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The volume of the carotid body and periadventitial type I and type II cells in the carotid bifurcation region of the fetal cat and kitten

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Summary. The bilateral distribution of carotid body type I cells was investigated in 6 fetuses (gestational age 95%) and 9 newborn kittens (aged 1 day to 4 days) by serially sectioning the carotid bifurcation regions. In most specimens type I cells occurred in close proximity to the wall of the occipital artery or one of its small proximal branches within a division of connective tissue with defineable but irregular borders. This combination of type I cells and connective tissue constituted the principal mass of the carotid body. Using an interacting image analysis system, the area of the carotid body in each serial section was measured by accurately contouring its perimeter. The volume of the carotid body was calculated by multiplying the sum of the areas of the serial sections by the thickness of the section. The volume of the carotid body was 0.052 ± 0.018 mm^3 in the fetuses and 0.025–0.117 mm^3 in the 1–4 day old kittens. A degree of symmetry in the values for the volume of the right and left carotid body was found. Caudally, and separate from the principal mass of carotid body type I cells, isolated groups of periadventitial type I cells were noted in the connective tissues around the occipito-ascending pharyngeal trunk, origin of the occipital artery and rostral end of the common carotid artery in 7 out of 12 specimens from fetal cats and 11 out of 18 specimens in newborn kittens. The volumes of the periadventitial groups of cells ranged between 25–1,365 μm^3 in fetuses and 10–1,351 μm^3 in kittens.

Key words: Carotid body – Carotid bifurcation – Cat fetus – Newborn kitten

Introduction

Recent evidence indicates that the carotid body is active and responsive before birth, but discharges at a lower frequency in the fetus than in the adult at the same arterial oxygen tension (Kondo 1976; Blanco et al. 1984). The change in hypoxic sensitivity of the chemoreceptors is reset from the fetal to the adult range within a few days of birth (Belenky et al. 1979; Blanco et al. 1984). There is little information about the histological appearances or size of the carotid body during the transition from fetal to early neonatal life. Early work was concerned with the general position of the adult and fetal cat carotid body and various hypotheses were put forward to explain its embryological development (Schaper 1892; Kohn 1900; Watzka 1937, 1943; Hollinshead 1943). More recently, detailed studies have been limited to the adult cat and were undertaken to determine the precise distribution of carotid body type I and type II chemoreceptor cells in the carotid bifurcation regions (Seidl 1976; Clarke and Daly 1983). In other studies a neural crest origin for type I cells has been postulated (Pearse and Pollak 1978; Stevens and Moore 1983). Another aspect of the development of type I cells is the increase in type II synapses on type I cells in the immediate postnatal period (Kondo 1976).

In the present paper we give a detailed account of the distribution and appearance of type I and type II cells comprising the principal mass of the carotid body in the fetal cat and newborn kitten. In addition, a determination of the volume of the organ has been made by an analysis of every histological serial section using an interactive image analysis system.

Previously, additional groups of cells with similar histological characteristics to carotid type I and type II cells, lying in the perivascular connective tissues outside the confines of the principal mass of the carotid body, were described in the carotid bifurcation regions of the adult cat, rabbit and dog; they were designated on a purely morphological basis as "periadventitial" type I and type II cells (Clarke and Daly 1981a, 1981b, 1982, 1983). They are described in this paper together with the results of determinations of their volume.

Materials and methods

Observations were made on 6 fetal cats from 2 mothers and on 9 newborn kittens from 2 litters. The fetuses were delivered by caesarian section at 95% gestation period (full term 66 days), under pentobarbitone sodium anaesthesia (Sagatal, May and Baker, Ltd.) 40 mg kg^{-1} body weight of the pregnant animal, intraperitoneally). The animals were of either sex and the weights are given in Table 1.

The kittens were sacrificed on day 1 (2 animals), day 2 (2), day 3 (2) and day 4 (3). The 9 animals were anaesthetized with intraperitoneal Sagatal, 3.5 mg·100 g^{-1} body

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Table 1. *Fetal cats.* Dimensions and volume of carotid body and periadventitial type I cells (means ±SD)

Specimen no. side	Body weight (g)	Distance of RP rostral to Cbn (μm)	Distance of CP rostral to Cbn (μm)	RP-CP length (μm)	Volume of carotid body (mm^3)	Periadventitial cells	
						R-C extent (μm)	Volume (μm^3)
F1R	77.0	325	−55	380	0.02	0	0
F1L		700	300	400	0.051	0	0
F2R	96.4	710	310	400	0.051	0	0
F2L		560	45	515	0.052	0	0
F3R	90.8	700	220	480	0.043	25	29
F3L		720	285	435	0.045	75	140
F4R	84.7	730	130	600	0.071	50	89
F4L		790	195	595	0.074	150	335
F5R	96.2	840	495	345	0.035	190	1,365
F5L		810	470	340	0.036	390	26
F6R	82.2	590	305	285	0.07	200	1,274
F6L		490	150	340	0.078	0	0
$N=6$	87.9 ±7.9	649.2 ±177.7 678.3 ±127.7	234.2 ±186.2 240.8 ±146.1	415 ±111.1 437.5 ±101.2	0.048 ±0.02 0.056 ±0.017	77.5 ±93 102.5 ±153	459.5 ±668 83.5 ±135
$N=12$		663.7 ±148.3	237.5 ±159.6	426.2 ±102	0.052 ±0.018	90 ±121	271.5 ±500

RP, rostral pole; CP, caudal pole; Cbn, carotid bifurcation; R-C, rostral-caudal; L, left; R, right. Age (gestation %) 95

Table 2. *Kitten.* Dimensions and volume of the carotid body and periadventitial type I cells (means ±SD)

Specimen no. side	Age (days)	Body weight (g)	Distance of RP rostral to Cbn (μm)	Distance of CP rostral to Cbn (μm)	RP-CP length (μm)	Volume of carotid body (mm^3)	Periadventitial cells	
							R-C extent (μm)	volume (μm^3)
K1R	1	125.0	755	130	625	0.089	0	0
K1L			870	135	735	0.108	60	509
K2R	1	124.5	640	235	405	0.038	0	0
K2L			505	230	275	0.025	370	1,062
K3R	2	126.6	645	315	330	0.059	90	382
K3L			520	110	410	0.068	235	575
K4R	2	126.8	660	285	375	0.051	0	0
K4L			700	270	430	0.048	85	185
K5R	3	110.0	755	225	530	0.092	175	306
K5L			625	225	400	0.062	85	105
K6R	3	118.0	795	295	500	0.055	15	10
K6L			785	220	565	0.063	0	0
K7R	4	127.0	895	225	670	0.092	0	0
K7L			845	240	605	0.061	50	109
K8R	4	127.0	680	200	480	0.117	0	0
K8L			675	270	405	0.112	0	0
K9R[a]	4	140.0	–	305	–	–	450	1,351
K9L[a]			815	365	450	0.085	105	904

[a] Specimen K9 was not included in paired analysis

RP, rostral pole; CP, caudal pole; Cbn, carotid bifurcation; R-C, rostral-caudal; L, left; R, right. No significant difference between rostral-caudal lengths or volumes of carotid bodies on the two sides ($P>0.7$ and $P>0.4$ respectively)

weight. The technique for perfusion and fixation of carotid bifurcation regions was the same for all animals and was similar to that described previously (Clarke and Daly 1981a, 1983). Briefly, the chest was quickly opened in the mid-sternal line and heparin (Weddel Pharmaceuticals Ltd.) 100 i.u. 100 g^{-1} was injected into the left ventricle to render the blood incoagulable. A cannula was inserted through the wall of the left ventricle into the aorta and tied in place. The escape of fluid into the pulmonary circulation was prevented by ties at the root of the lungs. Perfusion of the

systemic circulation was begun with sodium chloride solution (154 m·mole l^{-1}) at a temperature of 37° C to clear the blood, and then 3% glutaraldehyde in isotonic phosphate buffer (pH 7.3; temperature 37° C) was perfused for 3–5 min. The perfusion pressure used in the fetuses was 50 mm Hg and in the kittens it was the same as the mean femoral arterial pressure measured immediately after anaesthetizing the animals. The body weights of the kittens are given in Table 2.

The carotid bifurcation regions were block dissected 12–14 h later, freed from the base of the skull, and following removal from the body, were immersed in 3% glutaraldehyde in isotonic phosphate buffer (pH 7.3) at 2° C for 4–6 h. The tissues were prepared for light microscopy and ribbons of transverse serial 5 μm sections were cut commencing from the rostral end of the specimen and continuing caudally to the end of the rostral 1 mm of the common carotid artery. The principal mass of kitten (K) K9 was only found in part on the right side of the neck. Ribbons of sections were stained using a modification (Dawes and Hillier 1964) of the M.S.B. method for fibrin (Lendrum et al. 1962).

The volume of the principal mass of the carotid body was measured by an interactive image analysis system (Model IBAS 1; Reichert-Jung UK) as described previously (Clarke and Daly 1984). The area in every serial section was measured by contouring the well-defined perimeter of connective tissue embracing the type I and type II cells. The measured area excluded that of the connective tissue, blood vessels and nerve fibres lying external to the principal mass of the carotid body, the boundaries of which were defineable. The volume of the carotid body, expressed in mm^3, was calculated by multiplying the summed areas of the sections by the thickness of each section (5 μm).

The volume of tissue occupied by the groups of periadventitial type I and type II cells was determined in the same way.

Results

Histology of carotid body and periadventitial type I and type II cells

Serial transverse sections from the carotid bifurcation regions of fetal cats and newborn kittens revealed carotid body type I and type II cells in every specimen. These cells possessed classical cytological characteristics and were embedded in an easily defineable division of connective tissue. This combination of carotid body type I and type II cells and their immediately adjacent connective tissue represented the principal mass of the carotid body.

In addition, periadventitial type I and type II cells were observed lying caudal to the principal mass in oval and spherical groups of widely varying sizes; small clusters of less than 10 cells, and less commonly single cells in both fetal cats and newborn kittens. Collections of periadventitial cells appeared mainly in the periadventitial connective tissues of the occipito-ascending pharyngeal trunk, origin of the occipital artery and rostral end of the common carotid artery. These periadventitial cells showed similar cytological characteristics to carotid body cells, in that they stained a red-orange colour with the modified M.S.B. method, were associated with type II cells, and by light microscopy appeared to have a similar pattern of capillary supply to that observed in the principal mass of the carotid body.

Arteries of the carotid bifurcation region

In general the origin and course of arteries originating from the rostral end of the common carotid arteries in fetuses and newborn kittens conformed to the arterial patterns described previously in the adult cat (Clarke and Daly 1983).

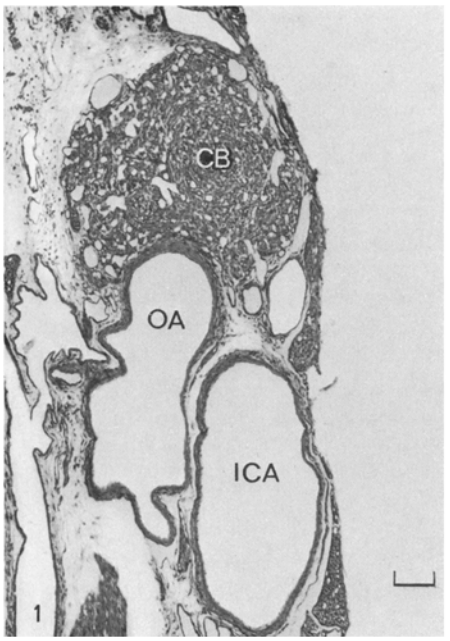
The arterial supply to the principle mass of the carotid body in fetuses and newborn kittens most commonly arose from the proximal part of the occipital artery or one of its small brances. In eight specimens (F2R, F4R, K1R, K3L, K4R, K5R, K7R, K8R) (fetus (F) or kitten (K), animal number, left (L) or right (R) side), an additional blood supply was contributed by the ascending pharyngeal artery. Less commonly the blood supply to the carotid body originated only from the ascending pharyngeal artery (F1L, F2L, K5L). The number of arteries entering the principal mass from the occipital artery or ascending pharyngeal artery varied considerably. It would appear from our material that either a single vessel or multiple closely arranged small arteries (specimen K3R) could enter the caudal pole of the principal mass of the carotid body. The occipito-ascending pharyngeal trunk contributed a small artery to the principal mass of specimen K1L in addition to its blood supply from the occipital artery.

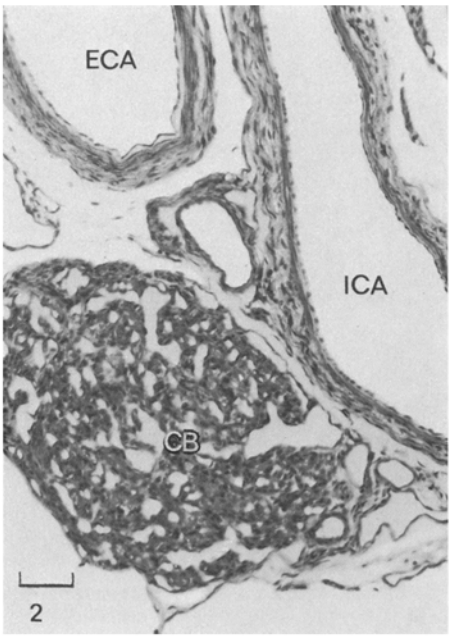
The internal carotid artery was patent in fetal cats and newborn kittens apart from specimens K6R and K6L in which the vessel was becoming fibrous at day 3 of postnatal life.

Some unusual features, however, were noted. In specimen F1R the rostral end of the right common carotid artery terminated in an arterial trifurcation. Two of the arteries arising from the trifurcation could be recognized as the internal and external carotid arteries, but the third vessel passed quickly from the area of the section in a lateral and slightly dorsal direction and could not be positively identified. A small vessel arose from this "third artery" and immediately entered the adjacent caudal pole of the principal mass of the carotid body. An additional complication occurred in the arterial arrangements in specimen F1R, since the occipito-ascending pharyngeal trunk originated from the external carotid artery at an atypical site rostral to the principal mass of the carotid body. These variations resulted in the proximal part of the right internal carotid artery having an unusually close relation to the right carotid body, which lay in a position dorsomedial to the vessel.

Microvascular bed of carotid bodies

An extensive examination of the serial sections showed that the capillary bed was patent in virtually all parts of the principal mass of the carotid body in fetuses and newborn kittens. Occasionally, small areas of closely arranged groups of type I and type II cells were seen which had no apparent accompanying capillary supply at the level of light microscopy. Such areas constituted less than 5% of the field and were not seen in every section. All the small veins at the perimeter of the principal masses had been filled by the perfusate, which could sometimes be recognised as a delicate pink precipitate in the lumen of the vessels, indicating that the blood had been washed out and that a sufficiently successful perfusion of the microvascular bed had been





Figs. 1, 2. Photomicrographs of fetal carotid body (*CB*). Internal carotid artery (*ICA*); external carotid artery (*ECA*). Stain M.S.B.

Fig. 1. Specimen F1L. Note dome-shaped carotid body ventral to occipital artery (*OA*). Scale = 100 μm.

Fig. 2. Specimen F1R. Note carotid body dorsomedial to internal carotid artery. Scale = 50 μm

made for meaningful volume measurements to be carried out on the carotid bodies.

Bilateral distribution of carotid body type I cells and periadventitial type I cells

Carotid body type I cells

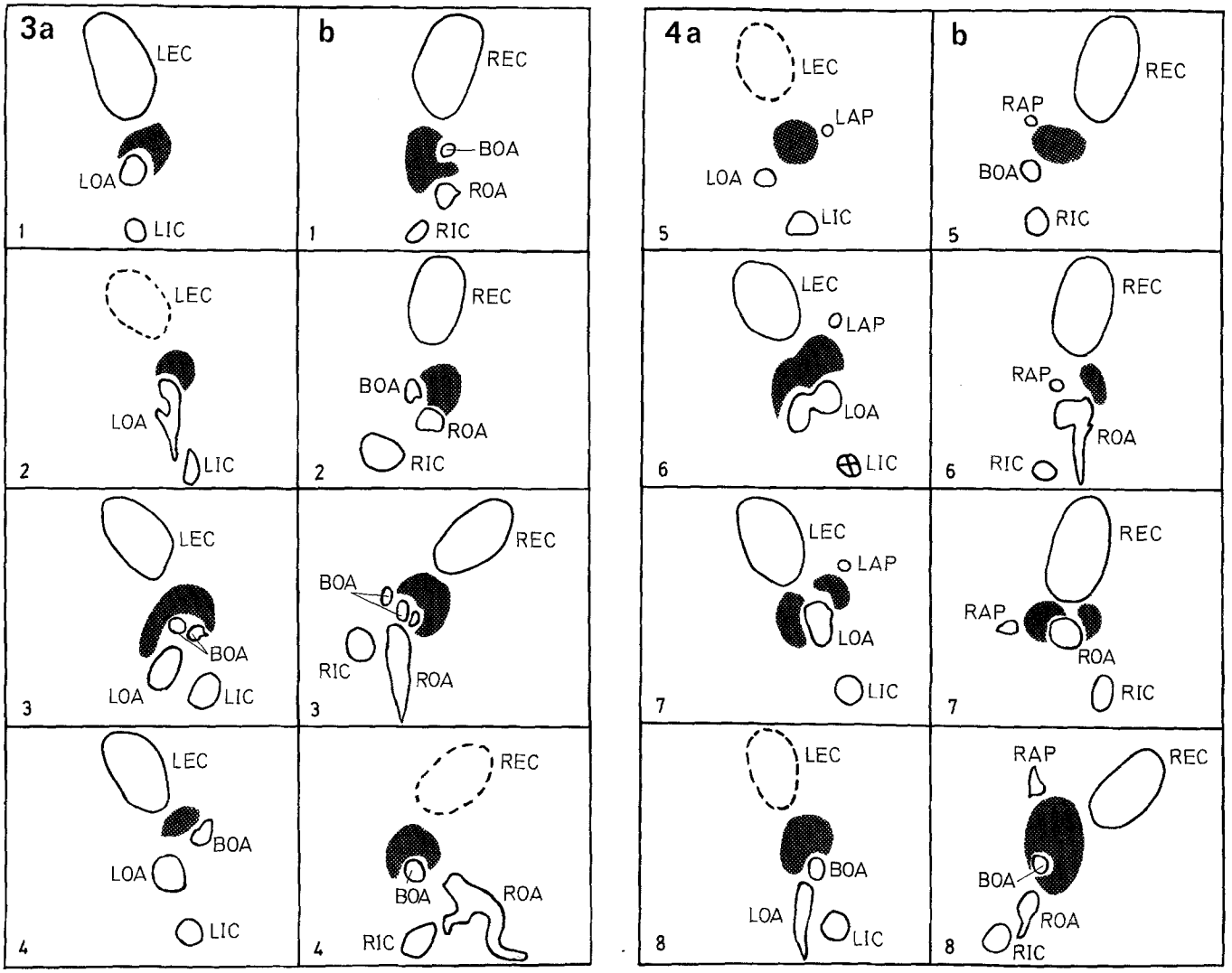
Fetal cats. Several distinctive features characterized the principal mass of the carotid body.

Each principal mass usually appeared as an ovoid- or crescentic-shaped structure with easily discernible rostral and caudal poles. All type I cells occurred within a distance of 1 mm from the rostral edge of the carotid bifurcation with an average mid-point distribution of 450 μm rostral to the bifurcation. While the majority of the type I cells lay in close proximity to the more ventral aspects of the proximal part of the occipital artery and its small branches (Fig. 1), a dorsal extension of type I cells encircled the occipital artery in specimen F3L so that the vessel briefly traversed the caudal part of the principal mass. Another example of a major artery passing through the carotid body was observed in specimen F4R, as the ascending pharyngeal artery pierced the caudal pole of the organ before inclining rostrally and medially away from the principal mass. The complex arterial arrangements of the carotid bifurcation region of specimen F1R resulted in type I cells being closely related to the dorsomedial aspects of the internal carotid artery (Fig. 2), and this was the only occasion in either fetal or postnatal specimens when the caudal pole of the principal mass extended caudal to the level of the carotid bifurcation to lie dorsomedial to the common carotid artery. A bipartite arrangement of type I cells occurred in the caudal part of the principal mass in specimen F4L and F6L and a tripartite distribution in specimen F4R. The rostral and caudal poles of the principal masses were discrete structures, with the exception of specimen F6L in which the rostral pole of the principal mass was bipartite. In 2 out of 12 specimens (F2L, F4R) the type I cells within the caudal pole of the principal mass extended sufficiently far caudally to come into relation with the occipital-ascending pharyngeal trunk.

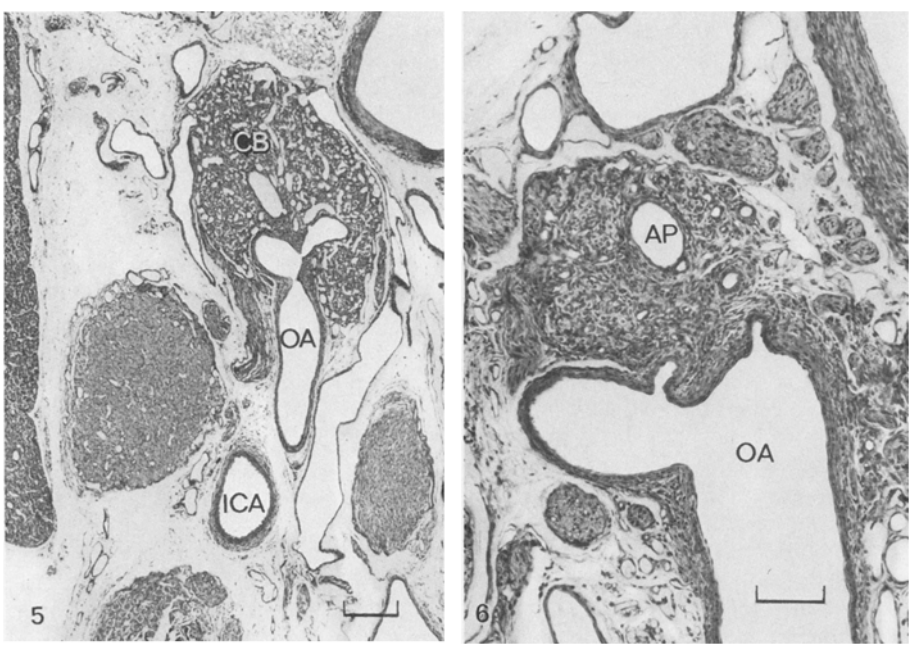
Part of the superior cervical ganglion was a prominent medial relation in many specimens, and in specimens F3L and F5R large nerve trunks supported the dorsal aspect of the principal mass. In the narrow track of connective tissue separating the principal mass from the dorsomedial aspect of the external carotid artery, small deposits of brown fat were observed in several specimens.

In keeping with our view that measurements of the principal masses were really only meaningful in a rostral-caudal plane, and taking all 12 specimens into consideration, the mean distance of the caudal pole rostral to the bifurcation was 237.5 ± 159.6 (SD) μm; mean distance of the rostral pole rostal to the bifurcation was 663.7 ± 148.3 μm, while the mean length of the principal mass of the carotid body between the poles was 426.2 ± 102 μm. The respective values for each of the 12 carotid bodies are shown in Table 1. A paired statistical analysis indicates that there is no difference between the two sides. Measurements of the volume of the principal mass of the carotid body ranged from a minimum value of 0.02 mm^3 (specimen F1R) to a maximum value of 0.078 mm^3 (specimen F6L), mean 0.052 ± 0.018 mm^3. The values for each of the 12 carotid bodies are shown in Table 1.

Newborn kittens. In general terms the histological picture of the cat carotid body closely resembled the appearance of the organ in the fetus. All type I cells were located within a distance of 1 mm from the rostral edge of the carotid bifurcation and each principal mass again appeared as an ovoid- or crescent-shaped structure with easily discernable rostral and caudal poles. The general positions and arrangements of the individual principal masses from 8 out of 9 carotid bifurcation regions are shown in Figs. 3 and 4, taking the reference level at the mid-point (470 μm) of the distribution of carotid body type I cells rostral to the carotid bifurcation. The rostral-caudal lengths of the principal



Figs. 3, 4. Paired transverse sections from 8 kittens of left (**a**) and right (**b**) internal carotid artery (*LIC, RIC*) and external carotid artery (*LEC, REC*) 470 μm rostral to carotid bifurcation. Note distribution of carotid body type I cells (*dark stippling*) comprising principal masses. Left and right occipital artery (*LOA, ROA*); branch of occipital artery (*BOA*); left and right ascending pharyngeal arteries (*LAP, RAP*). Occluded internal carotid artery only in 6L at this level(x). Outline of incomplete wall of external carotid artery (---)



Figs. 5, 6. Photomicrographs of kitten carotid body (*CB*). Occipital artery (*OA*); internal and external carotid artery (*ICA, ECA*). Stain M.S.B. Scale = 100 μm

Fig. 5. Specimen K8R. Note carotid body ventral to occipital artery being supplied with vessels

Fig. 6. Specimen K5R. Note carotid body ventral to occipital artery being supplied with two small arteries and ascending pharyngeal artery (*AP*) traversing the caudal pole

Table 3. *Kitten.* Rostral and caudal poles of the kitten carotid body

Specimen no. side	Rostral pole		Caudal pole			
	Arrangement type I cells	Relation to OA/BOA	Arrangement type I cells	Relation to		
				OA	AP	OPT
K1R	Discrete	V/M	Discrete	–	–	D/L
K1L	Discrete	V/M	Discrete	–	–	L
K2R	Discrete	V/L	Discrete	V/M	D/M	–
K2L	Discrete	V/M	Discrete	V/L	–	–
K3R	Discrete	V/L	Discrete	L	–	–
K3L	Discrete	L	Discrete	–	–	V/M
K4R	Discrete	V/M	Discrete	–	–	V/M
K4L	Discrete	V/M	Discrete	V/M	–	–
K5R	Discrete	V/L	Discrete	V/M	V	–
K5L	Discrete	V/M	Discrete	V/M	V	–
K6R	Discrete	V/L	Discrete	–	–	V/L
K6L	Discrete	V/M	Discrete	–	–	D/L
K7R	Discrete	V/M	Discrete	–	–	L
K7L	Discrete	V/M	Discrete	–	–	D/L
K8R	Discrete	V/L	Discrete	–	–	V/L
K8L	Discrete	V	Discrete	V/M; V/L	–	–
K9R	Not found	–	Discrete	V/M	D/M	–
K9L	Discrete	V/L	Discrete	V/M	–	–

Ventromedial and ventrolateral (V/M; V/L); ventral (V); lateral (L); dorsolateral (D/L); dorsomedial (D/M). Occipital artery, branch of occipital artery and ascending pharyngeal artery (OA; BOA; AP). Occipito-ascending pharyngeal trunk (OPT)

masses are shown in Table 2, while the arrangement of type I cells in the rostral and caudal poles of the principal masses and their major vascular relations are recorded in Table 3.

The arrangement of type I cells varied considerably and was largely dependent upon the branching position of the proximal part of the occipital artery (Fig. 5). In the majority of specimens the principal mass of the carotid body had a reasonably discrete appearance with type I cells being quite closely positioned around the occipital artery and its branches. In 5 out of 9 kittens (K1L, K3L, K4R, K5R, K6L), however, the caudal part of the principal mass became bipartite, while in specimen K7R the arrangement was tripartite. As sectioning proceeded caudally in these specimens, only one of the partitions remained leaving each principal mass with a discrete arrangement of type I cells within the caudal pole. The ascending pharyngeal artery briefly traversed the caudal pole of specimen K5R (Fig. 6) before inclining rostrally in close proximity to the medial aspect of the principal mass. More usually, the vessel could be recognized a short distance from the medial border of the carotid body lying ventromedial or dorsomedial to the type I cells. In the majority of specimens type I cells within a part of the medial border of the principal mass were very closely related to the superior cervical ganglion. Small veins occurred at the perimeter of the principal mass in many sections and sometimes these could be traced into adjacent larger veins in which cusps of venous valves were occasionally seen. No arterio-venous anastomoses were observed arising from arterioles with a diameter greater than 20 μm. In every specimen the major part of the carotid body was separated from the external carotid artery by a tract of connective tissue as it lay dorso-medial to the vessel. A small part of the principal mass of specimen K7L, however, was firmly attached to the periadventitial connective tissue of the external carotid artery. Measurements of the volume of the principal mass of the carotid body indicate a minimum value of 0.025 mm^3 for a day 1 kitten (specimen K2L) and a maximum value of 0.117 mm^3 for a day 4 kitten (specimen K8R). Because the kittens are of different age, no attempt has been made to average the data contained in Table 2. There is, however, no significant difference between the rostral-caudal lengths or the volumes of the carotid bodies on the two sides (paired analysis, $P > 0.7$ and $P > 0.4$ respectively). Linear regression analysis of the data by the least squared method for the rostral-caudal length of the carotid bodies on age gave a correlation coefficient of 0.18; for volume on age, 0.44.

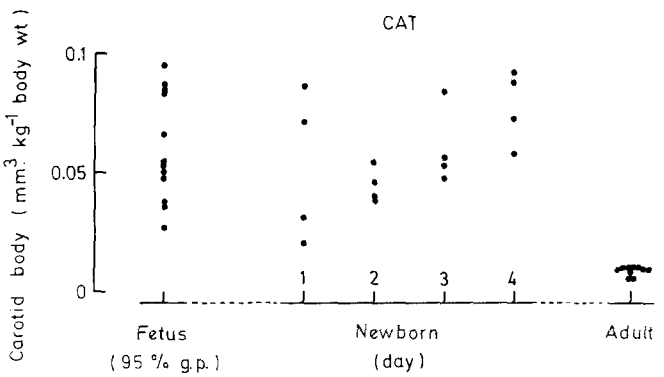


Fig. 7. The volume of the carotid body expressed in mm^3 per kg weight of animal in the fetus, kitten and adult cat. Data for adult cat from Clarke et al. 1985. Gestational age 95%, 100% = 66 days. Adult cats more than 1 year of age

A comparison of the rostral-caudal lengths and volumes of the carotid bodies in the fetus on the one hand, and in each neonatal group (day 1, 2, 3 and 4) on the other, revealed that a statistically significant difference occurred

Table 4. Relation of periadventitial type I cells to arteries in the carotid bifurcation region in the fetus and kitten

Specimen no. side	OA		OPT		ICA		Cbn		CC	
Fetus										
F1R	–		–		–		–		–	
F1L		–		–		–		–		–
F2R	–		–		–		–		–	
F2L		–		–		–		–		–
F3R	–		–		–		–		D/L	
F3L		–		L		–		–		–
F4R	–		–		–		–		M	
F4L		–		–		–		–		M; D
F5R	V/L; V/M		–		–		–		–	
F5L		–		L		–		–		D/L
F6R	–		–		–		–		D/M	
F6L		–		–		–		–		–
Kitten										
K1R	–		–		–		–		–	
K1L		–		V/M		–		–		–
K2R	–		–		–		–		–	
K2L		L		–		–		–		D/L
K3R	–		M		–		–		–	
K3L		–		–		–		–		D/M
K4R	–		–		–		–		–	
K4L		–		–		–		–		D
K5R	–		–		–		–		D/L; D/M	
K5L		–		–		–		–		D/L
K6R	–		L		–		–		–	
K6L		–		–		–		–		–
K7R	–		–		–		–		–	
K7L		–		–		–		–		D/L
K8R	–		–		–		–		–	
K8L		–		–		–		–		–
K9R	–		L		–		D/M; D/L		D/L	
K9L		–		–		V/L		D/L		D/L

Common carotid artery (CC); internal carotid artery (IC); absence of periadventitial type I cells (–); other abbreviations as in Tables 2 and 3

only in the day 4 kittens. Both the length and volume of the carotid body were greater than those in the fetal animals (unpaired analysis; $0.1 > P > 0.05$ and $P < 0.005$ respectively).

In Fig. 7 the volume of the carotid body, expressed in mm^3 per kg total body weight, has been plotted against the age of the animal.

Periadventitial type I cells

Periadventitial type I cells were observed in 7 out of 12 fetal cat specimens and 11 out of 18 kitten specimens lying outwith the confines of the principal mass of the carotid body mainly in the periadventitial connective tissues of the origins of the occipital artery and occipito-ascending pharyngeal trunk, and along the vessels themselves and rostral end of the common carotid artery. Viewed on a rostral-caudal plane periadventitial type I cells appeared either as intermittently dispersed groups of cells or as varyingly sized, single clusters of cells. Periadventitial type I cells were observed both rostral and caudal to the carotid bifurcation and the different positions these cells occupied in relation to the arteries of the carotid bifurcation region are recorded in Table 4, while the number of groups of periadventitial type I cells and their rostral-caudal extent in fetuses and in kittens are shown in Table 5. It would appear that the distribution of periadventitial type I cells caudal to the carotid bifurcation is around the dorsal aspect of the common carotid artery only. Measurements of the total volume of periadventitial type I cells in each specimen are noted in Tables 1 and 2.

Small arterioles, veins and myelinated nerves were often seen in close association with groups of periadventitial type I cells in both fetuses and kittens (Figs. 9–12).

Reconstruction of the carotid body

Three-dimensional reconstructions of the typical distribution of carotid body type I and periadventitial type I cells from the right and left carotid bifurcation regions of one fetal cat (F5) and one kitten (K7) are shown in Fig. 8. Each reconstruction incorporates the results of detailed observations taken at intervals of 5 μm from sections throughout the rostral-caudal extent of the specimens. The small proximal branches of the occipital artery have, however, been omitted in order to give an unobscured view of the carotid body, which has been presented on this occasion from a ventral view.

Discussion

In a previous paper (Clarke and Daly 1983) the bilateral arrangements and distribution of carotid body type I cells in the adult cat was considered in detail and the variety in the form of the carotid body was emphasized. The results from the present study underline these earlier observations in showing that, in the carotid body of the fetus and newborn, there is not only an inconstant distribution of type I and type II cells in both a rostral-caudal and medial-lateral plane, but also a variable pattern of arterial branching at the rostral end of the common carotid artery.

In previous descriptions of the irregular distribution of type I cells in the carotid bifurcation regions in the adult rabbit (Clarke and Daly 1981a, 1981b), dog (Clarke and Daly 1982) and cat (Clarke and Daly 1983), we adopted the terms "principal mass" of the carotid body to describe the main and largest conglomeration of type I cells encapsulated in a well defined division of connective tissue, and "periadventitial type I cells" to describe the other scattered groups of cells. The use of this terminology has again proved adequate to describe the distribution of type I cells in the carotid bifurcation regions of the cat fetus and kitten. As in the adult cat the principal mass of the carotid body was ovoid- or crescent-shaped, lying in close relation to the occipital artery and occipito-ascending pharyngeal trunk, or more rarely, the ascending pharyngeal artery. There was a variation too in the shape and size of the organ, not only between animals of the same age, but also

Table 5. Distribution of periadventitial type I cells in the fetal cat and kitten

Specimen no. side	Number of groups PT cells	Distance (μm) of rostral PT cells rostral to Cbn	Distance (μm) of caudal PT cells rostral to Cbn	Rostral caudal extent (μm) of PT cells
Fetus				
F1R	0	0	0	0
F1L	0	0	0	0
F2R	0	0	0	0
F2L	0	0	0	0
F3R	1	− 60	− 85	25
F3L	1	110	35	75
F4R	1	− 85	−135	50
F4L	2	− 55	−205	150
F5R	2	390	200	190
F5L	2	165	−225	390
F6R	1	−135	−335	200
F6L	0	0	0	0
Kitten				
K1R	0	0	0	0
K1L	1	245	185	60
K2R	0	0	0	0
K2L	2	135	−235	370
K3R	1	185	95	90
K3L	1	− 20	−255	235
K4R	0	0	0	0
K4L	1	−120	−205	85
K5R	1	−150	−325	175
K5L	1	− 15	−100	85
K6R	1	185	170	15
K6L	0	0	0	0
K7R	0	0	0	0
K7L	1	− 70	−120	50
K8R	0	0	0	0
K8L	0	0	0	0
K9R	4	200	−250	450
K9L	1	70	− 35	105

Periadventitial type I cells (PT), carotid bifurcation (Cbn)

on the two sides of the same animal, as found previously in the adult cat (Seidl 1976; Clarke and Daly 1983). The same variation was found in the present study with regard to the distribution of periadventitial type I cells, and these all lay outside the confines of the principal mass of carotid body. Histological examination of fetal and kitten carotid bodies did not reveal a strikingly different microvascular picture and we conclude, therefore, that an alteration in the vasculature of the carotid body does not occur at the time of birth, at least up to the fourth postnatal day.

The present study, in conjunction with our previous report on the adult cat carotid body (Clarke and Daly 1983), allows an opinion to be expressed about the appearances of the carotid body in a single species over a period of time extending from late gestation through the immediate neonatal period to the adult state. One of the most striking features about the cat carotid body is the increase in the extent of the rostral-caudal distribution of type I cells between birth and adulthood. The earlier analysis (Clarke and Daly 1983) of 10 carotid bifurcation regions in the adult cat showed that the mean group distance of the rostral pole of the carotid body rostral to the bifurcation was 1,944 μm, while the mean group rostral-caudal extent of type I cells was 1,159 μm and the average mid-point of type I cells distribution rostral to the carotid bifurcation was 1,305 μm. When these values in the adult cat are compared with the respective values in the fetal cat (664 μm; 428 μm; 450 μm), it can be seen that a considerable postnatal increase in the rostral-caudal extent of the carotid body occurs. In the context of this part of the discussion we have excluded the kittens because averaged results can not be obtained from this data as explained above. However, from Table 2 it would appear that this postnatal development must occur subsequent to day 4 after birth. Moreover, the distance the most rostral type I cells lie rostral to the carotid bifurcation in the adult cat (Clarke and Daly 1983) is greater than in the fetus by a factor of approximately 3. In addition, there is a considerable increase in the volume of the carotid body, from a mean of 0.052 mm^3 in the full-term fetus and between 0.025–0.117 mm^3 in the 1–4 day old kittens to a mean of 0.247 mm^3 in the adult cat (Clarke et al. 1985). Yet, when the volume of the carotid body is expressed in relation to the animal's body weight, the adult cat's carotid body is actually the smallest (Fig. 7).

The postnatal alteration in the size of the carotid body and its position in the carotid bifurcation has some interesting implications. Any embryological basis for morphological change in the carotid body after birth seems unlikely. Assuming the neural crest hypothesis (Pearse and Polak 1978) for the orgin of type I cells, and this has yet to be

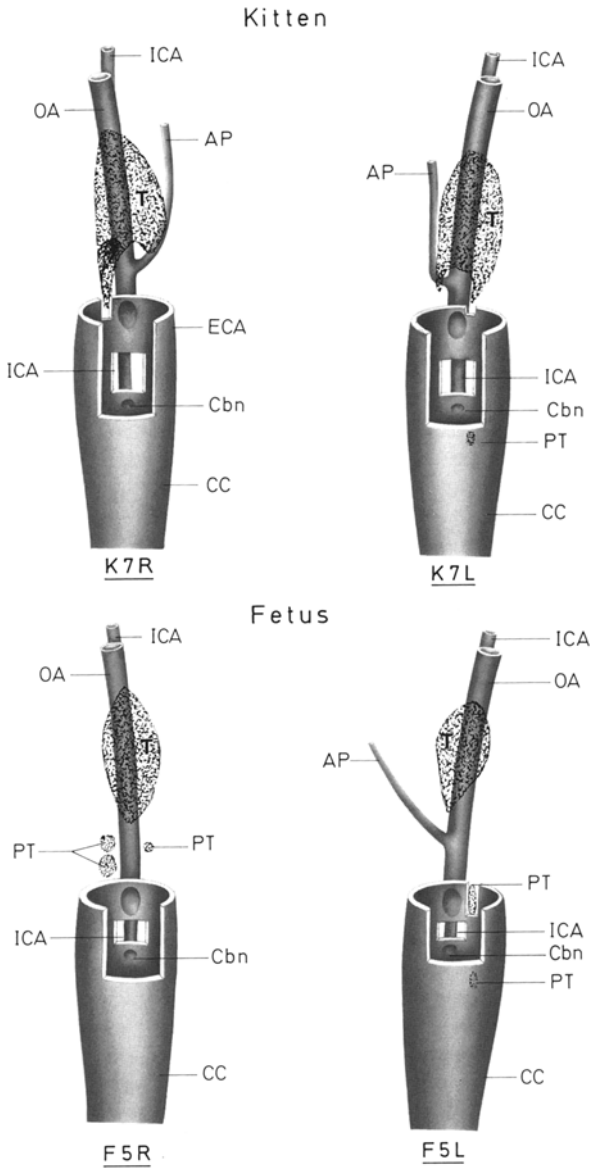


Fig. 8. Three-dimensional constructions of the distribution of carotid body type I (*T*) and periadventitial type I cells (*PT*) from the right and left carotid bifurcation regions of specimens K7 and F5. Common carotid, internal and external carotid arteries (*CC, ICA, ECA*); carotid bifurcation (*Cbn*); occipital and ascending pharyngeal arteries (*OA, AP*). Caudal group of periadventitial cells in F5L are dorsolateral to common carotid artery

proved in common laboratory animals, it would be expected that the migratory patterns of type I cells would not differ greatly in chronological terms from other well-known neural crest derivatives (e.g., adrenal medullary cells, Pearse 1976) and that the majority of type I cells would have arrived in the carotid bifurcation region before birth. While some late migration or even postnatal addition in type I cells cannot be ruled out, it is reasonable to presume that the carotid body will have received a high proportion of its complement of parenchymal cells at the time of birth. It would appear, therefore, that an alteration in size of the carotid body after birth is more likely to be due to internal changes within the organ. The major structural elements which have the capability of altering the morphology of the carotid body lie in the cytological and vascular compartments of the organ, although it must be remembered that the increase in type II synapses on type I cells, which have been reported in the rat in the immediate postnatal period, must have a smaller influence on the size of the carotid body (Kondo 1976).

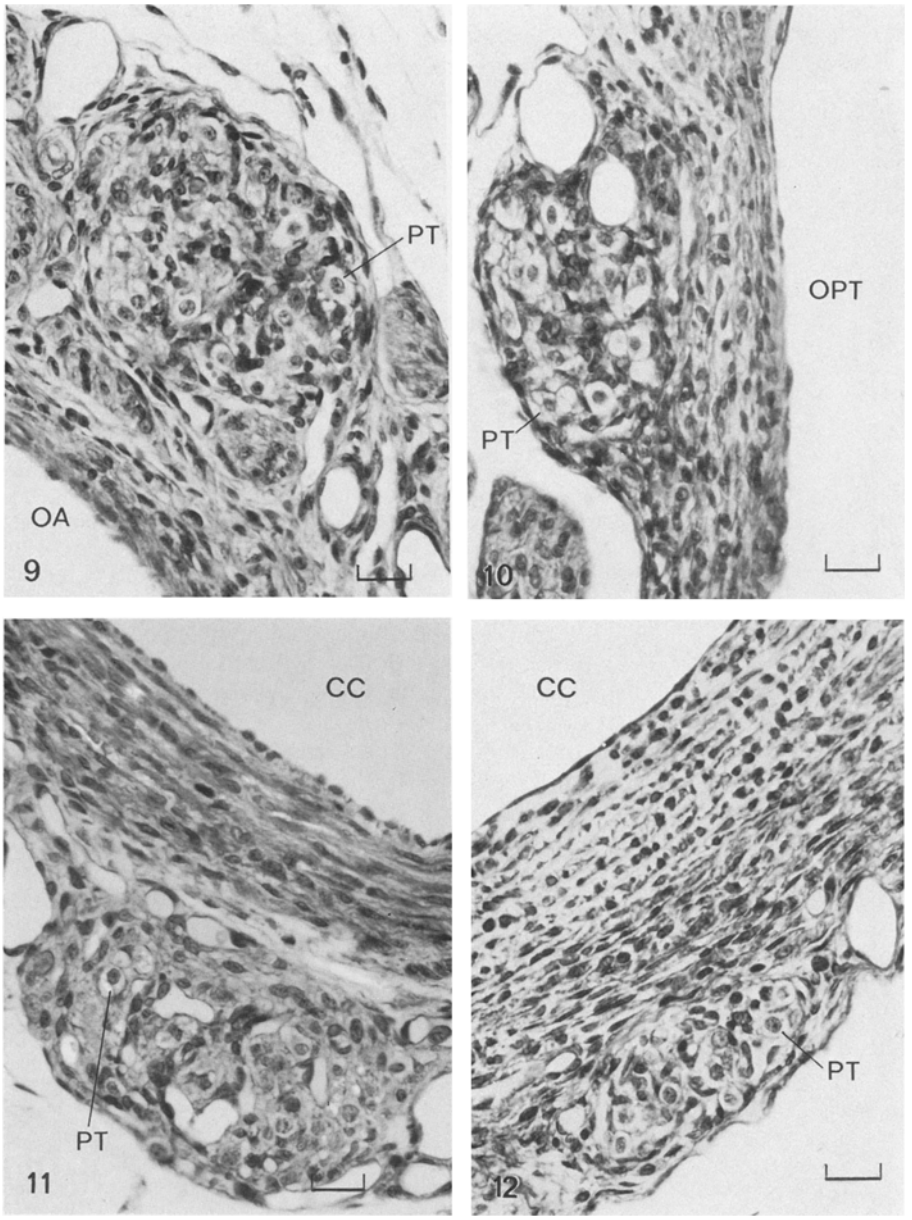
In the cytological compartment the most likely candidates for consideration are the type I and type II cells, since the connective tissue framework of the carotid body is stable and minimal in the cat. If type I and perhaps type II cells are neuroectodermal derivatives from the neural crest, it is unlikely that these cells would undergo extensive mitosis postnatally (Bock 1982). This rationale is borne out in practice, since few mitotic configurations have been reported in the nuclei of type I cells under normal physiological conditions. In this study mitotic figures were conspicuously absent in the nuclei of type I cells in both fetal and kitten carotid bodies. Reports of alterations in the appearance and size of type I and type II cells (Pfeiffer et al. 1984; Smith et al. 1984) have usually been associated with alterations in the physiological melieau of the animal (chronic alveolar hypoxia; systemic hypertension). As far as we are aware type II cells, as seen at light microscopy level, present a fairly constant appearance in the laboratory animal under normal physiological conditions.

It would seem, therefore, that the vascular compartment of the carotid body must contribute a controlling influence on the increased size of the carotid body with age. Such an expansion would inevitably produce a slow increase in the dimensions of the carotid body, which would be quite difficult to detect accurately. Nevertheless, an expansion of the vascular compartment, however gradual, would be reflected in a parallel increase in the total volume of the organ. For technical reasons it is not possible to estimate the volume of the vascular compartment separately using the present interactive image analyser.

Functionally, the carotid bodies of the fetus are active and responsive, while after birth their discharge frequency is diminished by the rise in arterial Po_2 and fall in Pco_2. The range of their sensitivity to changes in arterial Po_2 is reset to that of the adult within a few days of birth (Blanco et al. 1984). The mechanism by which this phenomenon takes place is still unknown. One possible explanation, however, which has a bearing on the present study, is that after birth a change in the distribution of blood flow takes place in the carotid body, which is oxygen sensitive in that it is dependent on the rise of arterial Po_2 (Acker et al. 1980).

Physiological studies in adult animals indicate that the blood flow through the capillary network adjacent to the type I and type II cells can be regulated independently of the total blood flow through the carotid body (Acker and O'Regan 1979), possibly by altering the flow through "arterio-venous anastomoses" (AVA). Alternatively, local changes in the morphology of the arterial wall could exert an influence and alter the blood supply to different groups of type I and type II cells. Such a mechanism could operate at birth to account for the resetting of the carotid bodies to arterial Po_2. On the other hand, there is still some doubt, not only about the existence of such anastomotic channels, but if present, their precise position and morphological nature within the framework of the organ.

Originally, De Castro and Rubio (1968) postulated AVA's outside the carotid of the adult cat. However, most of our material included a considerable portion of cervical



Figs. 9–12. Photomicrographs of periadventitial type I cells (*PT*); common carotid artery (*CC*). Stain M.S.B. Scale = 20 μm

Fig. 9. Specimen F5R. Note periadventitial type I cells ventrolateral to occipital artery (*OA*)

Fig. 10. Specimen F5L. Note periadventitial type I cells lateral to occipito-ascending pharyngeal trunk (*OPT*)

Fig. 11. Specimen F6R. Note periadventitial type I cells dorsomedial to common carotid artery

Fig. 12. Specimen K5R. Note periadventitial type I cells dorsolateral to common carotid artery

tissue surrounding the carotid body and we have been unable to identify AVA's in either the fetal cat or newborn kitten. Within the carotid body the presence of AVA's or straight-through capillaries as found in other tissues (Chambers and Zweifach 1944) have been either described or denied (e.g., McDonald and Haskell 1984; Seidl 1976; Bock 1982). In our specimens no AVA's or vessels with occluding devices in their walls were noted along arterial channels with a diameter greater than 20 μm. In vessels of smaller calibre, it would not be possible to identify with certainty small "shunt vessels" in 5 μm thick sections using light microscopy. In this context, a reconstruction, using a computerised image analysis method of the microvasular bed from 2 μm sections of the central part of the rat carotid body (extending over a rostral-caudal length of approximately 50 μm), did not reveal any cross connecting channels along the route of arterial channels less than 20 μm in diameter (Clarke et al. 1983) or small arterioles with constrictions in their walls. The slight irregularity noted in the luminal diameters of many of the small arterioles could be accounted for by the sinuous nature of their route, which could be traced in the serial sections of the carotid body. Even so it is possible that such an arrangement is to be found in a specific part of the carotid body; e.g., the caudal pole of the organ.

An unusual aspect of the concept of AVA's within the carotid body is the remarkable lack of published photomicrographic evidence of these structures, although diagrammatic representations and some casts are presented. Our interpretation of an AVA conforms with the comparative descriptions of Molyneaux and Bryden (1981) and as these authors point out AVA's often have a definite structure along the anastomotic portion of the vascular complex. This aspect of AVA morphology is frequently neglected in discussions of these vessels in association with the carotid body.

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