the control of carotid body blood flow and also may influence plasma skimming.

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However, the existence of arteriovenous anastomoses in addition to at least two types of capillaries indicates that blood flow to the chemoreceptive tissue can be regulated independently of total blood flow. Furthermore, the redundancy of venous connections may be related to the sensitivity of carotid body chemoreceptors to changes in venous pressure.

Introduction

The carotid body is an arterial chemoreceptor: its neural output reflects the partial pressure of oxygen (PO_2) and carbon dioxide (PCO_2) as well as the pH, osmolarity and temperature of arterial blood (Eyzaguirre & Fidone, 1980). The organ has an enormous blood flow for its size (2000 ml/100 g/min; Daly *et al.*, 1954). Consistent with the function of measuring the composition of arterial blood without changing it, the carotid body's venous blood has an O_2 content approaching that of arterial blood.

The high rate of blood flow has been considered the reason that carotid body chemoreceptors are comparatively unresponsive to anaemia and carboxyhaemoglobinaemia, which reduce the O_2 content of blood without diminishing the PO_2 (Comroe & Schmidt, 1938; Comroe, 1964). This explanation also fits with the extreme vascularity of the organ and the large calibre of its capillaries (de Castro, 1951).

However, there are reports that arteriovenous anastomoses are present in the carotid body (see Discussion), and simultaneous measurements of total blood flow and local flow within the organ suggest that some blood bypasses the chemoreceptive tissue (Acker & Lübbers, 1977). Furthermore, there is evidence that tissue PO_2 in the carotid body is appreciably lower than would be predicted from the arterial PO_2, blood and oxygen consumption (Acker & Lübbers, 1975; see also Lübbers *et al.*, 1977). One explanation for these phenomena is that plasma skimming diminishes the haematocrit of blood in some vessels supplying the chemoreceptive tissue, and the erythrocyte-rich blood is shunted into venules by arteriovenous anastomoses (reviewed by Acker, 1980). Such mechanisms also may participate in the carotid body's insensitivity to anaemia and carboxyhaemoglobinaemia (reviewed by Lahiri, 1980).

Some features of the carotid body's vasculature have been revealed by examining single or serial sections by light microscopy (de Kock, 1960; Serafini-Fracassini & Volpin, 1966; Seidl, 1975, 1976; Hesse & Böck, 1980) and by examining the organ after injecting opaque substances into its blood vessels (Muratori, 1943; de Castro, 1951; Chungcharoen *et al.*, 1952b; de Castro & Rubio, 1968; Schäfer *et al.*, 1973). Electron microscopic studies have provided descriptions of the ultrastructure of fenestrated capillaries near glomus cells (Biscoe & Stehbens, 1966; Al-Lami & Murray, 1968; Kobayashi, 1968; Böck, 1973). However, existing information on the morphology of the carotid body's vascular bed is not sufficient to explain the organ's distinctive functional properties.

The goal of the present morphological study of the rat carotid body was: 1. to analyse the source, course, branching pattern and ultrastructural features of the arterial supply of the carotid body; 2. to examine the ultrastructural diversity of blood vessels connecting arterioles with venules; 3. to elucidate the ultrastructure of venules and the

geometry, connections and destinations of veins that drain the carotid body; and 4. to determine the types and extent of interconnections of the blood supplies of the carotid body and carotid sinus.

We found that a large sphincter-like intimal cushion is located at the origin of the carotid body artery. The carotid body artery branches extensively within the carotid body but only a few branches end in the organ itself. There exist at least two types of capillaries, which are connected in parallel and are preceded by precapillary sphincters, and there are also direct arteriovenous connections. The venous side of the carotid body's vascular bed has a conspicuous redundancy of thoroughfares and is intimately linked to the blood supply of the carotid sinus. In a companion paper (McDonald, 1983a) we report the results of a quantitative electron microscopic study in which we classified various types of blood vessels in the rat carotid body according to objective morphological criteria and determined the density of autonomic nerve endings on different segments of the carotid body's vascular bed.

Methods

Our experiments were carried out on 41 female rats of the Long-Evans strain (body weight 200–280 g). All animals were anaesthetized with sodium methohexital (75 mg/kg injected intraperitoneally), then ventilated with oxygen via a tracheal cannula attached to a Harvard rodent respirator.

ULTRASTRUCTURE OF BLOOD VESSELS OF THE CAROTID BODY AND CAROTID SINUS

Preparation of specimens for transmission electron microscopy

Twenty rats were perfused through the left ventricle with two fixatives in succession. The perfusion pressure was 120 mm Hg. The first fixative (A) contained 3% glutaraldehyde and 22 mM hydrogen peroxide in 75 mM cacodylate buffer containing 2 mM calcium chloride, 30 mM sucrose and 4% polyvinylpyrrolidone (PVP, mol wt 40 000). The second fixative (B) differed only in that it lacked hydrogen peroxide. After the carotid bodies were removed and freed of such structures as the superior cervical ganglion, they were treated with 1.5% osmium tetroxide in 14 mM Veronal–Acetate–HCl buffer (pH 7.4) for 14–18 h at 4° C and 1.5% uranyl acetate in 25 mM sodium maleate buffer (pH 5.0) for 6 h at 4° C, then dehydrated and embedded as described in previous studies (McDonald & Mitchell, 1975; McDonald & Blewett, 1981).

Carotid bodies were embedded in aluminium weighing pans containing epoxy resin to a depth of 4 mm. Specimens sawed from discs of polymerized resin were mounted to transparent acrylic cylinders (7.9 mm × 12.7 mm, Ladd Research Industries, Inc., Burlington, Vermont) with cyanoacrylate adhesive. This procedure permitted us to study and photograph specimens with a compound light microscope before they were sectioned (example shown in Fig. 11). Carotid bodies were oriented so sections could be cut parallel to the organs' rostrocaudal axis.

Sections 0.5 μm in thickness were stained with toluidine blue for light microscopy, and sections approximately 50 nm in thickness were mounted on single slot grids and stained with lead citrate for electron microscopy. Sections were cut from various levels of the carotid body, from the surface to the centre. The diameter of blood vessel profiles was determined by measuring the broadest aspect of the smaller dimension of the lumen. Vessel wall thickness was measured as the

distance from the luminal surface of the endothelium to the innermost aspect of the adventitia (including pericytes but excluding fibroblasts). Wall thickness was expressed as the harmonic mean of twelve measurements made at 30° intervals around the perimeter of a vessel profile. The methods used to make these measurements are described in detail in a companion paper (McDonald, 1983a).

Preparation of vascular casts for scanning electron microscopy

Vascular casts, made of methacrylate resin (Mercox CL-2B, Japan Vilene Company Ltd, Tokyo), were prepared in ten rats according to our modification of the method described by Murakami (1975). After an intravenous injection of heparin (250 units), rats were perfused through the heart for 1 min with a buffered salt solution (McEwen, 1956), which contained 4% PVP, was heated to 37° C, and equilibrated with 95% O_2–5% CO_2. Thereafter, rats were perfused for 10 min with fixative B. Both solutions were perfused at a pressure of 120 mm Hg.

Following the fixative, Mercox (40 g resin containing 1 g catalyst) was infused through the heart for a period of 4–6 min. By the end of the infusion, the Mercox was solidified. In different rats the infusion pressure ranged from 120 to 200 mm Hg and the volume infused ranged from 7 to 32 ml.

Tissues in the region of the carotid bifurcation were excised and then corroded in 80% potassium hydroxide at room temperature. Specimens were corroded for a day and then washed in water for a day repeatedly until they were clean. Some casts were placed into an ultrasonic cleaner to remove small blood vessels (example in Fig. 2). Others were dissected to remove vessels overlying the carotid body. Casts were dried under vacuum after being frozen in water. Dried specimens were sputter-coated with gold immediately before they were examined with a scanning electron microscope.

MORPHOLOGY OF THE CAROTID BODY ARTERY

Scanning electron microscopy of vessel surfaces

Surface features of the intimal cushion at the origin of the carotid body artery were analysed by scanning electron microscopy in specimens from two rats. These animals were perfused with McEwen's buffer and then with fixative B as in the preparation of vascular casts. However, instead of infusing Mercox, we excised the carotid bifurcations, dehydrated them in cold ethanol, and then dried them in a critical point drier using liquid CO_2. Dried specimens were glued with conductive adhesive to aluminium stubs, dissected further to expose the orifice of the carotid body artery, and finally sputter-coated with gold.

Serial sections

The branching pattern of the carotid body artery and connections between arterioles and capillaries were studied by preparing serial sections of carotid bodies from six rats. In two cases, serial sections (0.5 μm in thickness) were cut from carotid bodies prepared for transmission electron microscopy (McDonald & Haskell, 1983). Four additional animals were perfused for 1 min with a solution containing 0.9% NaCl and 4% PVP and then perfused for 5 min with Bouin's fixative which also contained PVP. Serial sections (7 μm in thickness) cut from blocks of tissue embedded in paraffin were stained with iron haematoxylin and aniline blue.

Alcian blue staining of vessels

Additional information on the carotid body artery's branching pattern was obtained from three rats in which blood vessels were stained *in situ* with alcian blue. For these experiments we used our modification (McDonald & Blewett, 1981) of a method described originally by Anderson & Anderson (1975).

VENOUS CONNECTIONS OF THE CAROTID BODY

Connections between venules of the carotid body and major veins of the neck were elucidated by studying specimens described above and vascular injections made of silicone rubber. To prepare the silicone casts, two rats were perfused with McEwen's buffer containing 4% PVP, 0.02% sodium nitrite and 2 units of heparin/ml. After this, solution (at 42° C) was perfused for 2 min at a pressure of 120 mm Hg, 50 ml of silicone rubber injection compound (Microfil MV-112, Canton Bio-Medical Products, Inc., Boulder, Colorado) was perfused over 2–3 min at a pressure of 120–200 mm Hg and a temperature of 24° C. The preparations were stored for 24 h at 4° C during which the silicone solidified, and then blood vessels in the neck were removed in a block of tissue, fixed in 10% formalin for 24 h, and cleared either in glycerol or (after alcohol dehydration) in xylene followed by benzyl benzoate. Terms we used for naming veins are consistent with those used in the atlas by Greene (1935).

Results

CAROTID BODY ARTERY

The carotid body was supplied by branches of a single artery, the carotid body artery. The artery arose from the dorsolateral aspect of the external carotid artery between the carotid bifurcation and the origin of the occipital artery (Figs. 9, 10) or from the dorsolateral aspect of the proximal portion of the occipital artery (Fig. 2). In one specimen the artery arose from the external carotid artery at a point distal to the origin of the occipital artery. (In the rat the carotid bifurcation is oriented such that the course of the internal carotid artery diverges from and is dorsolateral to the external carotid artery.)

Intimal cushion at origin of carotid body artery

When viewed from the lumen of the parent artery, the orifice of the carotid body artery appeared as a hole with a sharply defined border (Figs. 1, 5). At the perimeter of the orifice, a sphincter-like intimal cushion reduced the diameter of the lumen by as much as 60%. In vascular casts the region of the intimal cushion caused such an abrupt narrowing at the origin of the carotid body artery as to create the illusion that the artery was not attached to its parent vessel (Figs. 2, 6).

The intimal cushion was composed of irregularly-shaped, circumferential smooth muscle cells enveloped by basal laminae, elastic laminae, collagenous fibres and other components of the extracellular matrix (Figs. 3, 4). The luminal surface of the endothelium was irregular, and on the abluminal surface, cytoplasmic extensions of endothelial cells projected toward the smooth muscle cells (Fig. 4).

The thickness of the cushion increased from apex to base, but did not exceed half the wall thickness of the external carotid artery (approximately 50 μm). The tip of the cushion was slightly everted into the lumen of the parent artery in the sectioned material (Fig. 3) and in some (Fig. 1) but not all (Fig. 5) specimens examined by scanning electron microscopy.

The lumen of the carotid body artery reached its greatest diameter just distal to the intimal cushion where it penetrated the wall of the parent artery (Figs. 3, 5). Beyond this

region, the lumen of the carotid body artery diminished somewhat and then remained uniform in calibre. Just beyond its origin, the artery typically made a 90° bend with respect to the orifice, and then followed a straight course to the carotid body (Figs. 2, 10).

Main portion of carotid body artery

Structure of wall. The wall of the artery consisted of a thin nonfenestrated endothelium, two or three layers of circumferential smooth muscle, internal and external elastic laminae, and an adventitial layer that merged with the dense connective tissue (ligament of Mayer) in the crotch of the carotid bifurcation (Figs. 7, 8).

The nuclei of endothelial cells, as viewed as depressions in the surface of vascular casts, were oriented with their long axis parallel to the direction of blood flow (Fig. 15). Near the origin of the artery, bands of longitudinally oriented smooth muscle cells were located outside the circumferential smooth muscle (Fig. 7). Elastic laminae in the tunica media of the parent artery were in continuity with the internal and external elastic laminae of the carotid body artery (Fig. 3). Bundles of axons were present in the adventitia (Fig. 8).

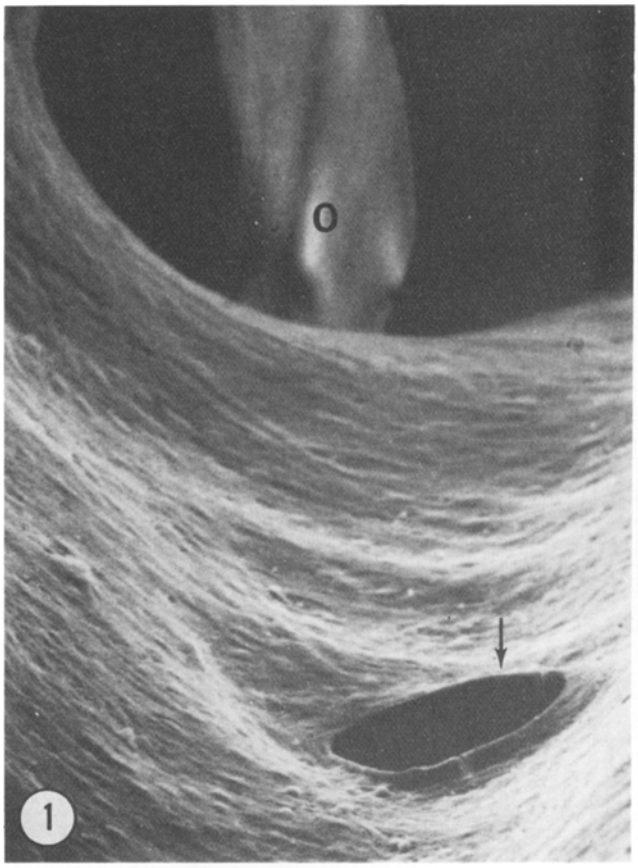
Pattern of branching. The carotid body artery followed a trajectory that brought it to the caudal or dorsomedial surface of the carotid body. The caudal pole of the carotid body was 0.5–2 mm rostral to the carotid bifurcation (Fig. 34). Near or just inside the carotid body, the main artery gave rise to three or four first-order branches (Figs. 9–12). Most first-order branches arose at approximately the same site (Figs. 2, 9, 12), and all arose within an interval of 0.5 mm. Near their origin most first-order branches gave rise to one or more second-order branches.

Fig. 1. The luminal surface of a right external carotid artery showing the origin of the carotid body artery (arrow). The sharp edge of the orifice results from a sphincter-like intimal cushion. This view is looking dorsally and rostrally toward the origin of the occipital artery (O). Scanning electron micrograph. × 390.

Fig. 2. A vascular cast showing a carotid body artery from its origin at the occipital artery (O) to the point at which it divides into multiple branches (arrow). The arrow also designates the location of the carotid body before it and other small blood vessels were removed to expose the major arteries. Scanning electron micrograph of the medial aspect of a left carotid artery bifurcation. E, external carotid artery; I, internal carotid artery. × 100.

Fig. 3. A section through the origin of the carotid body artery (C) illustrating the prominent intimal cushions projecting into the vessel lumen. The tips of the cushions (arrows) point toward the lumen of the external carotid artery (E). Elastic laminae of the carotid body artery are in continuity with those of the external carotid artery. Toluidine blue-stained 0.5 μm section. × 580.

Fig. 4. A portion of an intimal cushion of a carotid body artery (C) showing the irregular endothelial surface, circumferential smooth muscle cells cut in cross-section, and dense extracellular matrix. One endothelial cell has a process that projects into the interior of the cushion (arrow). E, lumen of external carotid artery. Transmission electron micrograph. × 1840.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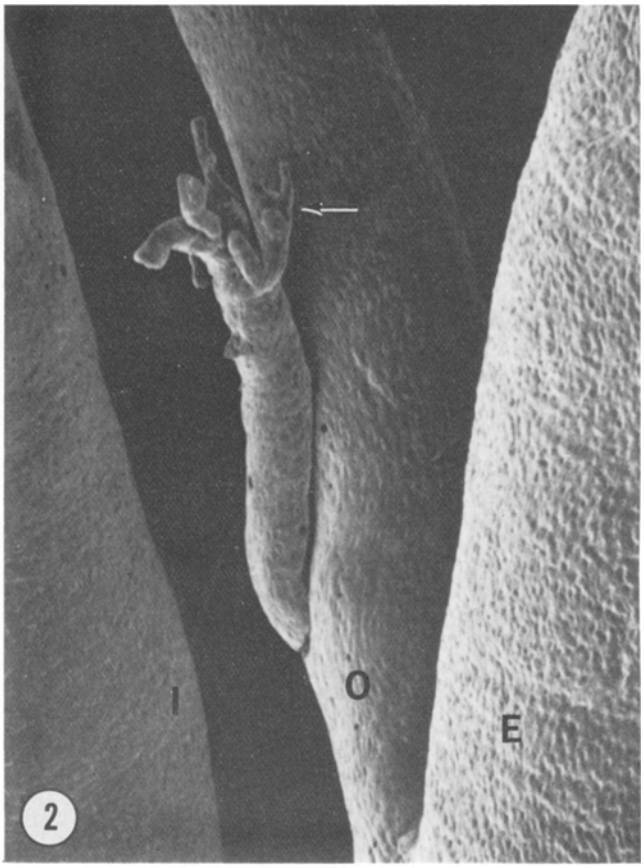
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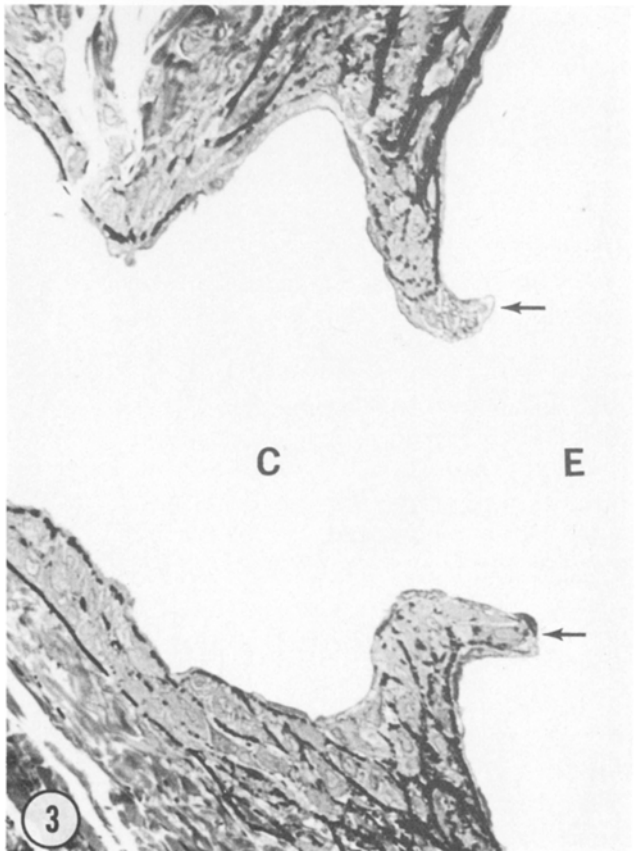
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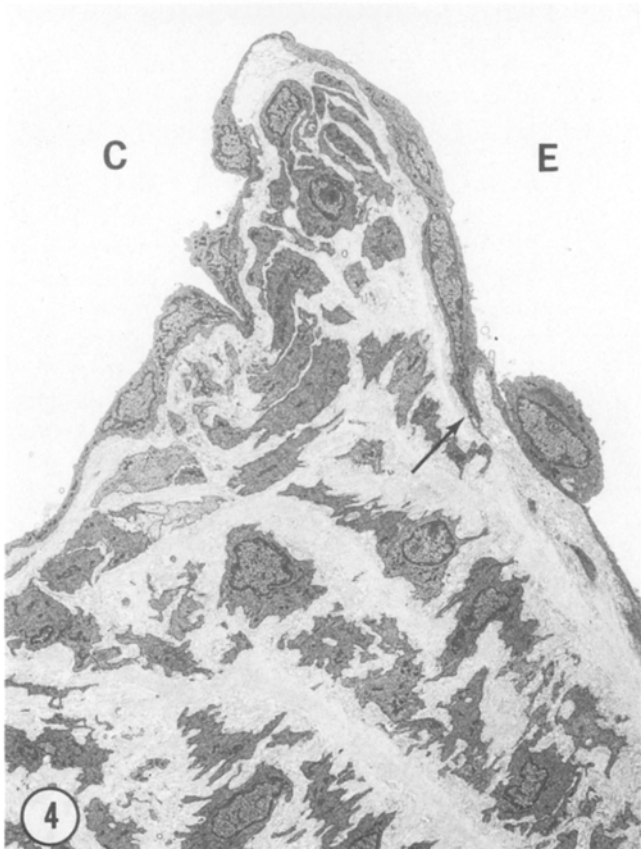
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By examining whole-mounts and serial sections, we learned that first-order and second-order branches of the carotid body artery had destinations that showed only minor differences from rat to rat. In most rats the first branch arose from the caudal or dorsomedial aspect of the artery (branch 1, Fig. 9; also Fig. 10), turned caudally, and traversed the dorsomedial surface of the carotid artery bifurcation. In some cases a second artery also followed this course (branch 2, Fig. 9). These vessels supplied the vasa vasorum of the carotid sinus, caudal part of the superior cervical ganglion, rostral sympathetic trunk, and cervical vagus nerve. They also provided a major portion of the blood supply to the superior laryngeal nerve as far distally as the larynx. A large branch typically arose from the ventrolateral aspect of the carotid body artery, passed over the ventrolateral surface of the internal carotid artery, and supplied the vascular bed of the nodose ganglion and rostral vagus nerve (branch 4, Fig. 9; also Fig. 33); in some cases it also supplied the vasa vasorum of the carotid sinus. Other branches of the main artery (branches 5, 7, 8, Fig. 9) contributed to the blood supply of the rostral part of the superior cervical ganglion, the pharyngeal branch of the vagus nerve and other structures nearby. The large branch leaving the rostral pole of the carotid body en route to the most rostral part of the superior cervical ganglion and adjacent structures (branch 7, Fig. 9) appeared to represent the continuation of the main artery (Fig. 11). This vessel usually followed a trajectory slightly medial to the organ's central axis (Figs. 9, 11, 13). Vessels traversing the interior of the carotid body were adjacent to glomus cells (Fig. 12).

CAROTID BODY ARTERIOLES

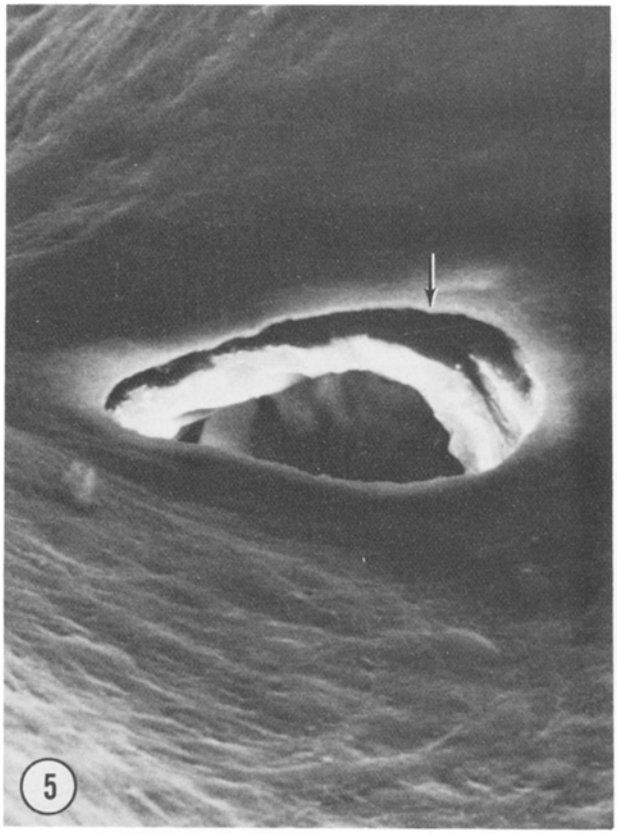
Most carotid bodies were supplied by three or four second-order branches of the carotid body artery. These vessels arose from the proximal portion of first-order branches passing through the carotid body (Figs. 9, 11, 13, 14). In specimens illustrated in Figs. 9

Fig. 5. The thin edge of the intimal cushion (arrow) at the origin of the carotid body artery is shown in a view of the dorsolateral luminal surface of a right external carotid artery. Just beneath the sphincter-like cushion is a short dilated segment of the carotid body artery that corresponds to the widest part of the vessel shown in Fig. 3. Scanning electron micrograph. × 900.

Fig. 6. A cast of a right carotid body artery (C) showing the constriction due to the sphincter-like intimal cushion at the vessel origin (arrow). O, Occipital artery. Scanning electron micrograph of dorsal surface. × 530.

Fig. 7. Cross-section of the carotid body artery (luminal diameter = 80 μm) near its origin showing bundles of longitudinally oriented smooth muscle (arrows) located outside 2–3 layers of circumferential smooth muscle. Elastic laminae are stained very darkly (arrow heads). Toluidine blue-stained 0.5 μm section. × 780.

Fig. 8. Electron micrograph showing components of the wall of the carotid body artery. Only two layers of circumferential smooth muscle are present here. Arrows designate bundles of axons in the adventitia. The lumen is at the right margin. × 4000.



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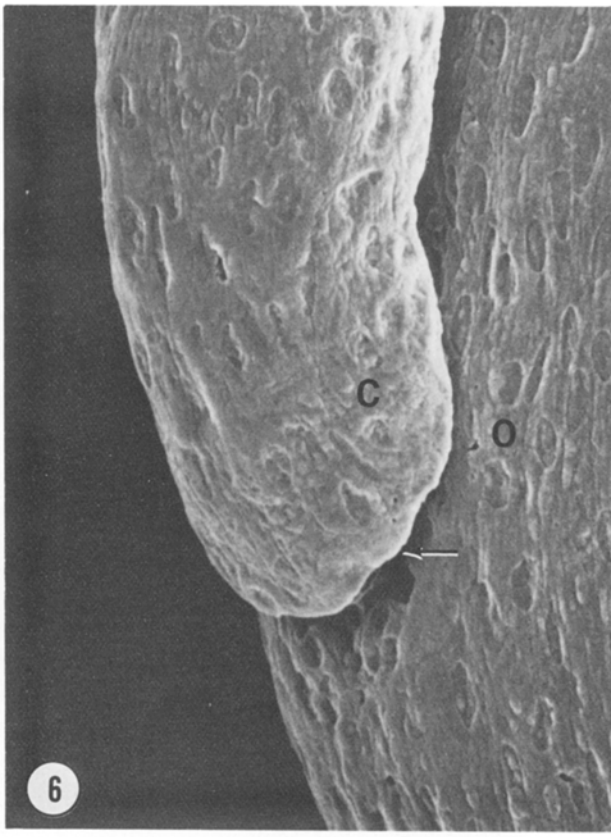

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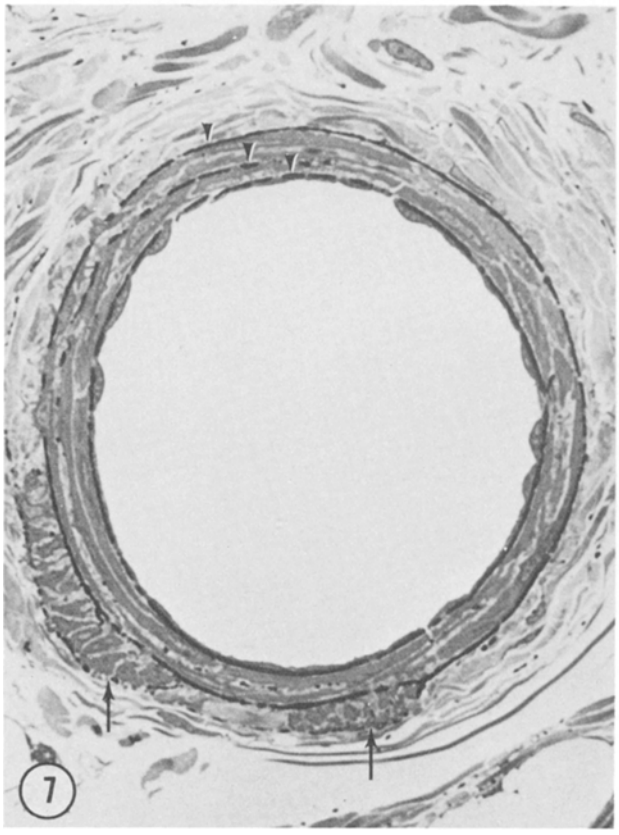

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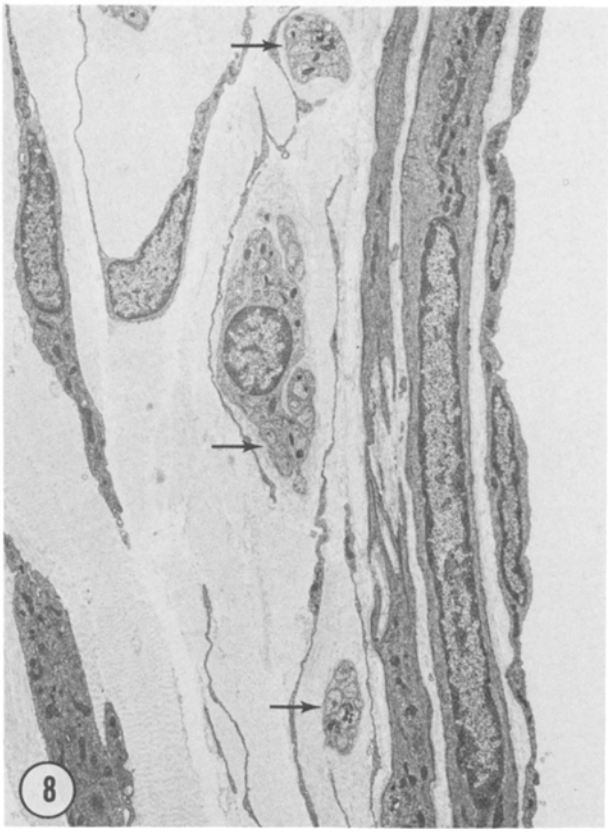

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and 11, the second-order arteries going to glomus tissue came from vessels en route to the superior cervical ganglion, nodose ganglion, vagus nerve and carotid sinus.

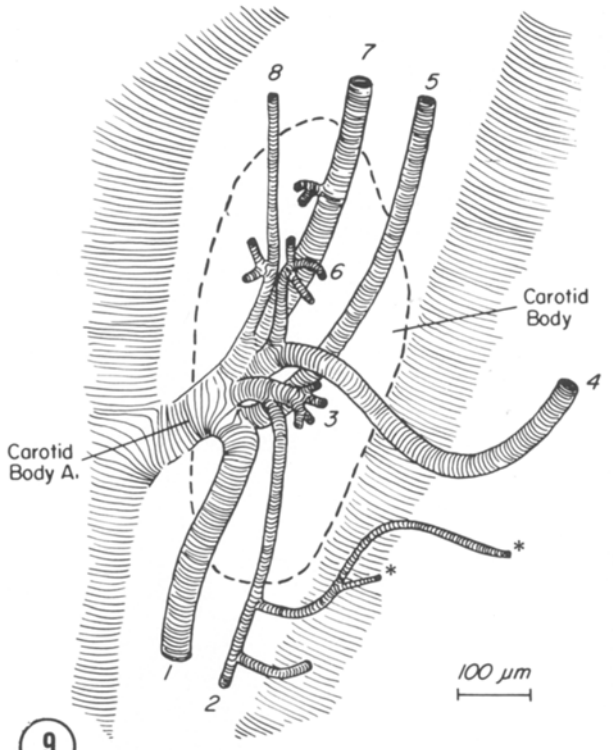
Second-order branches within the carotid body (Fig. 14) were morphologically similar to second-order branches that bypassed the organ (Fig. 15), and their wall structure resembled that of first-order branches. They had a continuous nonfenestrated endothelium with nuclei oriented longitudinally (Figs. 15, 30), one or two layers of smooth muscle wrapped around the circumference, and a thin ($<10\,\mu m$) adventitial layer

Fig. 9. A drawing of the ventrolateral aspect of a left carotid artery bifurcation showing the branching pattern of the carotid body artery reconstructed from serial sections. The carotid body artery arises from the dorsolateral aspect of the external carotid artery. The outer limits of the carotid body are designated by the dashed line. The destinations of three first-order and five second-order branches (numbered) are as follows: 1. First-order branch; emerges from dorsal surface of the carotid body (CB); traverses dorsomedial aspect of carotid bifurcation and terminates in caudal part of the superior cervical ganglion (not shown here). 2. Second-order branch arising with vessel 3; gives rise to two branches to the carotid sinus (asterisks); extends caudally over dorsomedial aspect of carotid bifurcation where branches supply the vagus nerve and the superior laryngeal nerve. 3. Second-order branch; *enters the CB* where it divides repeatedly following a medial to lateral course. 4. First-order branch; gives rise to vessel 6, then crosses ventrolateral surface of carotids sinus portion of internal carotid artery en route to the nodose ganglion and vagus nerve. 5. Second-order branch; arises from dorsal aspect of base of vessel 1; extends rostrally over dorsal surface of CB to the rostral part of superior cervical ganglion. 6. Second-order branch; arises from dorsal surface of base of vessel 4; *enters CB* and divides repeatedly. 7. First-order branch; extends rostrally giving rise to *branch to rostral CB*; ends in most rostral part of superior cervical ganglion, with branches going to adjacent structures. 8. Second-order branch; arises from proximal part of vessel 7; extends rostrally giving rise to a *branch to CB*; destination uncertain. Approximate magnification × 100.

Fig. 10. Dorsomedial aspect of a cast of a right carotid body artery which arises from the external carotid artery (E), gives rise to a large descending first-order branch (arrow), and then continues on toward the carotid body (CB). V, vein from carotid body. Scanning electron micrograph. × 180.

Fig. 11. Ventromedial view of an entire left carotid body showing three first-order branches (numbered) of the carotid body artery that pass through the organ. Branches 1, 2, 3 correspond to branches 1, 4, 7, respectively, in Fig. 9. Arrows mark two of the four second-order branches that end in the carotid body. The carotid body artery arises from the external carotid artery at a point just outside the lower margin of the micrograph. E marks proximal portion of external carotid artery; distal portion of artery has been removed. V, venules at the rostral pole of the carotid body. Specimen fixed by perfusion for electron microscopy, embedded whole in epoxy resin, viewed by transillumination and photographed with a compound microscope. × 130.

Fig. 12. Section of a left carotid body showing the region where three first-order branches (arrows) arise from the carotid body artery. Luminal diameter of the branches ranges from 32 to 50 μm. Asterisks mark two second- or third-order branches. V, venule receiving several tributaries inside the carotid body. Left cervical sympathetic trunk cut 10 days before fixation. Rat ventilated with 10% O_2–10% CO_2–80% N_2 for 10 min before the perfusion. Toluidine blue-stained 0.5 μm section. × 250.



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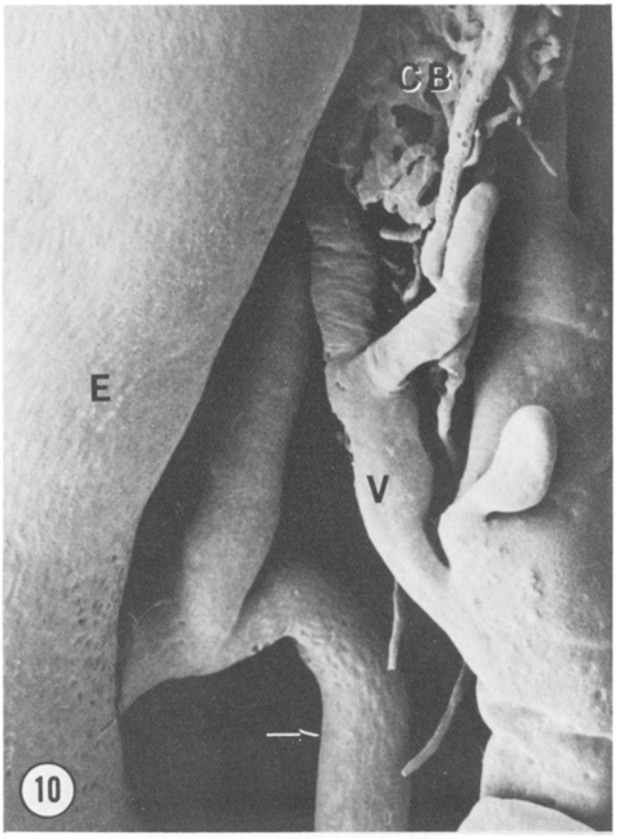

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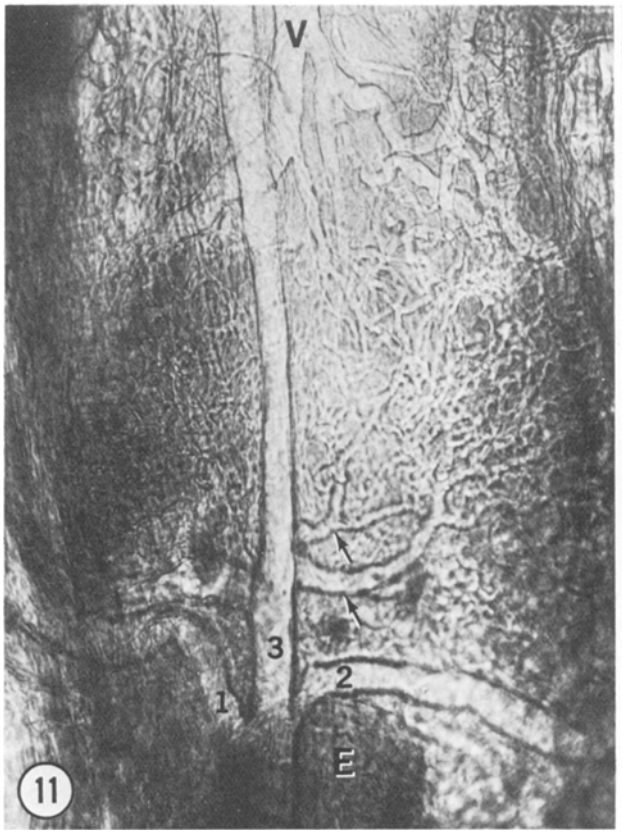

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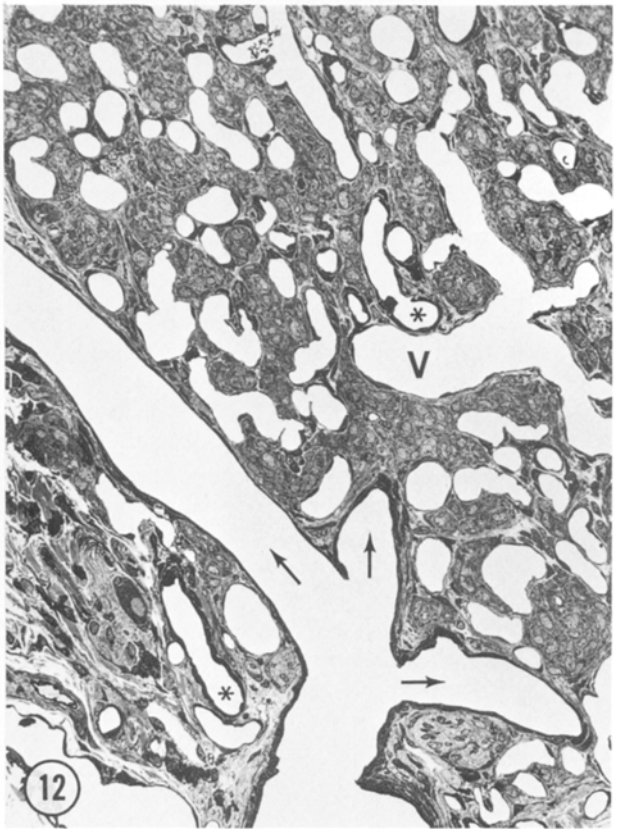

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bordering glomus tissue (Figs. 12, 14, 16). Small intimal cushions were present at the origin of some second-order branches ending in the carotid body (Fig. 14) but not at the origin of most first-order branches (Fig. 12).

Second-order branches that ended in the carotid body were rather short (<200 μm) vessels located near their first-order branches from which they arose (Figs. 11, 13, 14). At the periphery of the carotid body, most arterioles with a diameter exceeding 15 μm were destined for other organs.

The lumen of branches of the main artery diminished in calibre with each successive division (Fig. 14). In the wall of third- and fourth-order branches, smooth muscle cells had cytoplasmic processes that were thinner and more elaborately branched than those in the carotid body artery or its primary branches (compare Figs. 8 and 16). Yet the number of layers of smooth muscle (usually two) was the same as in most regions of the main artery.

TERMINAL ARTERIOLES AND PRECAPILLARY SPHINCTERS

Vessels with protruding endothelial cells

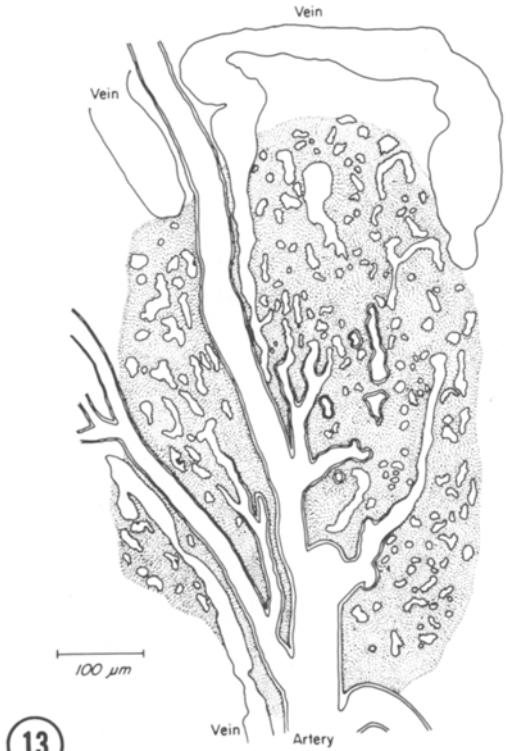
Terminal arterioles that supplied glomus tissue arose from third- or fourth-order branches of the main artery and had a diameter less than 15 μm (McDonald & Haskell, 1983). The segment of vessel at the junction of terminal arterioles and capillaries was characterized by a complete layer of smooth muscle cells or pericytes and protruding endothelial cells resembling those of vessels described by Rhodin (1967) as precapillary sphincters (Figs. 17, 18).

Fig. 13. Drawing based on whole mounts and sections of several carotid bodies showing the branching pattern of the carotid body artery. Branches that end in the carotid body (stippled) arise from first-order branches that continue on through the organ. A tapered venule that parallels a first-order arteriole and then joins a surface vein is shown at the rostral pole (top) of the carotid body. Approximate magnification × 120.

Fig. 14. Section of a right carotid body showing first to fourth-order branches (numbered 1 to 4) of the carotid body artery. Small intimal cushions are visible at some branching points (arrows). Several tributaries of a surface venule (V) also are shown. Rat ventilated with 10% O_2–10% CO_2–80% N_2 for 10 min before fixation. Toluidine blue-stained 0.5 μm section. × 250.

Fig. 15. Vascular cast illustrating luminal surface features of a portion of a left carotid body artery and one of its second-order branches (diameter 23 μm). Elliptical indentations due to endothelial nuclei are oriented longitudinally on both vessels. The second-order branch, which was located just outside the carotid body, arose from the large descending branch of the carotid body artery. Scanning electron micrograph. × 840.

Fig. 16. A cross-section of a second or third-order branch of the carotid body artery surrounded by glomus tissue of the carotid body. This arteriole has a luminal diameter of approximately 12 μm and has two layers of smooth muscle enveloped by an extensive extracellular matrix. A vessel (presumptive precapillary sphincter) with a protruding endothelial cell (arrow) is shown at the upper left. × 3400.



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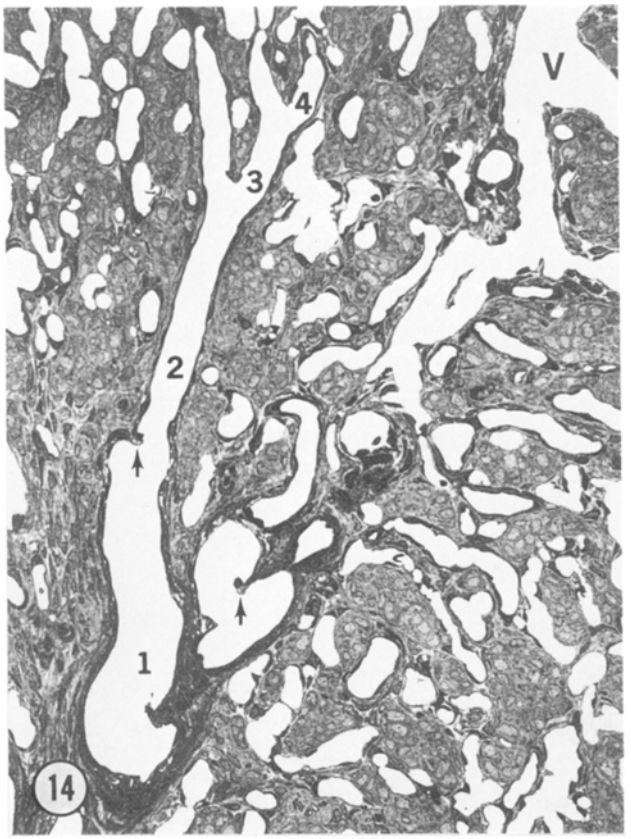

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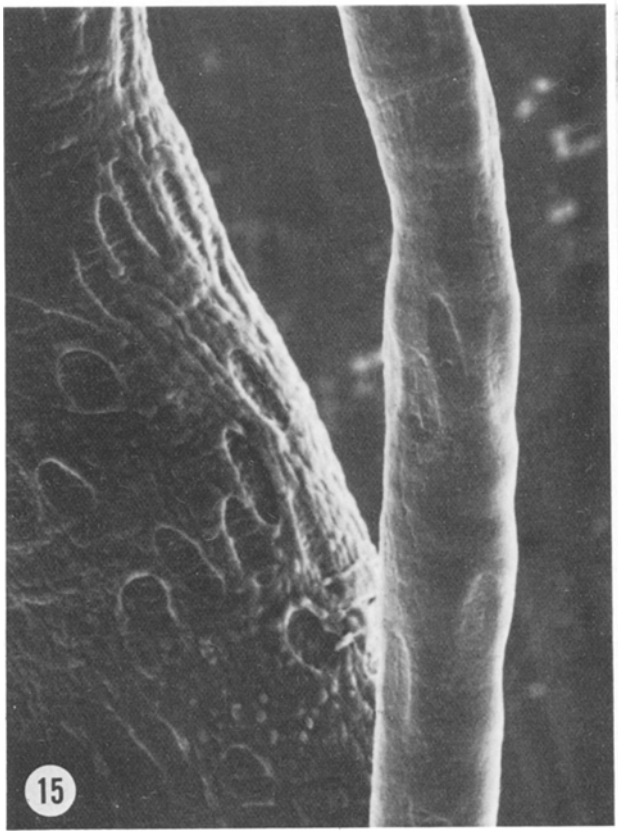

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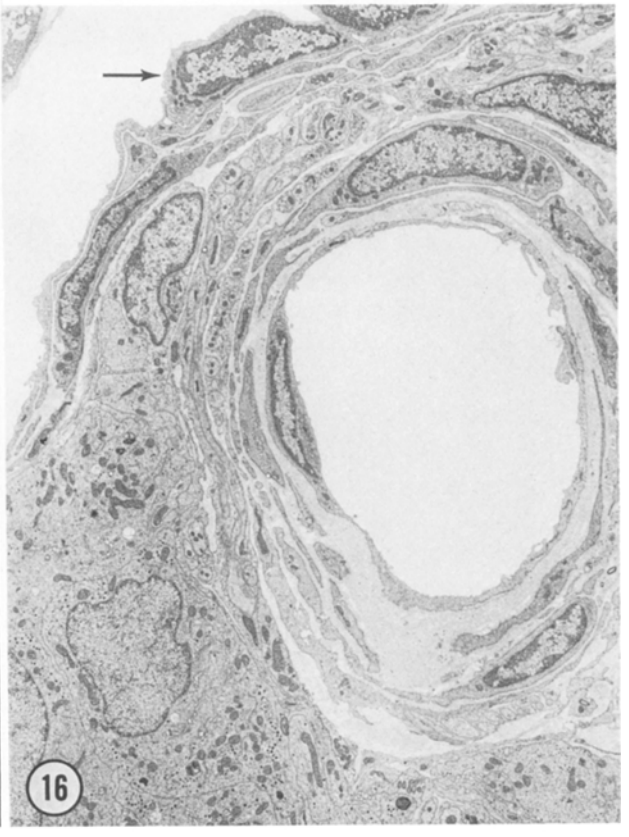

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Most terminal arterioles divided at their distal end giving rise to two types of capillaries (see below). A precapillary sphincter was located in each branch of a terminal arteriole just beyond the bifurcation. In vessels that did not divide, the boundary between terminal arteriole and precapillary sphincter was less clear, because protruding endothelial cells were interspersed with other endothelial cells throughout the length of the precapillary vessel (Fig. 17).

Examination of longitudinal sections of precapillary sphincters revealed that the upstream end of endothelial cells was thin, but the downstream end of the same cells was thick in the region of the nucleus and protruded into the vessel lumen (Figs. 17, 18). Just beyond the downstream extent of the nucleus, the cell ended abruptly. Junctions connecting adjacent endothelial cells of precapillary sphincters were located where the thick portion of one cell ended and the thin portion of the next cell began (Fig. 18). When the upstream cell overlapped the downstream cell (Figs. 17, 18), the junction was located beneath the overhang of the upstream cell.

Vessels with pericytes

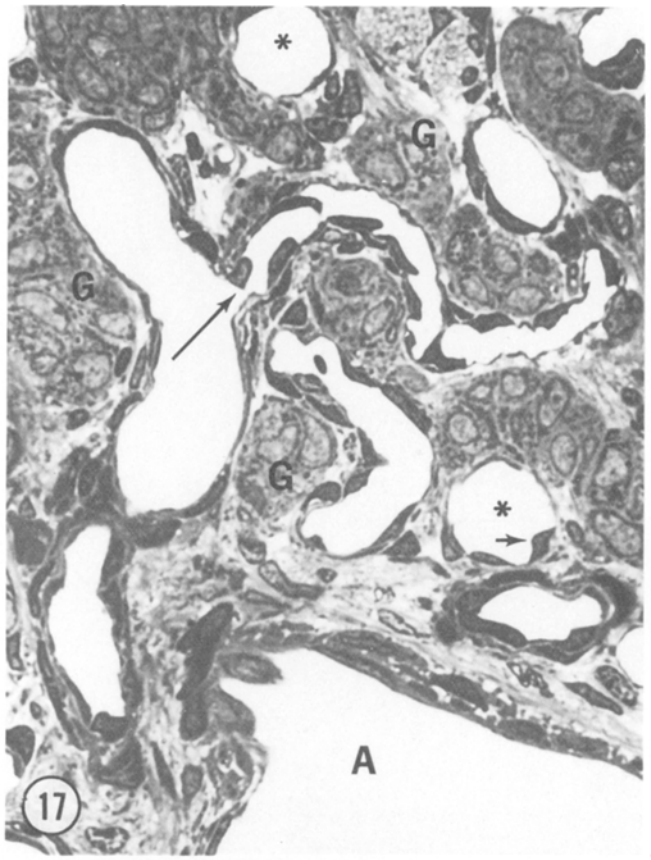
Pericytes were associated with all capillaries and venules and with the precapillary sphincters that were not enveloped by smooth muscle cells. Most pericytes were similar in ultrastructure to those in the basal lamina of capillaries in other organs (Majno, 1965;

Fig. 17. A section of carotid body showing an arteriole (diameter 18 μm) that gives rise to a terminal arteriole/precapillary sphincter (diameter 6 μm, long arrow), which has protruding endothelial nuclei. A type II capillary begins just beyond the sphincter. Compare this vessel with the large (11–19 μm) type I capillaries (asterisks), one of which has a protruding endothelial cell nucleus (short arrow). The parent arteriole is a branch of a larger arteriole, part of which is visible at the lower right (A). G, glomus cells. Toluidine blue-stained 0.5 μm section. × 750.

Fig. 18. Electron micrograph of the same carotid body shown in Fig. 17 illustrating here a small intimal cushion (long arrow) at the origin of a precapillary sphincter, which has a protruding endothelial cell (EC). This sphincter was proximal to a type I capillary (not shown). Junctions between this endothelial cell and the next cell downstream are located near the tip of the short arrow. A group of axons and axonal varicosities is positioned at the base of the intimal cushion. P, an elongated pericyte. × 8000.

Fig. 19. Cast of a heterogeneous group of capillaries which are interconnected with venules (V) near the surface of a carotid body. A small arteriole (A) also is visible. Most of the capillaries have distinctive surface undulations. Endothelial nuclear indentations on venules tend to be round (short arrows), whereas those on the arteriole are elliptical (long arrow). Scanning electron micrograph. × 580.

Fig. 20. The arterial end of a type II capillary (maximal luminal diameter 6 μm; mean wall thickness 1.1 μm) that is completely enveloped by processes of a pericyte (P) and has no endothelial fenestrations is shown next to a vessel (upper left) of similar morphology (mean wall thickness 2.0 μm). A thin process (long arrow) of a fibroblast-like veil cell (VC) separates pericytes on the two vessels. Short arrows mark pericyte–endothelial cell contacts. G, process of a glomus cell. × 7200.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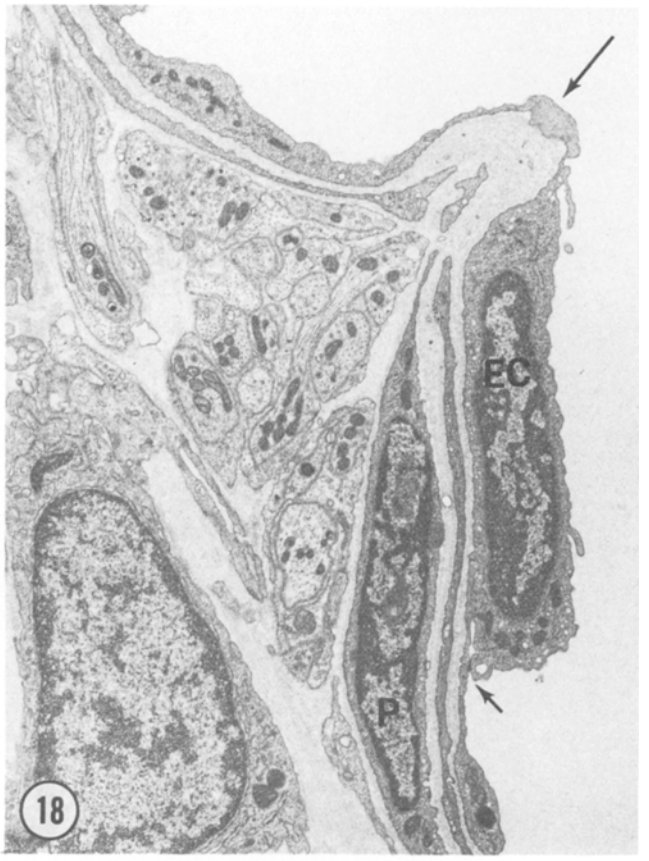
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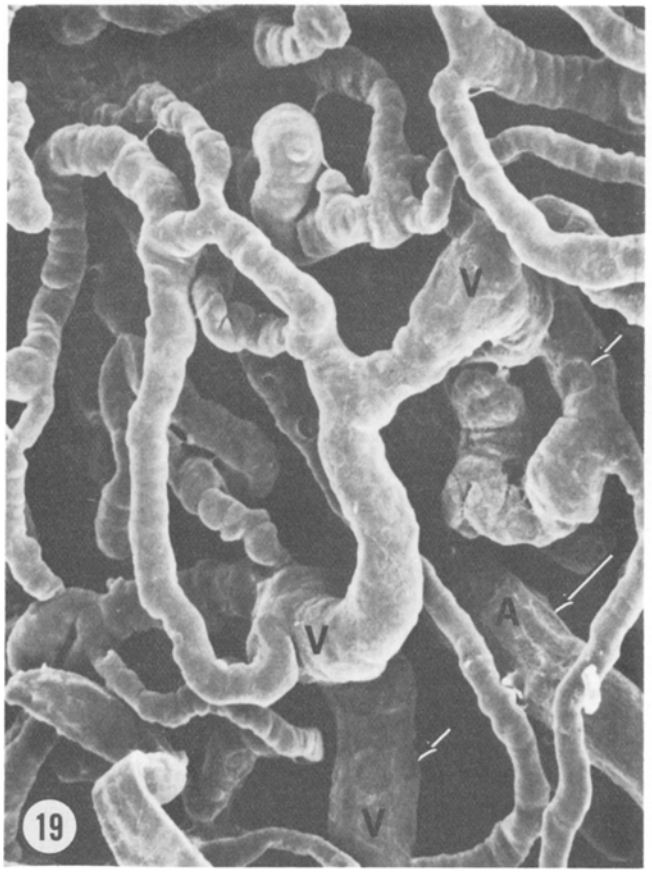
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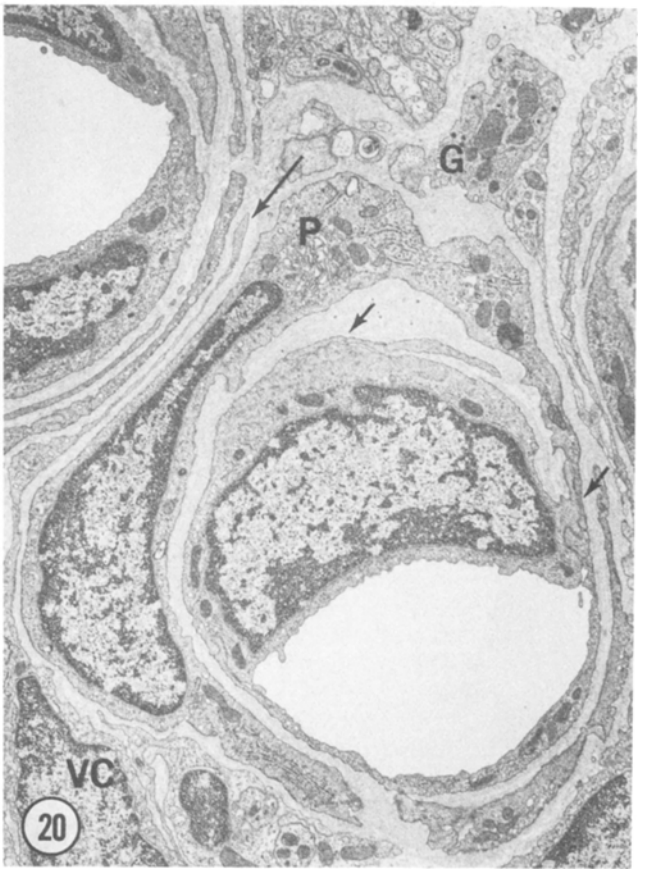
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Weibel, 1974; Stensaas, 1975), although some perivascular cells appeared to be intermediate forms between smooth muscle cells and pericytes. The principal cytoplasmic processes of pericytes extended along the length of vessels. Branches of these processes wrapped around the vessel circumference (Figs. 20, 21). Branching of cytoplasmic processes produced multiple layers of pericytes on some vessels (Fig. 23), and some pericyte processes were within 20 nm of the endothelium (Fig. 20). Most pericytes had a small rounded cell body that protruded into the adjacent tissue (Fig. 21), but some were flattened and elongated as in the case of smooth muscle cells (Fig. 18). Because of the small cell body and long processes, the region of pericytes containing the nucleus was not visible on most vessel profiles. Caveolae present on the plasma membrane of pericytes were most numerous on the abluminal surface (Fig. 20), but they were not restricted to this location (Figs. 22, 23). Bundles of filaments in the cytoplasm of pericytes were morphologically similar to but less numerous than those in smooth muscle cells on arterioles (compare Figs. 23 and 24). The cytoplasm of pericytes tended to be more electron dense than that of smooth muscle cells or perivascular connective tissue cells (Fig. 26).

CAPILLARIES

Capillaries in the carotid body were particularly difficult to analyse because they were extremely numerous, densely packed, contorted and morphologically heterogeneous (Figs. 14, 19). But most capillaries could be classified as one of two types.

Type I capillaries

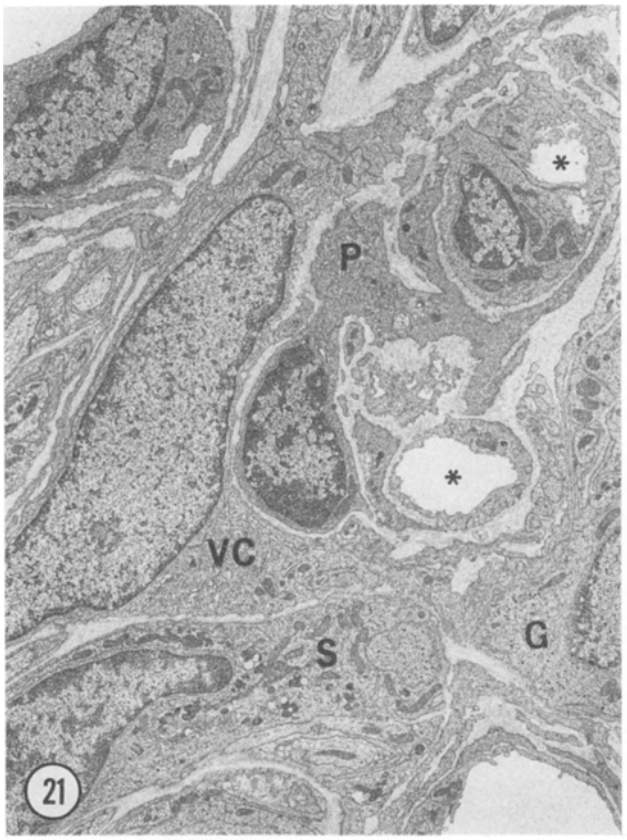
The type I capillary was large and had the characteristic feature of penetrating a cluster of glomus cells and then being associated with that cluster throughout its length (McDonald & Haskell, 1983). Vessels of this type followed a winding course with one or more U-shaped turns, but at least part of their wall always was within 3 μm (and usually was within 0.5 μm) of members of the same group of glomus cells (Fig. 25).

Fig. 21. A pericyte (P) with an irregularly-shaped process is shown in this grazing section through a blood vessel (asterisks) of the carotid body. VC, fibroblast-like veil cell; G, glomus cell; S, sheath cell (type II glomus cell). × 4500.

Fig. 22. A portion of the cell body of a pericyte (P) is shown next to an endothelial cell (EC) of a precapillary sphincter with a luminal diameter of 10.4 μm and mean wall thickness of 2.0 μm. Note that a thick basal lamina occupies much of the space around the pericyte. × 60 000.

Fig. 23. A branching process (P) of another portion of the pericyte illustrated in Fig. 22 is shown here in the thick basal lamina next to the endothelium (EC). Caveolae are present on the plasma membrane of both cells. × 60 000.

Fig. 24. A process of a smooth muscle cell in the wall of a carotid body arteriole (luminal diameter 11.2 μm; mean wall thickness 4.1 μm). Compare the bundles of filaments in this cell with those in the pericyte in Figs. 22 and 23. × 60 000.



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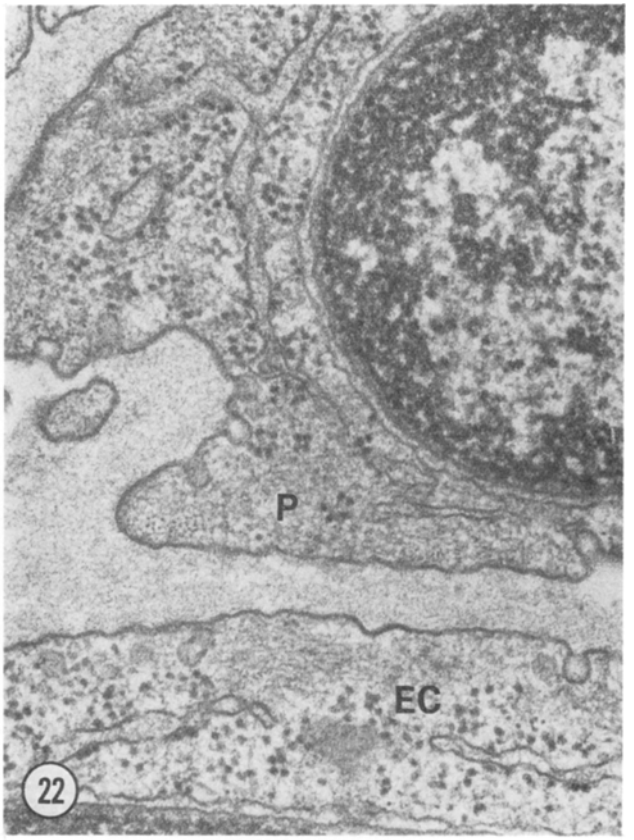

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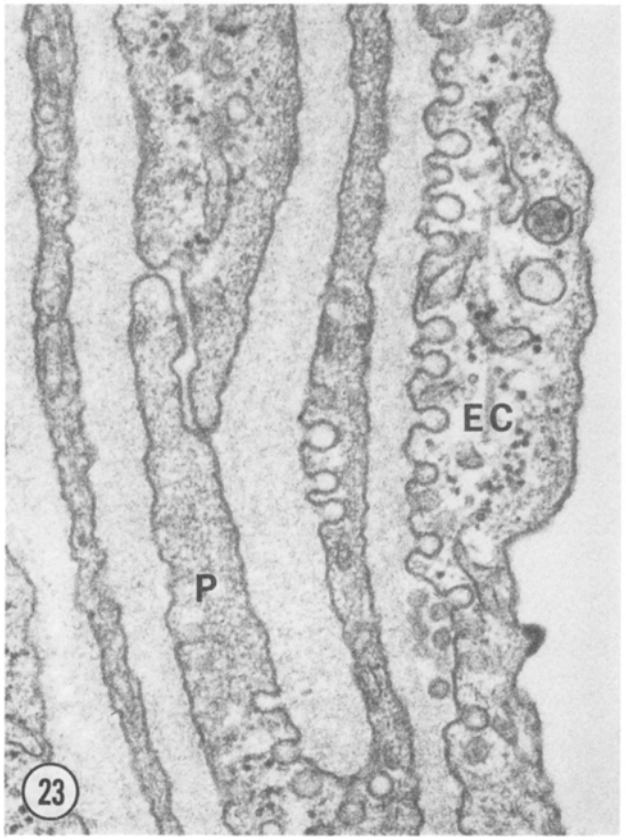

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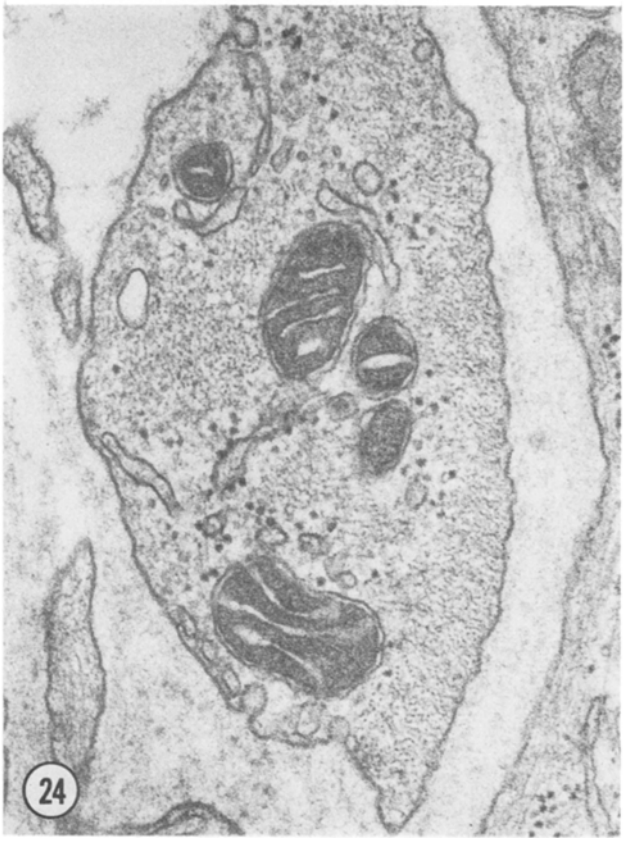

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Where they emerged from a glomus cell cluster, type I capillaries were connected to collecting venules by thin-walled channels narrower than the capillaries themselves. Most of these capillaries also had small side branches that were connected to venules, but there were no direct connections between different capillaries.

Type I capillaries varied in calibre along their length (Fig. 19). The smallest of these vessels had a minimal luminal diameter of about 8 μm and a maximal diameter of about 12 μm. The diameter of the largest type I capillaries ranged from 14 to more than 20 μm. The largest vessels tended to be located at the periphery of the carotid body (Fig. 29).

The capillary wall averaged less than 1 μm in thickness, was lined by a thin endothelium that had numerous fenestrations but few caveolae, and was only partially covered by pericytes (Figs. 25–27). Pericyte processes that were present usually were attenuated (Figs. 25, 26), and the basal lamina typically was thin (Fig. 27). The surface of the endothelium was irregular (Fig. 29) and in some regions had microvillous projections or prominent marginal folds (Fig. 25). Surface undulations, which gave capillaries a segmented appearance in vascular casts (Fig. 19), resulted from the protrusion of endothelial cells (Fig. 17) and from luminal narrowings overlying circumferential pericyte processes.

Because none of these blood vessels was completely surrounded by glomus cells, some regions of each vessel were next to stromal elements (Fig. 25). The distribution of endothelial fenestrations was not restricted to the region of the endothelium next to glomus cells (Fig. 26).

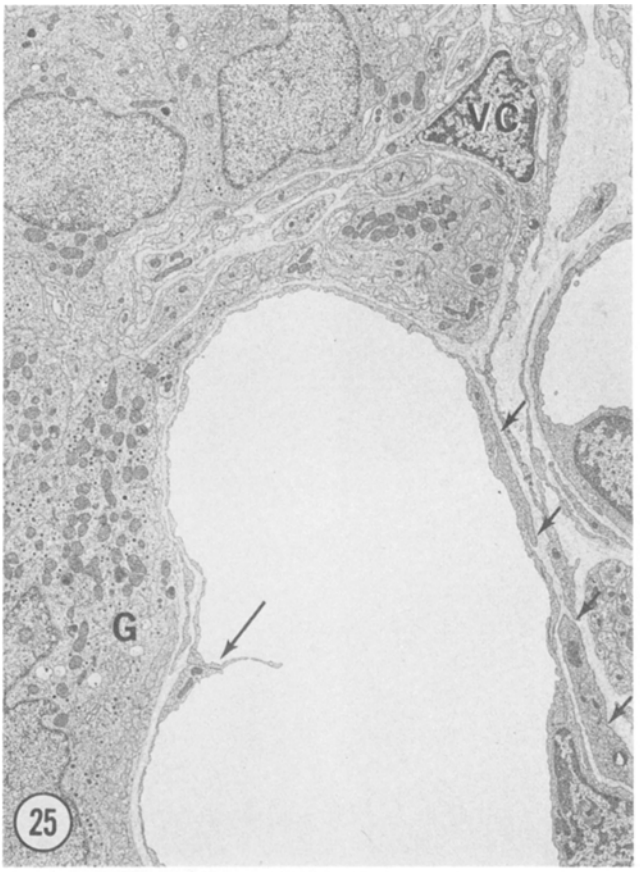
Fibroblast-like connective tissue cells (called *veil cells*, Majno, 1965) were located outside the layer of pericytes on most capillaries (Figs. 21, 26). Veil cells, which had a comparatively lucent cytoplasm and no basal lamina, were readily distinguished from pericytes (Fig. 26).

Fig. 25. A type I capillary (luminal diameter 10.1 μm; mean wall thickness 0.34 μm) is shown within 300 nm of glomus cells (G). The vessel's endothelium has numerous fenestrations and a prominent marginal fold at an intercellular junction (long arrow). Note on the right that a portion of the vessel does not border glomus tissue. Short arrows mark two pericyte processes next to the vessel; VC, fibroblast-like veil cell. × 4500.

Fig. 26. Pericyte processes (arrows) and a portion of a fibroblast-like veil cell (VC) are shown next to the fenestrated endothelium of a type I capillary. The basal lamina bordering the endothelium forms a distinct thin layer but that covering the pericyte processes is more dispersed. × 33 000.

Fig. 27. An exposed region of a glomus cell (G) is shown next to a pericyte process (P) that borders a fenestrated type I capillary. Note that the basal lamina (arrows) covering the endothelium, pericyte and glomus cell is much thinner than that present on vessels illustrated in Figs. 20, 22, 23. × 60 000.

Fig. 28. A vascular cast showing a type I capillary (diameter approximately 9 μm) that joins a venule (V) at two separate locations (arrows) near the surface of a carotid body. Note the irregular contour and rounded endothelial nuclear indentations of the venule (diameter about 23 μm). × 1400.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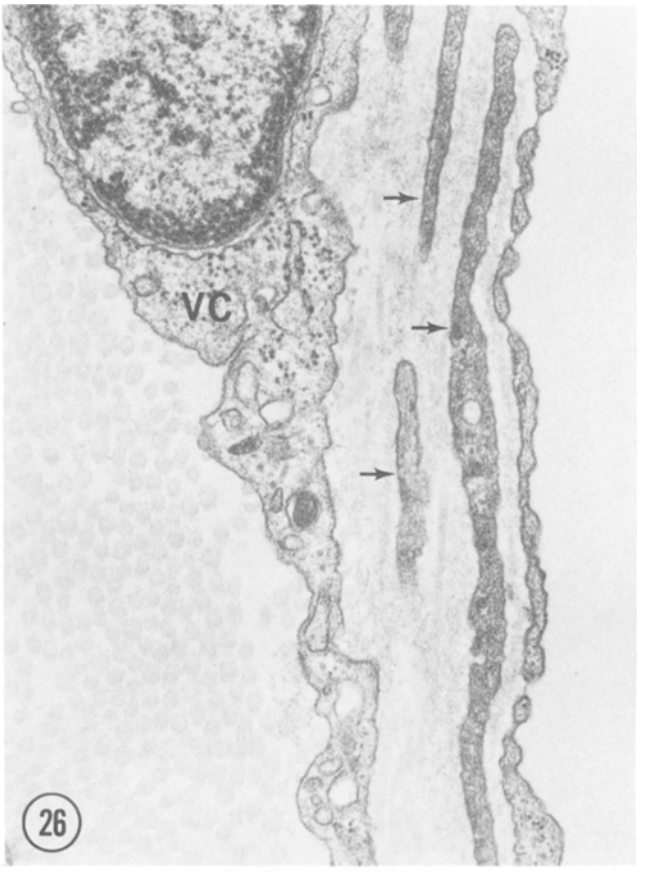
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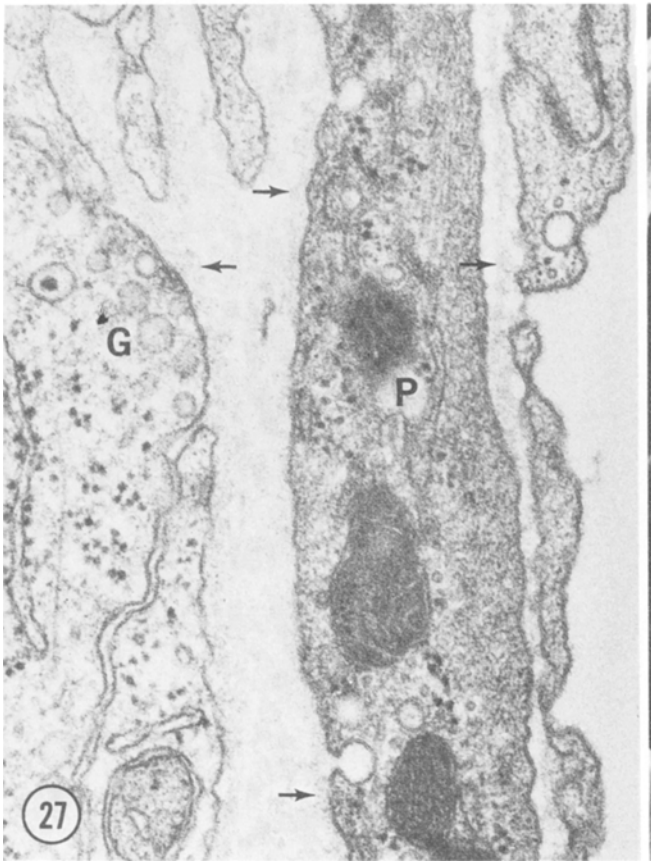
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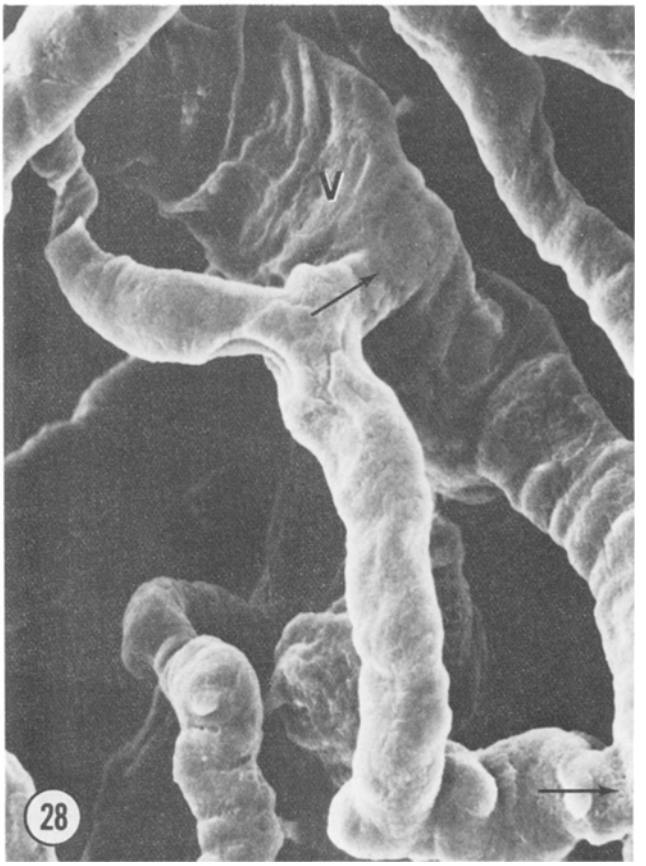
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Type II capillaries

These vessels usually arose as side branches of the same terminal arterioles that gave rise to type I capillaries (McDonald & Haskell, 1983). Like type I capillaries, type II capillaries were preceded by a precapillary sphincter (Fig. 17). However, unlike type I capillaries, these capillaries had a luminal diameter of less than 10 μm throughout their length, had straight as well as curved regions, and did not penetrate glomus cell clusters (McDonald & Haskell, 1983). The difference in the relationship to glomus cells of types I and II capillaries was not always evident in single sections. Yet an examination of serial sections revealed that most type II capillaries passed near the perimeter of several glomus cell clusters and were not associated with one cluster in particular. Curiously, some type II capillaries had no apparent relationship with glomus cells. Type II capillaries typically were connected to only one or two venules.

Near their arterial end, type II capillaries had a complete or nearly complete envelopment of pericytes, a smooth continuous endothelium with numerous caveolae but no fenestrations, a wall as much as 2 μm in thickness, and thick basal laminae covering the endothelium and pericytes (Fig. 20). Their walls became thinner and their endothelium fenestrated toward the venous end, but their lumen remained about the same size.

ARTERIOVENOUS ANASTOMOSES

In our analysis of serial sections, we found that two out of a group of 14 terminal arterioles connected directly to the side of small venules, bypassing both types of capillaries (McDonald & Haskell, 1983). In the region of the connection, endothelial cells protruded into the vessel lumen, as in precapillary sphincters. Near the anastomosis the venules were about the same size as the arterioles (10 μm), but these small venules were tributaries of larger vessels.

VENULES, VEINS AND LYMPHATICS OF THE CAROTID BODY

Venules and lymphatics of the carotid body interior and surface

Connections between capillaries and venules were much easier to analyse than those between arterioles and other vessels, because the former were more numerous and were visible in most planes of section through the carotid body. Furthermore, the superficial location of many venules in the carotid body facilitated their examination in vascular casts and whole mounts. Most type I capillaries had multiple connections with venules (Figs. 19, 28). Capillaries were not only connected to venules near the surface of the organ (Figs. 19, 28, 33), but they also joined interior collecting venules (Figs. 13, 14), which in turn were tributaries of larger venules at the surface (Figs. 13, 29). The smallest venules were about 8 μm in diameter. The largest were as much as 125 μm. A large funnel-shaped venule paralleled one branch of the main artery within the carotid body and drained into a surface venule (Figs. 11, 13).

In single sections examined by light microscopy, venules with a diameter less than

20 μm resembled type I capillaries. However, reconstructions from serial sections revealed that venules did not come as close to glomus cells as did capillaries. Furthermore, venules were interconnected with one another and followed a course that was not confined to the territory of a single cluster of glomus cells (Figs. 12, 33).

Despite their thin walls and thin basal lamina, venules had a wall structure which differed in several respects from that of capillaries (compare Figs. 26 and 31). Venules 1. had an endothelium that was thicker and had fewer fenestrations, 2. had more numerous caveolae on the abluminal surface of the endothelium, 3. had a more extensive layer of pericytes, and 4. in the larger vessels contained collagenous fibres within the basal lamina between endothelial cells and pericytes (Fig. 31).

In vascular casts, venules had surface undulations, a noncylindrical shape and variations in calibre along their length, and therefore were readily distinguished from arterioles of comparable size (Fig. 30).

Lymphatics were numerous at the surface of the carotid body, but none was found within the organ except in the region near the main carotid body artery. Unlike venules, lymphatics near the carotid body had a discontinuous basal lamina and no endothelial fenestrations (Fig. 32). Furthermore, the lumen of lymphatics was not emptied of its contents by the vascular perfusion. In sections stained with toluidine blue for light microscopy, lymph appeared as a homogeneous, faint blue material that filled the lumen of lymphatics (Fig. 29). Lymph had a flocculent appearance by electron microscopy (Fig. 32).

Venous drainage of the carotid body

The plexus of venules and veins at the surface of the carotid body was enormous considering that the organ is supplied by branches of only one comparatively small artery (Figs. 33, 34). The largest veins of the plexus were located at the rostral and caudal poles of the carotid body outside the glomus tissue (Fig. 33). These vessels had thicker walls than the interior venules. Veins from the carotid body joined those from the superior cervical ganglion and nodose ganglion en route to larger veins in the neck (Fig. 34).

Though the anatomy of the venous drainage was more variable than that of the arterial supply, several features were constant in all rats examined. Some veins at the caudal pole of the carotid body were connected to the pharyngeal vein. Others were tributaries of a vein that traversed the ventral surface of the carotid artery and joined the internal jugular vein (usually small in the rat). Veins from the rostral pole of the organ, which were more numerous than those caudally, joined the rostral portion of the pharyngeal vein (Fig. 34).

On the lateral and posterior surfaces of the pharynx, the rostral end of the pharyngeal vein was connected by large anastomoses to the pharyngeal plexus that formed tributaries of the external jugular vein (very large in the rat) via the posterior facial vein (Fig. 34). Caudally the pharyngeal vein was a tributary of the internal jugular vein.

VASCULAR INTERCONNECTIONS OF THE CAROTID BODY AND CAROTID SINUS

A complex network of vasa vasorum was found in the adventitia of the carotid sinus. By contrast, few vessels were located in the wall of other portions of the internal carotid artery. This system of vessels was sufficiently conspicuous that it marked the location not only of the carotid sinus but also the carotid body, which invariably was located at the ventromedial surface of the sinus (Figs. 35, 36). Specimens in which sensory nerves were stained with methylene blue verified that the vasa vasorum coincided with neural elements of the carotid sinus (McDonald, 1983b).

The arterial supply to the vasa vasorum consisted of one or two arterioles about 15 μm in luminal diameter. These vessels usually arose from branches of the carotid body artery that supplied the nodose ganglion, vagus nerve and superior laryngeal nerve. The arterioles, which had a uniform cylindrical shape, traversed the ventral surface of the carotid sinus in the same plane as the other vessels but were not immediately adjacent to the largest venules (Fig. 35, 37). During this traverse, several smaller arterioles arose from the parent vessel (Fig. 35).

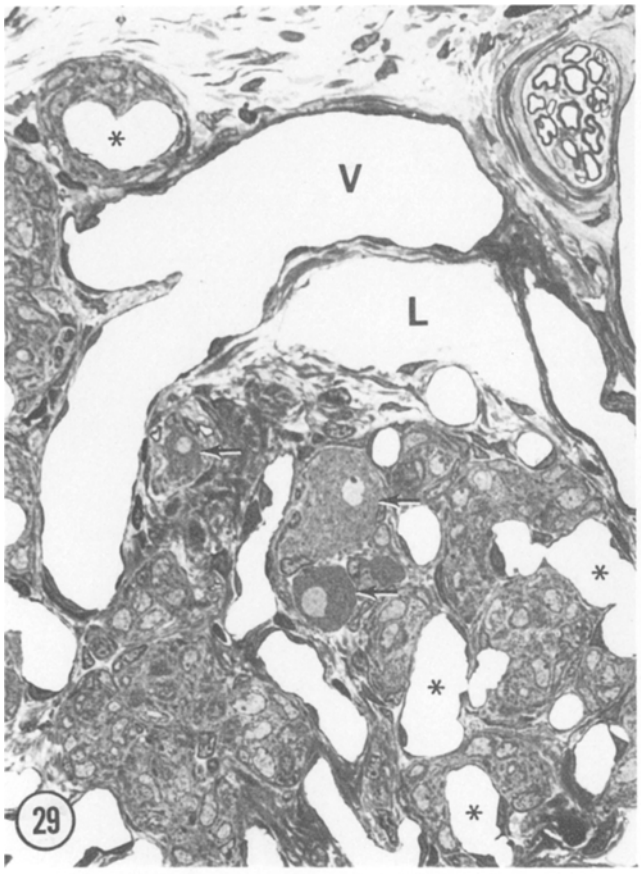
Fig. 29. Type I capillaries (asterisks) next to glomus cells, a surface venule (V) 40 μm in diameter, and a lymphatic vessel (L) are shown in this light micrograph of structures at the rostral pole of a carotid body (right side). Visible in the original section but not apparent here, lymph in the lymphatic vessel was stained faintly blue, whereas blood vessels were emptied of their contents by the perfusion. Arrows designate ganglion cell bodies. Rat ventilated with 10% O_2–10% CO_2–80% N_2 for 10 min before fixation. Toluidine blue-stained 0.5 μm section. × 440.

Fig. 30. A cast showing a first-order branch (A, diameter 44–50 μm) of the carotid body artery and a venule (V) of comparable size at the surface of a right carotid body. Scanning electron micrograph. × 860.

Fig. 31. The wall of a venule near the surface of a carotid body showing the comparatively thick endothelium (EC) with short villous processes on its luminal surface, a pericyte process (P) covered by a thin basal lamina, and a portion of a fibroblast-like veil cell (VC). Note that collagenous fibres (unstained structures at arrows) are located between the endothelium and the pericyte. × 33 000.

Fig. 32. The wall of a lymphatic vessel at the surface of a carotid body showing amorphous material in the lumen (left) and a thin endothelium bordered by a discontinuous basal lamina (arrow). × 33 000.

Fig. 33. Vascular cast showing interconnections of venules (the larger ones are numbered) and capillaries near the ventrolateral surface of a right carotid body. Note that some capillaries (pairs of short arrows pointed in same direction) connect to both venule 1 and venule 2, that venules 1 and 2 are joined via venule 3, and that venule 3 is connected to larger venules both rostrally (via venule 4) and caudally (via venule 5). The long arrow marks a first-order branch of the carotid body artery en route to the nodose ganglion. At the tip of the long arrow, this arteriole gives rise to a second-order branch directed caudally. I, internal carotid artery; O, occipital artery; E, external carotid artery. Scanning electron micrograph. × 220.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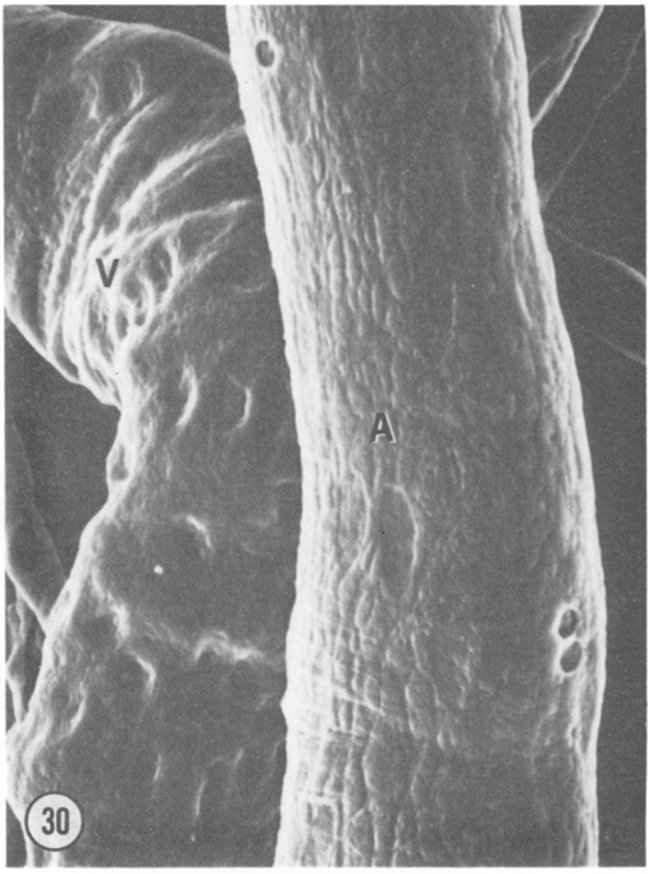
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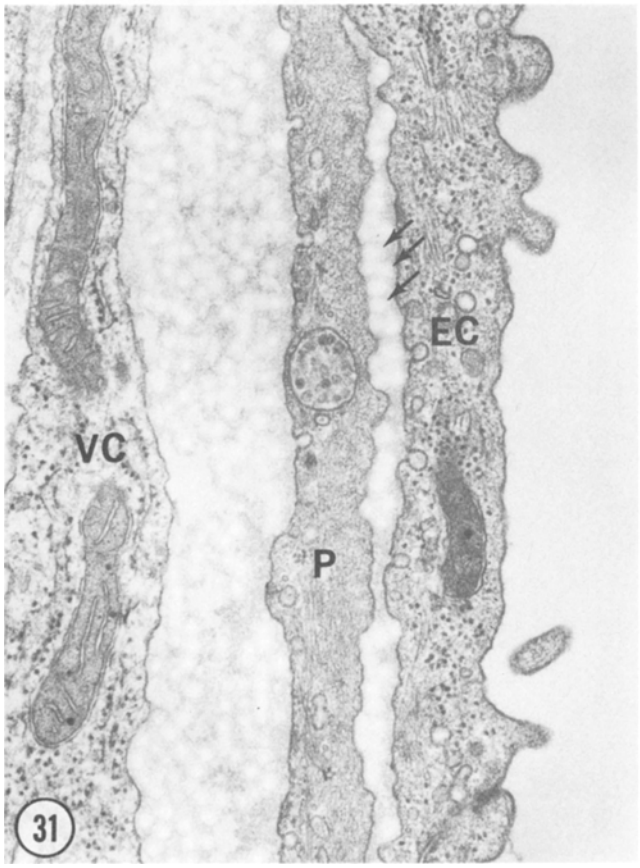
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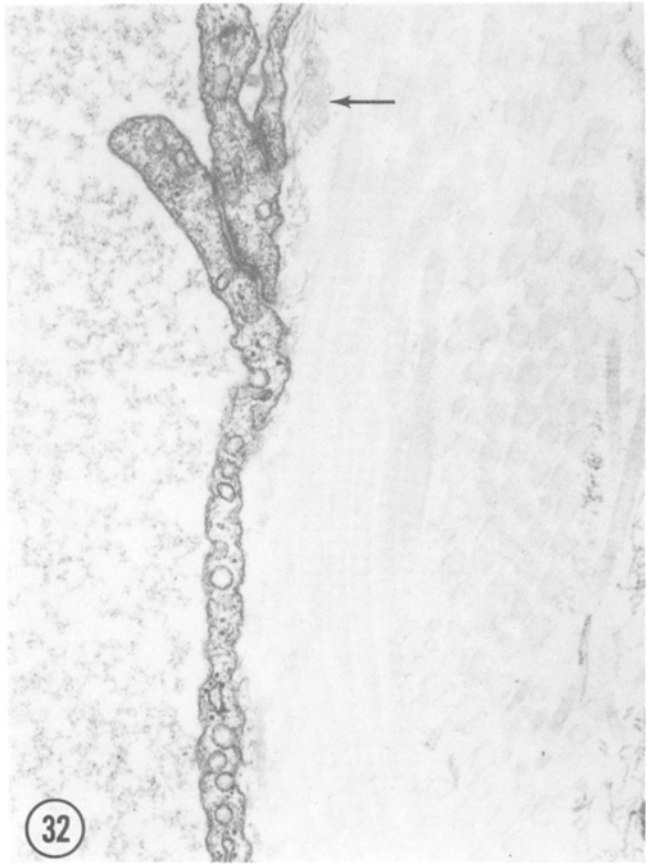
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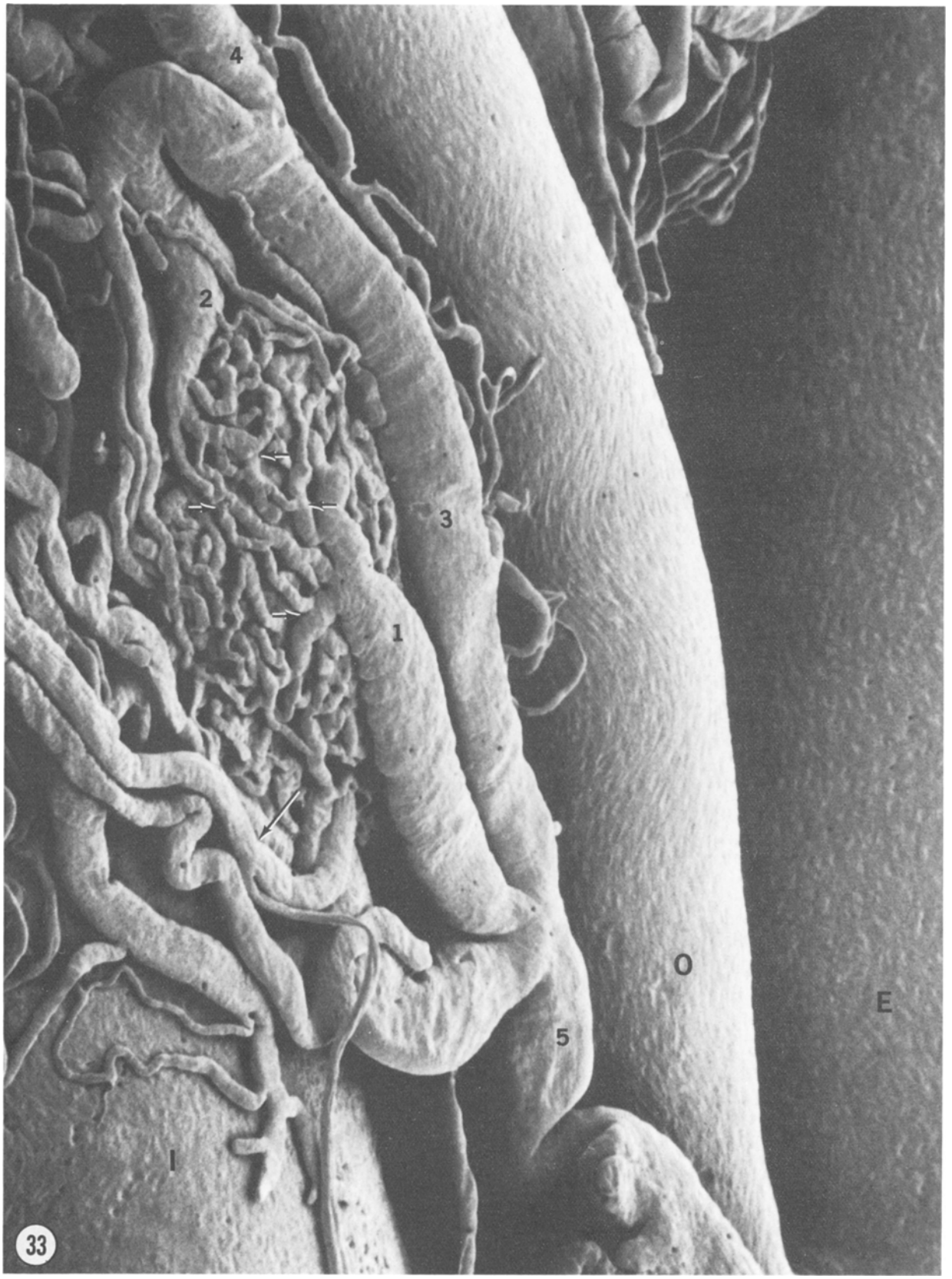
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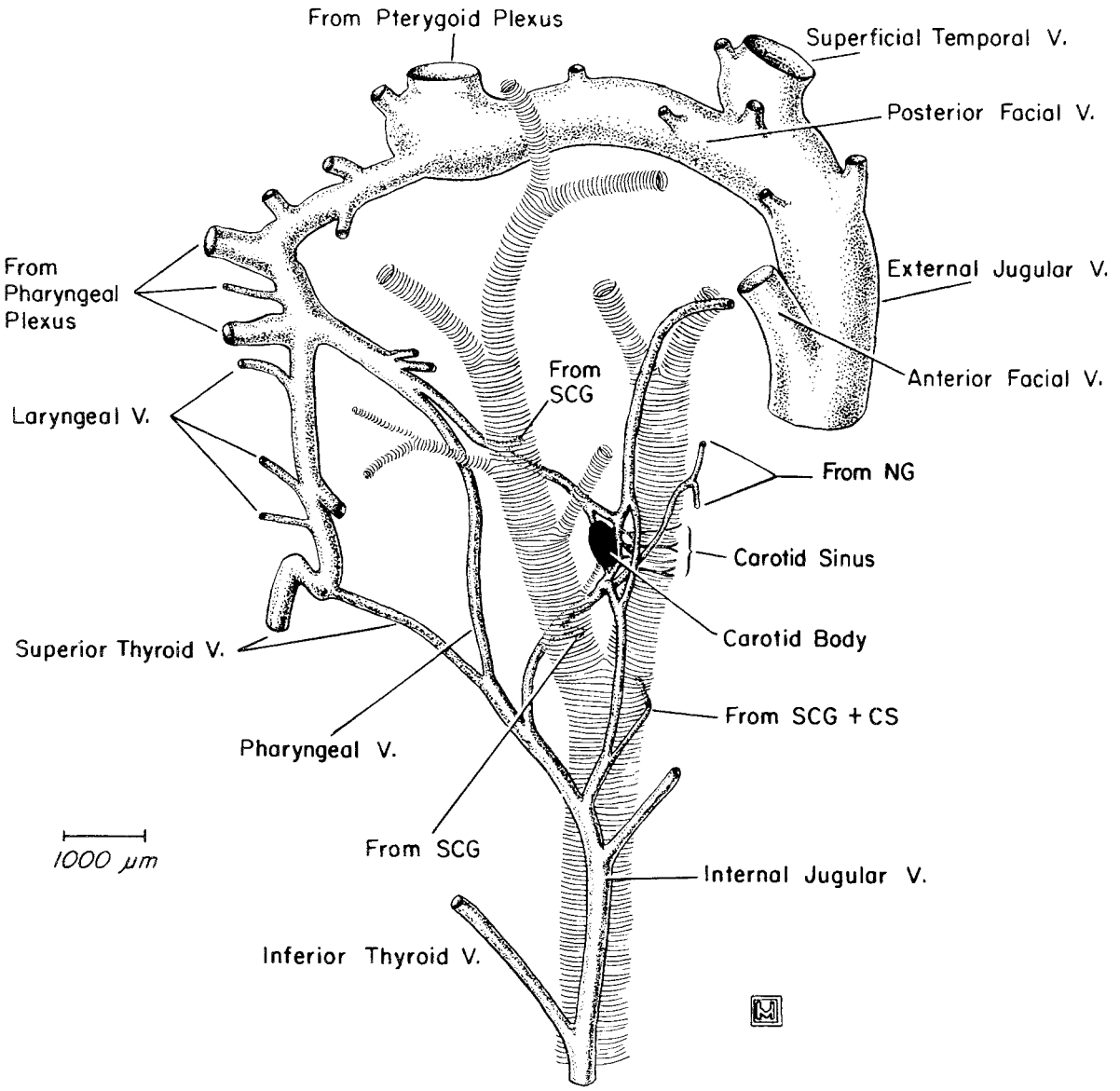


Fig. 34. A drawing showing the arterial supply (contoured vessels) and venous drainage stippled vessels) of the carotid body on the left side. Note that venules from the carotid body are connected to the external jugular vein via veins of the pharyngeal plexus and the posterior facial vein and also are connected to the internal jugular vein. V, vein; SCG, superior cervical ganglion; CS, carotid sinus; NG, nodose ganglion. Approximate magnification × 13.

Short capillaries arose at approximately right angles from the sides of the distal (most lateral) portion of the arterioles (Fig. 37). Most capillaries had a luminal diameter of 5–8 μm. Some were less than 100 μm in length. The capillaries resembled type II capillaries of the carotid body but were smaller and more uniform in contour than type I capillaries. Capillaries of the carotid sinus were tributaries of an elaborately branched and interconnected system of venules in the adventitia of the internal carotid artery.

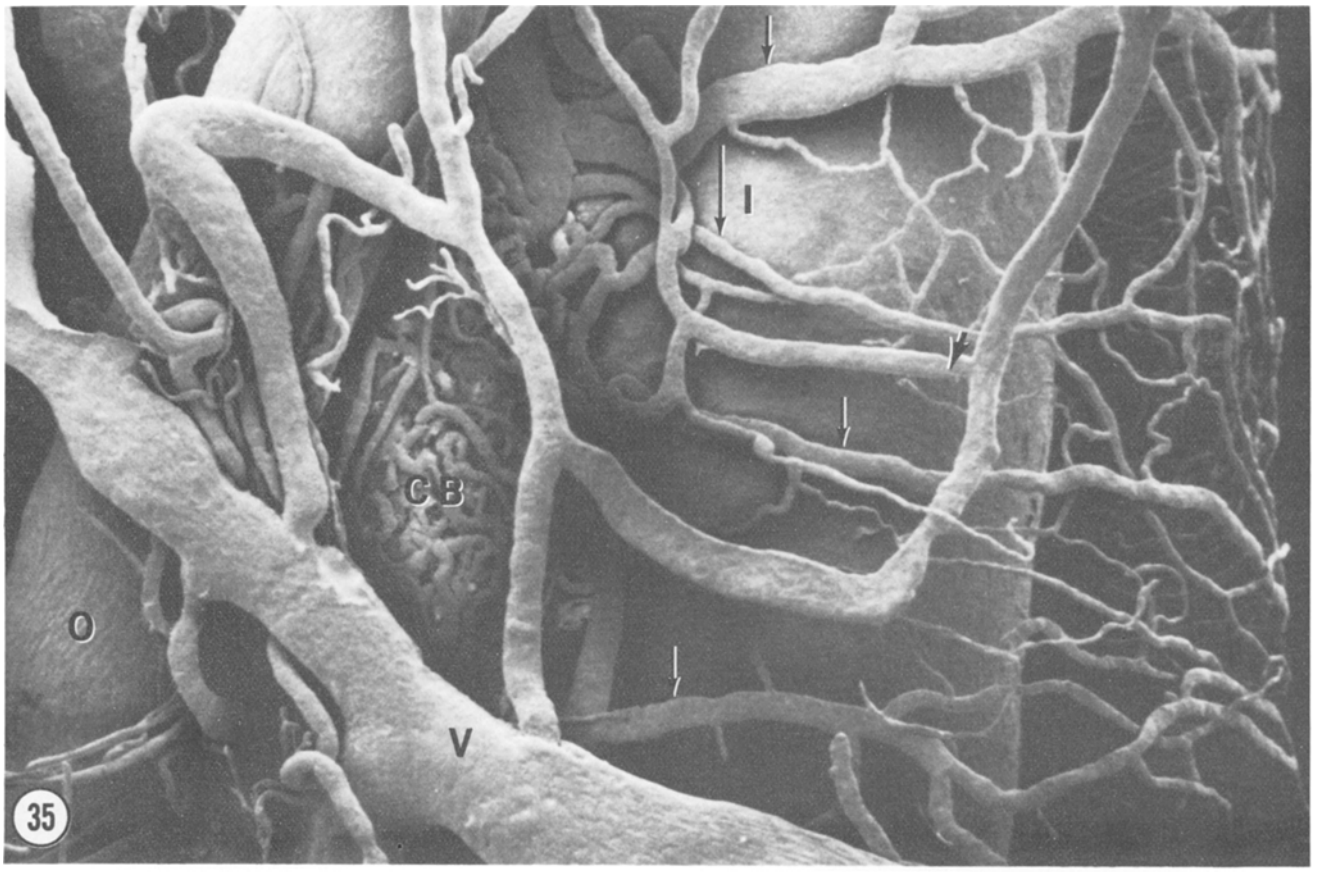
The largest venules, like the arterioles, tended to be oriented circumferentially around the carotid sinus. Yet venules 1. outnumbered arterioles several-fold, 2. were larger (luminal diameter 15–60 μm) and more variable in shape than arterioles, 3. had numerous anastomotic channels that interconnected different portions of the venous network, and 4. were abundant all around the carotid sinus. On both the ventral and the dorsal surfaces of the sinus, venules increased in size as they came closer to the carotid body, and then they joined the venous plexus at the interface of the carotid body and carotid sinus (Figs. 34–36).

In the vascular cast illustrated in Fig. 35, one arteriole supplies the entire system of vasa vasorum. This vessel gives rise to five smaller arterioles (9–11 μm in diameter) and then is the source of seven capillaries. Four of the smaller arterioles give rise to numerous capillaries in the wall of the sinus, and the fifth is a thoroughfare vessel that connects directly to a venule. The capillaries and small venules are tributaries of three collecting venules on the ventral surface of the sinus and an equal number of venules on the dorsal surface.

Fig. 35. A cast showing the blood vessels located near the ventrolateral surface of a left carotid body (CB) and in the wall of the carotid sinus (right half of picture). One arteriole (long arrow), which was derived from the carotid body artery, is visible among the vasa vasorum of the carotid sinus. The other large vasa vasorum are venules (short arrows), whereas the smallest vessels are capillaries. I, internal carotid artery enveloped by vasa vasorum; O, occipital artery; V, superficial venule receiving tributaries from the carotid body and carotid sinus via a connection marked by the *large arrow head*. Scanning electron micrograph. × 120.

Fig. 36. A drawing illustrating the common venous connections of the left carotid sinus and carotid body. Connections between rostral and caudal portions of the carotid body's venous plexus also are shown. The view is similar to that shown in Fig. 35, but this drawing was based on a composite of features of several specimens. Approximate magnification × 50.

Fig. 37. A portion of the cast of carotid sinus vasa vasorum illustrated in Fig. 35 is shown here at higher magnification and from a more lateral perspective. The lower arrow marks the region at which a capillary arises from the arteriole (A). The upper arrow shows where the same capillary joins a venule (V). The left margin of the picture coincides with the lateral margin of the lumen of the internal carotid artery. × 410.



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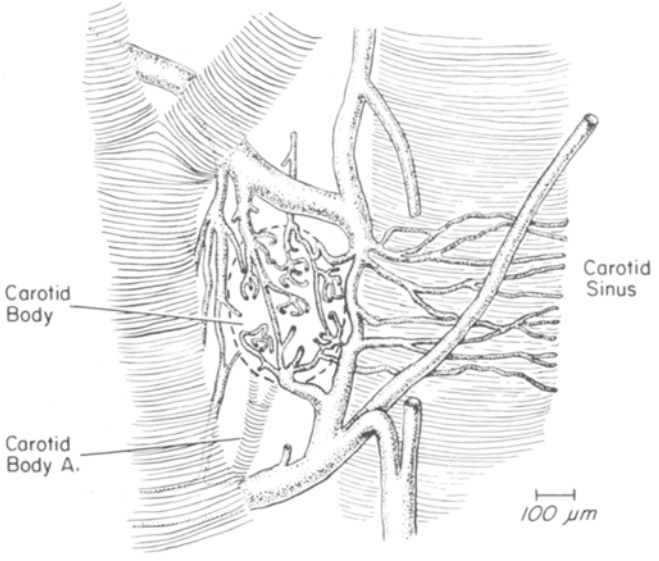

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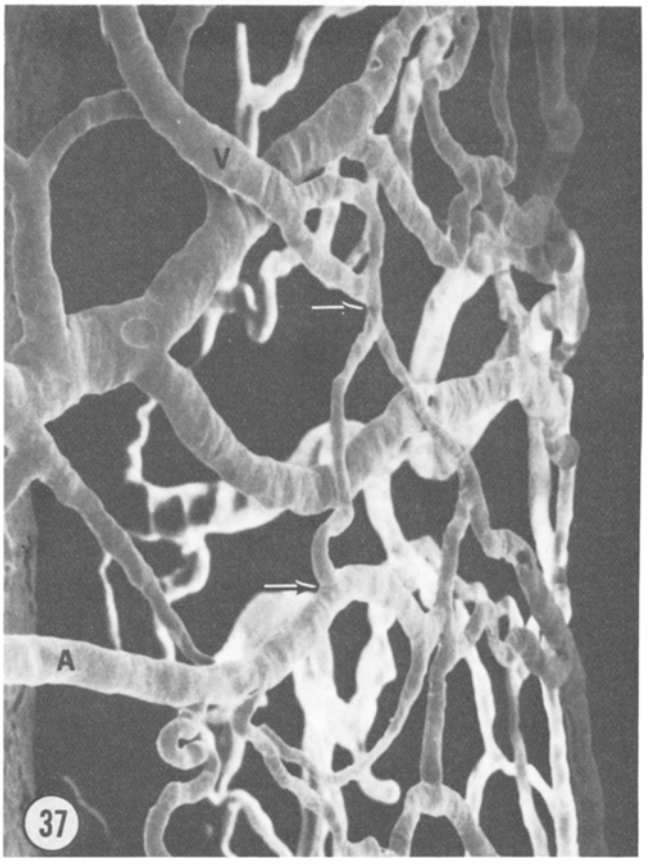

37

Discussion

ARTERIES AND ARTERIOLES OF THE CAROTID BODY

Intimal cushions

Cushions of the carotid body artery. In the rat the orifice at the origin of the carotid body artery is rimmed by a large sphincter-like intimal cushion. In some of our preparations this sphincter reduced the diameter of the orifice by more than half. Similar structures have been found at this location in the dog (Serafini-Fracassini & Volpin, 1966), mouse (Böck, 1973), and rat (Hesse & Böck, 1980; Habeck *et al.*, 1981). However, they were not described in a study of the human carotid body artery (Heath & Edwards, 1971), and Hesse & Böck (1980) mentioned that they are not present at this site in the rabbit or cat.

Intimal cushions (also known as intra-arterial cushions) have been identified in many different arteries and in numerous species (reviewed by Moffat, 1969; Stehbens, 1981). Cushions located at branch points of some arteries of the kidney, heart and eye have a sphincter-like structure similar to that of the carotid body artery (Moffat & Creasey, 1971; Yohro & Burnstock, 1973; Jellinger, 1974). However, intimal cushions in arteries of the brain and 'flow dividers' at bifurcations and branch sites of most large arteries do not completely surround the orifice (Hassler, 1962; Takayanagi *et al.*, 1972; Gorgas & Böck, 1975; Reidy, 1979; Langille & Adamson, 1981; Stehbens, 1981).

Terminal arterioles and precapillary sphincters. In the carotid body, intimal cushions are located also at branch points of some arterioles, in particular those supplying glomus tissue. The smallest of these vessels have endothelial cells that protrude into the vascular lumen, and thereby resemble precapillary sphincters. Although our preparative techniques may have exaggerated some features of these vessels, Rhodin (1967) identified protruding endothelial cells as a characteristic of precapillary sphincters, and others too have observed them in association with intimal cushions and at presumptive sites of vessel constriction (Moffat, 1969; Moffat & Creasey, 1971; Yohro & Burnstock, 1973; Addicks *et al.*, 1979). Morphological studies of arteries and arterioles have shown that endothelial cells protrude into the vascular lumen much more in constricted vessels than in dilated vessels (Hayes, 1967; Majno *et al.*, 1969; Phelps & Luft, 1969).

Functions of intimal cushions and precapillary sphincters. Some intimal cushions are thought to be involved in the regulation of blood flow through vessel side branches (reviewed by Moffat, 1969). In this regard, Yohro & Burnstock (1973) suggested that precapillary sphincters represent the intimal cushions of terminal arterioles. This concept is consistent with Zweifach's (1939) (see also Chambers & Zweifach, 1944) initial description of precapillary sphincters as valve-like folds of endothelium surrounded by smooth muscle. Zweifach observed that such sphincters are located at the origin of capillaries and control the flow of blood from arterioles into capillary branches.

Another intriguing function postulated for intimal cushions relates to the phenome-

non of plasma skimming. Fourman & Moffat (1961) (see also Moffat, 1959) concluded that the haematocrit of blood in a branching vessel was influenced by the configuration of the intimal cushion at its orifice. When the cushion elevated the branch orifice into the cell-rich axial stream of blood in the parent vessel, the haematocrit in the branching vessel tended to increase. When the cushion was absent or formed a sphincter-like projection into the lumen of the branch (as in the case of the carotid body artery), the haematocrit in the branching vessel tended to decrease as a result of plasma skimming.

Sphincter-like intimal cushions of the carotid body artery and other arteries may be controlled by blood temperature or humoral substances, but they have a low density of innervation (Moffat & Creasey, 1971; Hesse & Böck, 1980; McDonald, 1983a). However, cushions on terminal arterioles and precapillary sphincters in the carotid body (McDonald, 1983a) and certain other organs (reviewed by Burnstock, 1975) are more densely innervated.

Branching pattern of the carotid body artery

In the rat, eight to ten first- and second-order branches arise from the carotid body artery in the region of the carotid body, but only three or four branches actually end in the carotid body itself. Consequently the term 'carotid body artery' is misleading, and the vessel probably would be better described as the glomo-ganglionic artery or the glomo-sino-ganglionic artery. Previous studies have shown in several species that the carotid body artery supplies structures adjacent to the carotid body (de Boissezon, 1943; Muratori, 1943; Chungcharoen *et al.*, 1952b; Seidl, 1976; McDonald, 1981); yet the large proportion of vessels that bypass the carotid body has not been described heretofore.

The distinctive pattern of branching of the carotid body artery raises several issues pertaining to carotid body function. 1. Blood within branches passing through the carotid body is likely to flow at high velocity, a feature that would tend to augment plasma skimming in side branches going to glomus cells. 2. Most of the branches going to other structures pass through the carotid body, and thereby may be exposed to amines or other vasoactive substances produced within the organ. 3. Branches bypassing the carotid body may be routes for retrograde blood flow when there is insufficient flow in the carotid body artery (Chungcharoen *et al.*, 1952a; Rossatti & D'Agostini, 1953). In this regard, we observed one case in which clotted blood completely occluded the carotid body artery; yet the microvasculature of the carotid body was washed free of blood by perfusion of fixative, and glomus cells were well preserved.

VESSELS CONNECTING ARTERIOLES WITH VENULES

Capillaries

Our study identified two types of capillaries arranged in parallel that connect terminal branches of the carotid body artery with venules. Type I capillaries have a thin fenestrated endothelium, a total wall thickness less than 1 μm, and a luminal diameter ranging from 8 to more than 20 μm. Vessels of this type are among the most abundant

vascular channels in the carotid body (McDonald, 1983a). These vessels comprise the carotid body's glomerular capillaries or 'sinusoids' (the latter being an incorrect appellation), which have been described by many authors and are considered a characteristic feature of chemoreceptive tissue (reviewed by McDonald, 1981, p. 229). Such large thin-walled vessels have the most intimate association with glomus cells of any vessels in the carotid body.

Type II capillaries provide an alternate route for blood flow through the carotid body. These vessels have a luminal diameter averaging about 6 μm and thereby tend to resemble capillaries of muscle, heart, lung and certain other organs (Majno, 1965; Bruns & Palade, 1968). However, type II capillaries are distinctive in that they have a complete or nearly complete envelopment of pericytes near their arterial end and endothelial fenestrations near their venous end.

Our observations are consistent with those made by de Castro (1951) (see also de Castro & Rubio, 1968), who identified two types of capillaries in the cat carotid body. De Castro described the smaller vessels as being 6–12 μm in diameter and the larger vessels as having a diameter of 14–28 μm and being more tortuous, anastomotic and abundant than the smaller vessels. Another finding made by de Castro & Rubio (1968) was that some large capillaries become narrower abruptly before joining a venule, an observation consistent with Goormaghtigh & Pannier's (1939) report of sphincters at the junction of capillaries and venules. A narrowing of type I capillaries at points they join venules also was observed in the present study (see also McDonald & Haskell, 1983) and was found in reconstructions of vascular elements of the rabbit carotid body (Seidl, 1975).

Arteriovenous anastomoses

The issue of whether arteriovenous shunts exist in the carotid body has been long-debated. Evidence that they are present (Schumacher, 1938; Goormaghtigh & Pannier, 1939; de Castro, 1951; Serafini-Fracassini & Volpin, 1966; de Castro & Rubio, 1968; Schäfer *et al.*, 1973; Seidl, 1975) is brought into question by assertions that they are not present (Niedorf, 1970; Seidl, 1976).

Our data support the view that shunt vessels are present in the interior of the carotid body (McDonald & Haskell, 1983) as well as in the wall of the carotid sinus (see below). However, we obtained no evidence of arteriovenous anastomoses at the surface of the rat carotid body that would correspond to those described in the dog by Serafini-Fracassini & Volpin (1966) or cat by de Castro & Rubio (1968). In the rat, superficial arterioles give rise to capillaries or continue on to other organs; none was found to join a venule directly.

Goormaghtigh & Pannier (1939) considered all large-calibre, thin-walled vessels near glomus cells as 'pseudo-capillary' segments of arteriovenous anastomoses. According to their model based on a study of the cat carotid body and supracardiac paraganglia, total blood flow through the organ is regulated by sphincters at the arterial and venous ends

of the thin-walled vessels. The authors also described small arterial branches that bypassed glomus tissue; although such vessels had an unknown destination, they were thought not to control blood flow to glomus tissue. Against the interpretation of Goormaghtigh & Pannier are several lines of evidence from physiological studies which suggest that arteriovenous anastomoses bypass glomerular capillaries and that total blood flow is regulated independent of flow to the metabolically active chemoreceptive tissues (Purves, 1970; Acker & Lübbers, 1977, Acker & O'Regan, 1981). Both of these models assume that at least two types of parallel channels connect arterioles with venules and that one type bypasses glomus tissue. However, the models differ with respect to which vessels are the arteriovenous shunts involved in the control of total blood flow.

VENULES AND VEINS

The enormous venous plexus around the carotid body seems to be out of proportion to the size of the organ. Glomerular capillaries connect with venules both at the surface and in the interior of the carotid body, and venules emerge from the rostral and caudal poles and certain other regions of the organ as well. The numerous venules are tributaries of not only the internal jugular vein but also the external jugular vein. In sum, redundancy is a feature of all parts of the carotid body's venous system.

This vascular arrangement may be related to the sensitivity of carotid chemoreceptors to changes in venous pressure. Bingmann *et al.* (1975) discovered that a 2 cm H_2O rise in venous pressure abolishes the excitatory response of chemoreceptors to asphyxia. Mitchell made similar observations and in addition determined that the phenomenon is not mediated by nerves or by dopamine from glomus cells, as it persists after surgical denervation of the carotid body and is not affected by drugs that block muscarinic and dopaminergic receptors (see Bingmann *et al.*, 1975, p. 355).

RELATION OF CAROTID BODY AND CAROTID SINUS BLOOD SUPPLIES

Our observations of well-developed vasa vasorum in the wall of the carotid sinus and a close relationship of these vessels to arterioles and venules of the carotid body are consistent with the common embryological origin of the carotid sinus and carotid body (Boyd, 1937). Yet this arrangement differs from the source of most vasa vasorum, which arise from branches of the arteries whose walls they vascularize (Robertson, 1929; Wolinsky & Glagov, 1967).

In humans and other mammals weighing more than a few kilograms, arteries with a diameter exceeding one millimeter are supplied by vasa vasorum (Wolinsky & Glagov, 1967; and reviewed by Higginbotham *et al.*, 1963; Heistad & Marcus, 1979). But such vessels generally are not present on major arteries of rats and other small animals (Wolinsky & Glagov, 1967).

Several investigators have reported the association of carotid body vessels with vasa

vasorum of the carotid sinus (reviewed by Adams, 1958, p. 152). However, we are not aware of previous studies that have shown the extent of this relationship or the distinctive distribution of vasa vasorum in the adventitia of the carotid sinus.

We infer from our observations that carotid sinus baroreceptors have an extensive, specialized blood supply and have the same source of blood as the carotid body. Furthermore, the geometry of the vasculature indicates that: 1. blood from the carotid body artery can enter carotid body veins via arteriovenous shunts or via thoroughfare channels present in the wall of the carotid sinus; (arteriovenous anastomoses in vasa vasorum are not unique to the carotid sinus; Clara, 1956, p. 47); and 2. substances entering the blood in the carotid body could be carried to sensory nerves and smooth muscle cells of the carotid sinus (or vice versa) if a venous portal system exists.

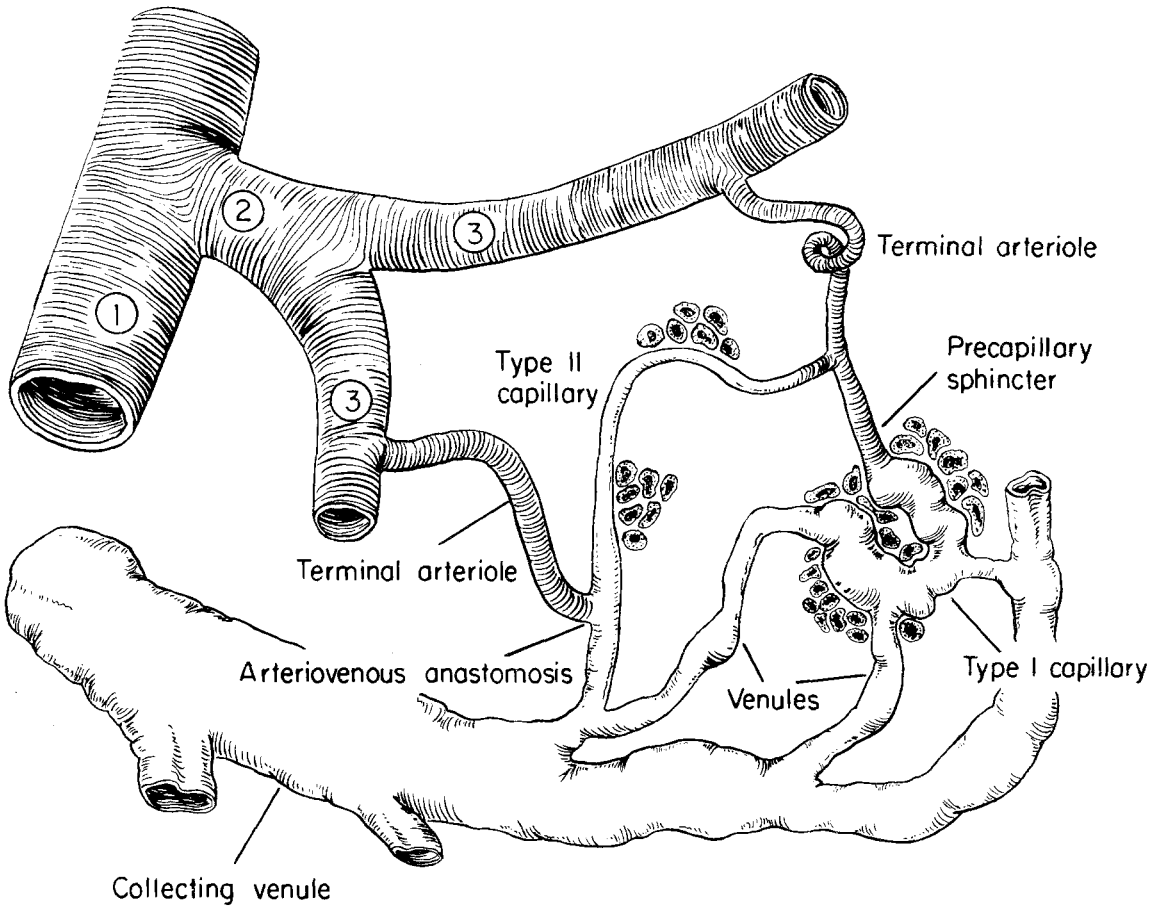

Fig. 38. A summary of connections of blood vessels within the rat carotid body. Among the vessels shown are first (1) to third (3) order branches of the carotid body artery and terminal arterioles giving rise to a type I capillary, type II capillary and an arteriovenous anastomosis. Glomus cells are located near both types of capillary. Small venules connect the capillaries and the arteriovenous anastomosis to a collecting venule.

Conclusions

A summary of our observations on the connections of blood vessels in the rat carotid body is presented in Fig. 38. From these observations we make the following conclusions. First, sphincter-like intimal cushions at the origin of the carotid body artery and some of its branches may regulate flow and influence the haematocrit of blood going to the carotid body. Second, because first-order branch-arteries pass through the carotid body, blood is likely to flow at high velocity at the origin of second-order branches that are the source of blood vessels going to glomus cells, another phenomenon that may influence plasma skimming. Third, there are at least two types of capillaries in the carotid body, and flow through them may be governed by precapillary sphincters. Fourth, arteriovenous anastomoses are present within the interior of the carotid body. Fifth, venous connections of the carotid body exhibit considerable redundancy and provide multiple pathways for blood to leave vessels supplying chemoreceptive tissues. And sixth, interconnections of the blood supplies of the carotid body and carotid sinus may permit the transfer of substances between the organs.

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