OBSERVATIONS ON THE FINE STRUCTURE AND HISTOCHEMISTRY OF THE CAROTID BODY IN THE CAT AND RABBIT

By J. D. LEVER, P. R. LEWIS and J. D. BOYD

Department of Anatomy, University of Cambridge.

The fine structural appearances of carotid body glomus cells, first illustrated by Lever and Boyd (1957), have since been further reported on by Garner & Duncan (1958) and by Hoffman & Birrell (1958). In this earlier description (Lever & Boyd, 1957) attention was directed to the presence within the glomus cells of (0·05–0·15μ) dense membrane-bound granules the population of which varied from cell to cell; and a parallel was drawn with appearances in the adrenal medulla (Lever 1955), the cells of which contain comparable but larger granules. In fact it was postulated that these granules in the carotid body glomus cell might represent the stored form of some humoral agent subserving a local physiological role, possibly as an activator of the Hering's nerve terminals. In the present paper we present evidence which gives further support to this view.

Good evaluations of the earlier writings on the histology and cytology of the carotid body may be found in the works of Hollinshead (1940), De Castro (1951) de Kock (1954) and Adams (1958).

MATERIALS AND METHODS

The rabbit carotid body lies medial to the upper end of the common carotid artery and is closely applied to the adventitia of this vessel; in the cat the relatively larger carotid body is loosely associated with the internal carotid artery immediately above the bifurcation of the common carotid artery.

The general histology and fine structure of the normal carotid body was studied in 6 rabbits and 2 cats, the material being prepared for microscopy as follows. After osmic fixation (see below) tissues were dehydrated through increasing concentrations of ethanol and then methacrylate-embedded. Sections exhibiting silver-gold interference colours were examined by a Siemens electron microscope: thicker (1–2 μ) sections from the same block were examined by phase contrast microscopy in order to identify tissue components. Some of these thicker sections were also attached to slides by albumen coagulation and after removal (in benzene) of the plastic were stained with haematoxylin and eosin.

During most of this investigation carotid bodies were removed under light nembutal or ether anaesthesia supplemented if necessary by local injection of 2 % procaine. Fixation for electron microscopy was by immersion in Dalton's 1 % dichromate osmic solution for two hours. It was later realized that better preservation of certain organelles could be obtained by direct perfusion of fixatives into the common carotid artery of the living animal immediately on the establishment of anaesthesia

by intravenous nembutal and with the minimum of delay or operative interference: and after its subsequent removal, fixation of the carotid body was completed by immersion in Dalton's fluid or other fixative.

Unilateral excision of the superior cervical ganglion and upper sympathetic chain was performed on 6 rabbits and after a week the animals were sacrificed and both carotid bodies removed and prepared for electron microscopy.

The effect of reserpine on carotid body cytology was investigated as follows. Fur clipping and other skin preparation was carried out on two male adult sibling rabbits on the day preceding the experiment. The animals were kept in a quiet place and received $\frac{1}{2}$ mg. largactil/lb. body wt. some 6 hours before operation. One animal then received (intravenously) $\frac{1}{2}$ mg. reserpine in a mixture of ethanol, propylene glycol and water (1:1:2) while the other (control animal) was given an equal volume of this vehicle alone. 30 min. after injection both animals were anaesthetized by a large intravenous dose of nembutal; and rapid carotid perfusion of Dalton's fixative was performed before the carotid bodies were removed.

For the histochemical studies a total of 24 carotid bodies was used, 16 from rabbits, 6 from cats or kittens and 2 from calves. As in the preparation of material for electron microscopy, some rabbit carotid bodies, used in the later stages of the investigation, were fixed by perfusion. Most of this material was fixed as follows: either in 10 % neutral formalin or in a modified Orth's fluid containing 2·25 % $K_2Cr_2O_7$, 0·25 % K_2CrO_4 and 10 % AnalaR formalin which was added immediately before use. A few carotid bodies were fixed specially for particular histochemical tests, and these are noted where relevant in the observations. Most specimens were embedded in paraffin wax, but a few were embedded in Nonex 63B (Miles & Linder, 1952) and some in ester wax (Steedman, 1947). All the histochemical tests used to study the carotid body were also performed on similarly prepared sections of intestine and/or adrenal.

1. *Basophilia.* The staining of sections by dilute solutions of methylene blue was followed over the pH range of 1·8–6·0. The procedure used was essentially that discussed by Singer (1952) except that appropriate mixtures of hydrochloric acid, formic acid and sodium acetate (Lewis, 1959) were used to obtain the desired pH values.

2. *Carbohydrates.* The procedure recommended by Lillie (1954) was used for the periodic acid-Schiff test. The original suphation technique of Kramer and Windrum (1954) and the recent modification of Lewis & Grillo (1959) were also used.

3. *Lipids* were visualized by treating sections with solutions of fat-soluble dyes in propylene glycol according to the procedure of Chiffelle & Putt (1951). Best results were obtained with Sudan Black B and with Fettrot 7B. In addition to the routine formalin-fixed material, use was also made of material fixed in Zenker-formol and post-chromed for two days.

Baker's acid haematin solution was used both on frozen sections as prescribed by him for the detection of phospholipids (Baker, 1946) and on paraffin sections of the post-chromed Zenker-formol material which had been mordanted for an extra hour in dichromate-calcium after taking to water.

4. *Silver reduction* was studied by both the Masson and the Gomori-Burtner silver methods quoted by Lillie (1954) as being suitable for the demonstration of argentaffin

cells. Gold toning and counterstaining were not always carried out and the incubation of some of the carotid body sections was prolonged. The methods developed for the staining of nerve fibres by Bodian (1936) and by Holmes (1947) were also used.

5. *Ferricyanide reduction.* Sections were incubated at room temperature for 15–20 min. in solutions containing 0·5 % ferric chloride and varying amounts of potassium ferricyanide. Molar ratios of ferric to ferricyanide ions between 2:1 and 15:1 were tried as recommended by Lillie and Burtner (1953).

6. *Coupling with diazonium salts.* Sections were incubated at room temperature with a solution of the stable diazotate of 5-chloro-*o*-toluidine (1 mg./ml.) in M/10 tris (hydroxymethyl) aminomethane buffer at a pH of 8·1 for a period of 10–15 min. (intestine) and for up to an hour (carotid body). The diazo-safranin method of Lillie, Burtner & Henson (1953) was also used.

7. *Chromaffin reaction.* Several chromate-containing fixatives were used in attempts to demonstrate this reaction. Their actual composition and mode of use are most appropriately discussed together with the results obtained. Some sections of this chromate-fixed material were mounted unstained, or only lightly stained with haematoxylin. Other sections were stained by the modified Sevki method of Nordenstam & Adams-Ray (1957).

OBSERVATIONS

Histology of rabbit and cat carotid bodies

The rabbit carotid body is lobulated (Pl. 1, figs. 1, 3; Pl. 2, fig. 8), each lobule consisting of parenchymal cells grouped around blood sinusoids and a small amount of fine connective tissue which, for the most part, is perisinusoidal in distribution. Around each lobule is a stroma of connective tissue containing relatively large blood vessels and nerve bundles, but the ultimate distribution of nerve fibres in the lobules is not discernible in haematoxylin and eosin preparations. Many of the small arteries at the periphery of the lobules display 'epithelioid' thickenings of their walls and electron micrographs indicate that in these special areas their endothelium is surrounded by a cuff of somewhat rectangular-shaped elements which are, or resemble, smooth muscle cells. By comparison the cat carotid body exhibits a less noticeable lobulation but is permeated by a similar neuro-vascular stroma which is freely admixed with parenchymal elements: these are grouped around blood sinusoids as in the rabbit.

The parenchymal glomus cells of the rabbit carotid body (Pl. 1, fig. 1) show a light-dark variation in cytoplasmic staining (with haematoxylin and eosin): the darker cells in general were smaller than the lighter ones. The cytoplasm of the latter cells is often 'foamy' or vacuolated in appearance (Pl. 1, fig. 1). While this light-dark cell variation in the parenchymal cells has been observed with regularity in the normal rabbit carotid body it is not such an obvious feature in the cat. The exact relationships of the parenchymal cells to the blood vessels in the carotid body of each species are obscure by light microscopy. In some situations the glomus cells are directly opposed to the walls of the sinusoids (Pl. 2, fig. 6; Pl. 1, fig. 1): in other regions, however, these walls appear thickened by an additional cell with an elongated

nucleus (Pl. 1, fig. 1 *b*). Whether the cells of this last type are part of the vascular endothelium or constitute adventitial elements lying outside this layer, cannot be determined with certainty by light microscopy.

Electron microscopy of normal rabbit and cat carotid body glomus cells

There is a wide range of appearances of the normal glomus cells in both cat and rabbit but particularly in the latter: perhaps this has its counterpart in the light-dark staining variation observed in these cells by light microscopy (Pl. 6, fig. 15).

Some glomus cells (Pl. 3, fig. 9; Pl. 4, fig. 10; Pl. 6, fig. 15) contain a relatively electron-opaque background cytoplasm in which are compact mitochondria, some of the small (± 150 Å) particulates probably indicative of ribonucleic acid (Palade 1955) and numbers of larger osmiophile granular bodies to which more attention will be given later.

In contrast are cells (Pl. 6, fig. 15) of much lower general electron opacity which contain vacuolated distended mitochondria and only a small number of these osmiophile granular bodies. There are a number of cell forms intermediate between these two extremes which are generally characterized by some degree of mitochondrial disorganization and a moderate to scant granule content. We also observed a range of cells with a very variable granule content but in which the mitochondria are normal and compact. This category extends from a virtually agranular cell with a low density cytoplasm to one with a high general density and granule content (Pl. 5, fig. 14; Pl. 6, fig. 15).

These various cell forms may be an expression of different phases in a cyclical cell process perhaps of a secretory nature, a suggestion which receives further attention in the Discussion.

The endoplasmic reticulum (Palade & Porter, 1954) of the glomus cell is usually simple in form. It consists of small rough-walled sacs and tubules. However in the cells containing compact mitochondria and few or no osmiophile granules some of the endoplasmic reticulum is arranged in bilaminar sheets (Pl. 4, fig. 11). In the cat glomus cell the outer lamina of the nuclear membrane is often observed to sacculate towards the cytoplasm, the outer wall of these nuclear blebs bearing numbers of cytoplasmic Palade (1955) granules. As in the parathyroid (Lever, 1957, 1958), in which similar appearances have been reported, the nuclear blebs resemble the larger saccular elements of the endoplasmic reticulum. Compact and seemingly discrete paranuclear collections of Golgi membranes are a normal feature of both the light and dark glomus cells.

A constant finding in all carotid bodies examined has been the presence of the osmiophile granular elements previously referred to (Pl. 3, fig. 9; Pl. 4, figs. 10, 12; Pl. 5, fig. 14); these are quite distinct from the much smaller ribonucleic acid particulates of Palade (1955). As already stated, the population of these granules per unit area of section is variable from one glomus cell to another (Pl. 6, fig. 15) but in general is scant in cells of low electron density. Variations in electron density within individual granules and from granule to granule are common (Pl. 4, fig. 12). Very often granules are seen to be surrounded by a continuous thin (100 Å) line; an appearance suggestive of a membranous investment (Pl. 4, fig. 12; Pl. 3, fig. 9). It has already been suggested (Boyd, Lever & Griffiths, 1959) that these membranes are golgiform.

Granules of comparable electron microscopic appearance have already been described in the rat adrenal medulla (Lever, 1955); but whereas the adreno-medullary granules range in size from 0·05–0·4 μ, those in the carotid bodies examined in the present series are between 0·05 and 0·15 μ in size. The carotid body granules are readily distinguishable from such larger droplets (Pl. 3, fig. 9; Pl. 6, fig. 15), which have a sparse and apparently random distribution in the glomus cell and are very likely of a lipide nature.

The most striking cytological differences between the carotid glomus tissue of the rabbit and cat are: (1) the greater preponderance in the latter of agranular (or seemingly agranular) glomus cells; (2) the darker, more electron dense cell with a heavy content of granules is more commonly observed in the rabbit carotid body; (3) the blebbing of the outer lamina of the nuclear membrane commonly observed in the cat glomus cell has only infrequently been found in the rabbit.

The sinusoids and perisinusoidal spaces

The endothelium of the sinusoids conforms to Palade's (1953) description of the fine structure of capillary endothelium in many other tissues. Imbrication or overlap of contiguous endothelial cells is a frequent appearance as are areas of extreme endothelial thinness in the sinusoid walls, and inward projections of endothelium in the form of 'baffles' are commonly seen (Garner & Duncan, 1958). A well marked space surrounds the sinusoids (Pl. 4, fig. 10; Pl. 5, fig. 14). In general the glomus cells are separated from the endothelium of the sinusoids by this perisinusoidal space containing collagen (Pl. 4, fig. 10) and lined on both sides by a basement membrane applied to the contiguous walls of glomus and endothelial cells. In such perisinusoidal spaces there may be found myelinated and unmyelinated nerves (Pl. 5, fig. 13; Pl. 4, fig. 10), numerous collagen bundles and fibroblasts, in addition to cells of questionable identity which are seen to be in close apposition to the outer endothelial surface (Pl. 5, fig. 14). Although occurring in both cat and rabbit carotid bodies such cells (pericytes) have been more often observed in the latter. The pericytes have an elongated nucleus, a relatively lucid cytoplasm containing few mitochondria and an inconspicuous form of endoplasmic reticulum. They possess none of the osmiophile granules associated with the typical glomus cell. These pericytes differ further from the typical glomus cell in that they are more closely related to the vascular endothelium, being in contact with the endothelial basement membrane (Pl. 5, fig. 14); but sending out cytoplasmic processes between the glomus cells to an unknown varying extent. Collagen fibres are present not only in the perisinusoidal space but are also found to a variable extent between the glomus cells, which may thus be separated by collagen fibres, fine cytoplasmic processes from the pericyte cells and at times by nerve bundles: but very often their plasma membranes are opposed across a narrow intercellular interval containing no defined structure. Dovetailing and interdigitation between cytoplasmic processes of adjacent glomus cells is commonly seen.

Nerve fibres in the carotid body. Bundles of myelinated and unmyelinated axons are frequently seen in perisinusoidal and intercellular spaces (Pl. 3, fig. 9; Pl. 4, fig. 10; Pl. 5, fig. 13). There is no evidence of nerve terminations in relation to vessel walls or pericytes but in a number of instances direct apposition of axonal and glomus cell

plasma membranes have been observed across a narrow (300 Å) interval (Pl. 3, fig.9). At or near such situations the axoplasm may contain a concentration of mitochondria and numbers of microvesicles, features which are now generally regarded as indicative of nerve terminations.

The rabbit carotid body after sympathectomy. No changes were observed in the parenchymal elements of the carotid body nor in the nerve endings upon the glomus cells following sympathectomy.

The rabbit glomus cells after reserpine administration. Comparison of the fine structure of these cells in the normal control animal with appearances in the identically treated animal which had received reserpine (Pl. 6, figs. 15, 16) strongly suggests that this drug results in a rapid depletion of the specific granular bodies from the glomus cells. It may be significant to recall that Dontas (1957) has demonstrated a marked chemoreceptive stimulation of the carotid body mechanism following reserpine administration.

Histochemical observations

None of the structures in the cat or rabbit carotid body shows marked basophilia. At pH values between 5 and 6 the cytoplasm of many of the glomus cells stains faintly with methylene blue, but this rapidly decreases as the staining pH is reduced below 5. In one carotid body examined, from a cat which was older than any of the other animals studied, there were occasional cells containing granules which stained metachromatically even at the most acid pH used: these were probably mast cells. There are many other reports of mast cells occurring in the perilobular connective tissue of the carotid body (*see* Adams, 1958). With the periodic acid-Schiff technique many of the connective tissue elements were well stained, but the glomus cells remained unstained. Metachromatic staining after sulphation shows up the glomus cells in high contrast—as completely clear areas in the midst of the densely staining connective tissue. Since this reaction is an extremely sensitive test for polysaccharides such substances must be virtually absent from the cytoplasm of the glomus cells.

It is clear from their electron-microscopic appearance (Pl. 3, fig. 9; Pl. 6, fig. 15) that the glomus cells contain appreciable amounts of lipid. Relatively little of this lipid appears to survive paraffin embedding after the ordinary formalin fixation. However, in the post-chromed, Zenker-formol fixed material, most of the glomus cells do show obvious staining both with Sudan Black B and with Fettrot 7B (Pl. 2, fig. 5). Treatment with acetone very rapidly removes all this coloration which must therefore be due to physical staining of lipids. Though their sudanophilia was much less than that of cells in many other tissues, such as the intestine and adrenal cortex, it was sufficient to show up quite clearly the cords of glomus cells against the virtually unstained background of the other carotid body tissue components except, of course, the myelin sheaths of nerve fibres which were intensely stained. There was some variation in the sudanophilia between the individual glomus cells in a single section, and under high power this lipid staining often gave a granular or particulate appearance to the cytoplasm. With post-chromed material (both the Zenker-formol paraffin sections and the formol-calcium frozen sections), treatment with the acid haematin solution gave some staining of the glomus cells which was, however, not detectable

after differentiation in borax-ferricyanide for the 18 hours prescribed by Baker (1946) in his method for the identification of phospholipids. After such differentiation, in fact, myelin was the only lipid component which remained stained.

Phenolic amines in the glomus cell cytoplasm. The osmiophile granules in the cytoplasm of the glomus cells have an electron-microscopic appearance reminiscent of two other types of cytoplasmic granules whose chemical identity is fairly firmly established: (*a*) those of the adrenal medulla (Lever, 1955) known to contain catechol amines or their precursors (Blaschko, Hagen & Welsh, 1955) and (*b*) those of the argentaffin cells of the intestine (Christie, 1955) thought to contain 5-hydroxytryptamine or some closely related substance (Erspamer, 1954). An attempt was therefore made by histochemical methods to discover if any of these or similar substances were present in the glomus cells of the carotid body.

Phenolic amines, such as adrenaline, noradrenaline and 5-hydroxytryptamine, are reducing substances and should give a positive reaction with a number of histochemical tests. Thus they should reduce ferricyanide to give a positive Schmorl reaction and silver ions to give a deposit of metallic silver. Adrenal medullary cells and certain cells in the intestinal mucosa give both these reactions under the appropriate conditions and can thereby be rendered clearly visible in tissue sections. In material fixed by immersion in formalin the glomus cells gave only a faintly positive Schmorl reaction, but after perfusion and fixation with chromate the cords of glomus cells showed up quite clearly amongst the much more faintly stained connective tissue (Pl. 2, fig. 7). With the Masson and the Gomori-Burtner silver methods only faint staining of the glomus cells was obtained in a period sufficient to produce marked blackening of cells in the adrenal and in the intestine. However, with the Bodian technique, the glomus cells were often intensely stained (Pl. 2, fig. 4).

Another histochemical test with a high specificity for phenolic compounds is the reaction with diazonium salts at an alkaline pH to give brightly coloured azo dyes. With the diazonium salt of 5-chloro-*o*-toluidine, argentaffin cells in the intestine rapidly acquire a fiery orange colour which is rather less intense after chromate fixation than after neutral formalin: under the same conditions adrenal medullary cells stain a deep brownish-orange after chromate fixation but only a much fainter yellowish-orange after neutral formalin, and glomus cells of the carotid body stain a definite orange-yellow after chromate but only a very faint yellow after formalin. Although the staining of the glomus cells was quite distinct (Pl. 1, figs. 2, 3) it was considerably less intense than that of adrenal medullary cells fixed under the same conditions and was more yellow in colour.

A very sensitive, though somewhat unspecific, test for phenolic amines is the production of yellow or brown pigment by chromate-containing fixatives—the classic chromaffin reaction. With this test the glomus cells give a definite yellow or brownish-yellow coloration which is, however, much fainter than in adrenal medullary cells and lacks visible granularity. Several fixation procedures were tried, both with carotid body and with adrenal. The buffered chromate-dichromate mixture recommended by Hillarp & Hokfelt (1955) gave good pigment formation but poor preservation of cytological detail. The fixative finally chosen, that given in *Material and Methods*, was a modified buffered Orth's fluid and its use resulted both in good pigment formation and cytological preservation. In carotid bodies perfused with

this fixative, staining of the glomus cell cytoplasm is marked and is particularly obvious when sections are viewed or photographed with blue light (Pl. 2, fig. 8). With the modified Sevki method of Nordenstam & Adams-Ray (1957) Giemsa gives good differential staining of the glomus cells (Pl. 2, fig. 6). The mechanism of this staining is not known but it appears to be related to the presence of catechol amines since the staining is much less in adrenals from reserpine-treated animals (Lever & Lewis, 1959).

DISCUSSION

The results of the various histochemical tests strongly suggest the presence within the glomus cell cytoplasm of a phenolic amine or some closely related chemical substance. Admittedly, some of the most positive indications were obtained with tests which probably have only a poor specificity for phenolic amines, but this is to be expected from the known sensitivities of the various tests used. In fact, the relative order of staining intensities obtained with these tests was strikingly similar in the glomus cells of the carotid body and in the medullary cells of the adrenal. The absolute intensity of staining, however, was much less in the carotid body than in the adrenal; it is obviously dangerous to make a quantitative estimate from purely visual comparisons, but the staining of the glomus cells appeared to be less by a factor of at least ten. Because of this faint staining of the glomus cells it is therefore not possible to identify the phenolic amine present with certainty by existing histochemical methods. The available evidence, however, appears to be more in favour of its being adrenaline or noradrenaline rather than 5-hydroxytryptamine.

However likely may be the relationship of this histochemical staining in the glomus cells to the presence of granular bodies (as observed in electron micrographs) it cannot be directly proved, since owing to their very small size—none being larger than 0·15 μ—these bodies are not discretely observed in the light microscope, a fact which would account for the general coloration and absence of 'granularity' in both the chromaffin and diazonium staining. It is also not certain whether these osmiophile granules are identical with the fuchsinophilic and siderophilic granules observed by Hollinshead (1943), and Ross (1957). It is possible that ordinary histological processing may cause the small osmiophile granules to swell or to aggregate sufficiently to become observable with the light microscope.

The cytological changes observed in the glomus cells after reserpine treatment might also be regarded as evidence of a 'chromaffin' nature for their inclusion granules. As in adreno-medullary cells (Lever & Lewis, unpublished observations) so also in the carotid body glomus cells, this drug produces a striking disappearance of inclusion granules. This is not to imply that these cell types are necessarily analogous but merely to stress that both give a chromaffin reaction and show a similar response to reserpine. Indeed it is not clear that the carotid body glomus cells should properly be called a *chromaffin tissue*, in the usually accepted sense of the phrase, since following sympathectomy no changes were observed in their cytology or in the nerve terminals upon their surfaces. From this one can infer that the glomus cells do not have a sympathetic innervation and should thus not be classed with the adrenal medulla and paraganglia as typical chromaffin tissues.

As a result of these observations we are unable to agree with Garner & Duncan (1958) that the glomus cell granules are an index of faulty fixation. On the contrary we consider they are a normal cell inclusion and one which is very likely concerned in the specific function of the glomus tissue. We hold this view for the following reasons: (i) These granules have been consistently observed under very favourable conditions of preparation. In fact there are always many more of them present in the glomus cells of carotid bodies which are vitally perfused with the osmic fixative before removal than in those (carotid bodies) which are removed after a lengthy operative procedure and secondarily fixed. (ii) In the Golgi region and elsewhere in the cytoplasm the glomus cell granules are frequently found to be individually enclosed by a delicate smooth-walled investing membrane and thus present an appearance similar to that of the secretory granules in the adrenal medulla (Lever, 1955), the endocrine pancreas (Lacy, 1957) and the adenohypophysis (Farquhar & Wellings, 1957). (iii) There is a variation in the cytoplasmic concentration of these granules from one glomus cell to another, a fact which might be interpreted as an indication of different functional levels between these cells. Such heterogeneity is common within secretory tissues and has been described in the parathyroid (Lever, 1958) and the adrenal medulla (Lever, 1959, in press). (iv) Following reserpine injection the glomus cell granules are greatly reduced in number as compared with the untreated normal control.

It is not clear what is the nature of the pericytes which are situated in the perisinusoidal spaces and which send cytoplasmic processes to an undetermined extent between the glomus cells. They probably correspond to the type II parenchymal cells described by de Kock (1954) and in their cellular-vascular relationship at any rate they resemble the 'stellate' cells observed by Rhinehart & Farquhar (1955) in the adenohypophysis. These pericytes have a cytoplasm of very 'watery' appearance (Pl. 5, fig. 14) and do not resemble glomus cells, muscle cells, fibroblasts, or neurones. Electron-micrographic comparison between a typical Schwann cell (Pl. 5, fig. 13) and a pericyte (Pl. 5, fig. 14), both within a perisinusoidal space, does not suggest an identity between them, but this must remain an open question. From their position the pericytes might conceivably perform a conductive role from sinusoid to glomus cell.

Without a clearer conception of the significance of these pericytes it would certainly be unwise to make any comprehensive speculations on the cellular mechanism underlying carotid body activity, but we believe that the results of our investigations do point to a humoral or local endocrine role for the carotid body glomus cell, a concept formerly presented by Moulon (1904). Conceivably the membrane-bound cytoplasmic granules in the glomus cell represent 'packets' of a transmittor substance —a phenolic amine, which might be responsible for initiating nerve impulses in the Hering's nerve terminals which are closely applied to the glomus cell walls and have not been found in relation to blood vessel walls or to the pericytes. We consider that the contents of these membrane-bound granules are released from the glomus cell in response to reserpine administration, during operative handling of the carotid arteries, and also in anoxia and hypercapnia, both of which states are certainly encountered during the initial stages of inhalation anaesthesia. Until we appreciated these last points our histochemical and electron microscopic findings were extremely

variable. Consistent results were obtained in the normal living rabbit carotid body only if this organ was perfused with the fixative after cannulation of the common carotid artery with the minimum delay and operative disturbance immediately on effecting anaesthesia by intravenous nembutal. Unless this procedure was followed the results of these staining techniques were inconsistent, a fact which may well account for the differences in opinion on the occurrence or otherwise of a true chromaffin reaction in the carotid body.

Something should perhaps be said about the possible role of acetylcholine in the initiation of chemoreceptor impulses—a topic which has been very adequately reviewed by Heymans & Neil (1958). Intracarotid injection of acetylcholine will initiate chemoreceptor impulses in Hering's nerve and anticholinesterases will potentiate this effect. A number of ganglioplegic drugs will abolish this response to acetylcholine (and that to such alkaloids as lobeline) without, however, blocking the normal chemoreceptive response to anoxia: so it seems unlikely that acetylcholine can be directly concerned in the transmission of the normal chemoreceptor response. It is quite possible that the glomus cells and/or the afferent terminals of Hering's nerve are, incidentally, responsive to acetylcholine, as are many other sensory structures, and that the cholinesterases known to be present in the carotid body (Hollinshead & Sawyer, 1945; Koelle, 1950) are present to guard against the dangers of this responsiveness. Such pharmacological studies do not exclude the possible role of an adrenaline-like substance in the initiation of the normal chemoreceptor response; and the observation by Dontas (1957) that intracarotid injection of small doses of reserpine cause a prolonged burst of chemoreceptive impulses in Hering's nerve would appear to strengthen this view.

Finally, a recent observation of Comline & Silver (1958) is perhaps relevant. They found that the *denervated* adrenal of the foetal sheep secretes noradrenaline in response to anoxia. This direct anoxic response of at least some medullary cells is lost late in foetal life. If the glomus cells of the carotid body retained this property, however, and if the liberated noradrenaline stimulated the neighbouring sensory nerve endings, an explanation would be provided for much of the known physiology of the chemoreceptor mechanism.

SUMMARY

1. It was possible to demonstrate positive staining of glomus cell cytoplasm by the chromaffin, Giemsa's ferric-ferricyanide and diazonium reactions, especially after fixation by intravital perfusion. These histochemical results suggest the presence of phenolic amines in carotid body glomus cells.

2. Parallel electron-microscopic investigation has demonstrated the presence of 0·05–0·15 μ membrane-bound granular bodies in the glomus cell cytoplasm of both rabbit and cat carotid bodies.

3. The population density of these granular bodies varies between glomus cells, and this variation and that of certain other cell features may be indicative of differing states of secretory activity within the glomus.

4. Reserpine administration results in a general depletion of these granular bodies throughout the glomus, a finding in parallel with the reserpine depletion of the 'adrenaline' bodies from the adrenal medulla.

5. Nerve endings have been seen in relation to glomus cells but terminals have not been found on sinusoid walls or in relation to the pericytes which in places separate the vascular endothelium from glomus cells and send processes to an unknown extent between glomus cells.

6. Sympathectomy produces no changes in the glomus cells or the nerve terminals associated with them.

7. These findings are discussed and a local humoral role is tentatively suggested for the glomus cells: possibly the electron-dense membrane-bound granular bodies contained by them represent (as in the adrenal medulla) a stored form of some catechol amine which on release in response to various stimuli (reserpine, anoxia, etc.) may stimulate adjacent Hering's nerve terminals.

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EXPLANATION OF PLATES

PLATE 1

Fig. 1. Rabbit carotid body (c.b.) lobule. A light-dark cytoplasmic staining variation is observed between glomus cells: some light cells show a 'foamy' cytoplasm (*a*). Occasional spindle shaped cells (*b*) are related to sinusoid walls. Osmic-fixed, methacrylate-embedded specimen stained with haematoxylin and eosin after removal of plastic. × 1500.

Figs. 2, 3. Rabbit carotid body, chromate-fixed 10 μ section treated with diazonium salt, no nuclear stain, photographed with blue light. Note cytoplasmic staining of glomus cells. Fig. 2 × 280; Fig. 3 × 1040.

PLATE 2

Fig. 4. Rabbit carotid body, formalin fixed, stained by modified Bodian silver proteinate technique to show the cytoplasmic argyrophilia of the glomus cells, nerve fibres and connective tissue unstained. × 1600.

Fig. 5. Cat carotid body, Zenker-formol fixed and postchromed material, 3 μ section stained with Sudan Black B in propylene glycol to show the sudanophilia of the glomus cell cytoplasm. × 1040

Fig. 6. Rabbit carotid body, formol-chromate fixed, 10 μ section stained by the modified Sevki technique. Erythrocytes (*R*) are stained pink and glomus cells (*G*) purple. × 1040.

Fig. 7. Rabbit carotid body, formol-dichromate fixed, stained by the ferric-ferricyanide technique. Note staining of the two groups of glomus cells (*G*). × 1040.

Fig. 8. Rabbit carotid body, formol-chromate fixed, nuclei lightly stained with haematoxylin, photographed with blue light. Note the faint but definite cytoplasmic 'chromaffin' staining of glomus cells (*G*). × 780.

PLATE 3

Fig. 9. Rabbit carotid body—electron micrograph of a group of glomus cells surrounded by collagen fibres (*c*) and fibroblasts (*F*). Note: Schwann cell (*W*) containing an unmyelinated axon (*a*), nerve endings on glomus cells (*n*), lipid bodies (*L*) and small membrane-bound granular bodies (*g*) in the glomus cell cytoplasm. × 15000.

PLATE 4

Fig. 10. Note general tissue arrangements in this electron micrograph of rabbit carotid body: sinusoid, *S*; glomus cells, *G*; collagen, *C*; nerve fibres, *n*; endothelial cells, *e*; pericyte, *P*; Schwann cells, *W*. × 3000.

Figs. 11, 12. Electron micrographs of glomus cells. Note membrane-bound cytoplasmic granular bodies (*g*) and vacuolated mitochondria (*M*) in fig. 12. Figure 11 contains numerous cytoplasmic sacs (*s*) but no obvious granules similar to *g* in fig. 12. Granular endoplasmic reticulum (*ER*) is particularly obvious in fig. 11. Both at × 40000.

PLATE 5

Both figures are electron micrographs depicting typical contents of the perivascular space in the rabbit carotid body.

Fig. 13. Note unmyelinated axon: *A* suspended by mesaxon (*m*) from surface of Schwann cell (*W*); collagen, *C*; sinusoid endothelium, *E*. × 27500

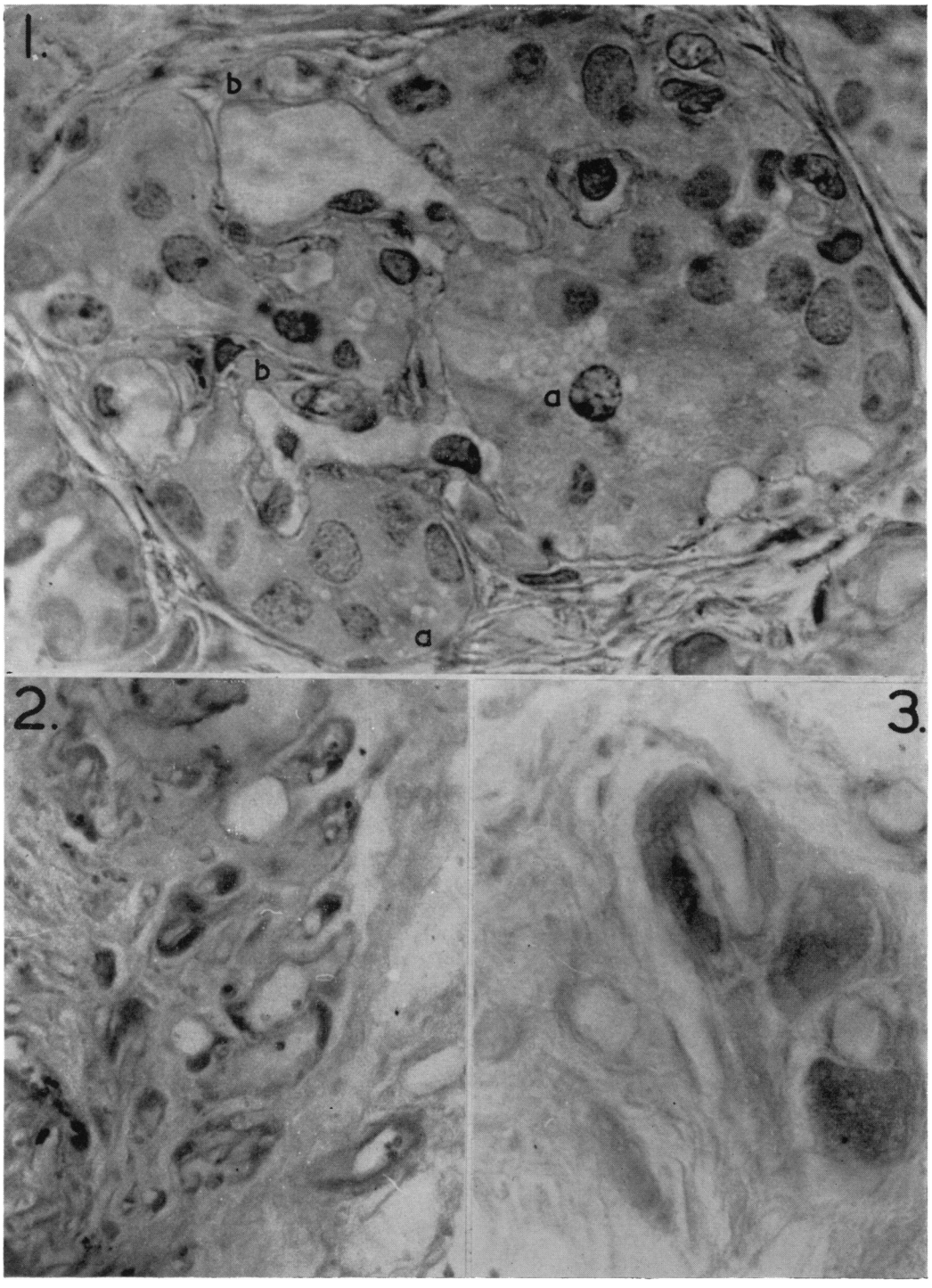
Fig. 14. Note glomus cell (*G*) with contained membrane-bound granular bodies: collagen, *C*; a pericyte of questionable nature, *P*; sinusoid endothelium, *E*. × 15000.

PLATE 6

Two sibling rabbits were prepared by basal narcosis for operation on the following day. One was given reserpine 0·5 mg. (in suitable vehicle) intravenously (fig. 16) while to the other (fig. 15) animal the vehicle only was administered (by i.v.i.).

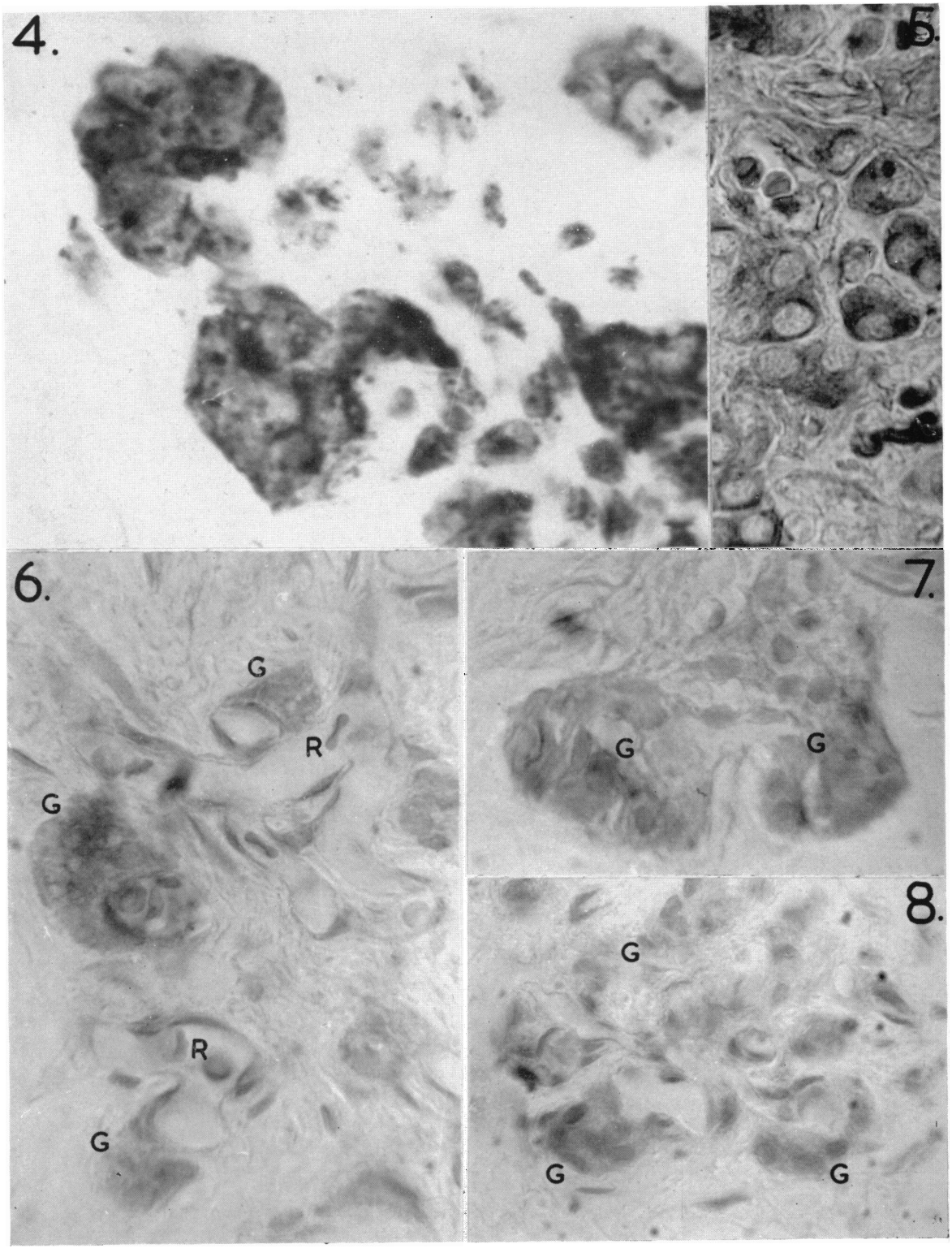
Fig. 15. Shows the normal cytological variation of the glomus cells. Some, more electron dense, cells (G_1) contain numbers of osmiophile granular bodies (*g*) as already depicted in figs. 9, 12, 14 while other glomus cells (G_2) contain fewer granular bodies: in further glomus cells (G_3) also containing few granular bodies many of the mitochondria are vacuolated. × 14000.

Fig. 16. After reserpine the normal cytological variations as seen in fig. 15 are not so apparent and most of the glomus cells contain few or no granular bodies. × 14000.

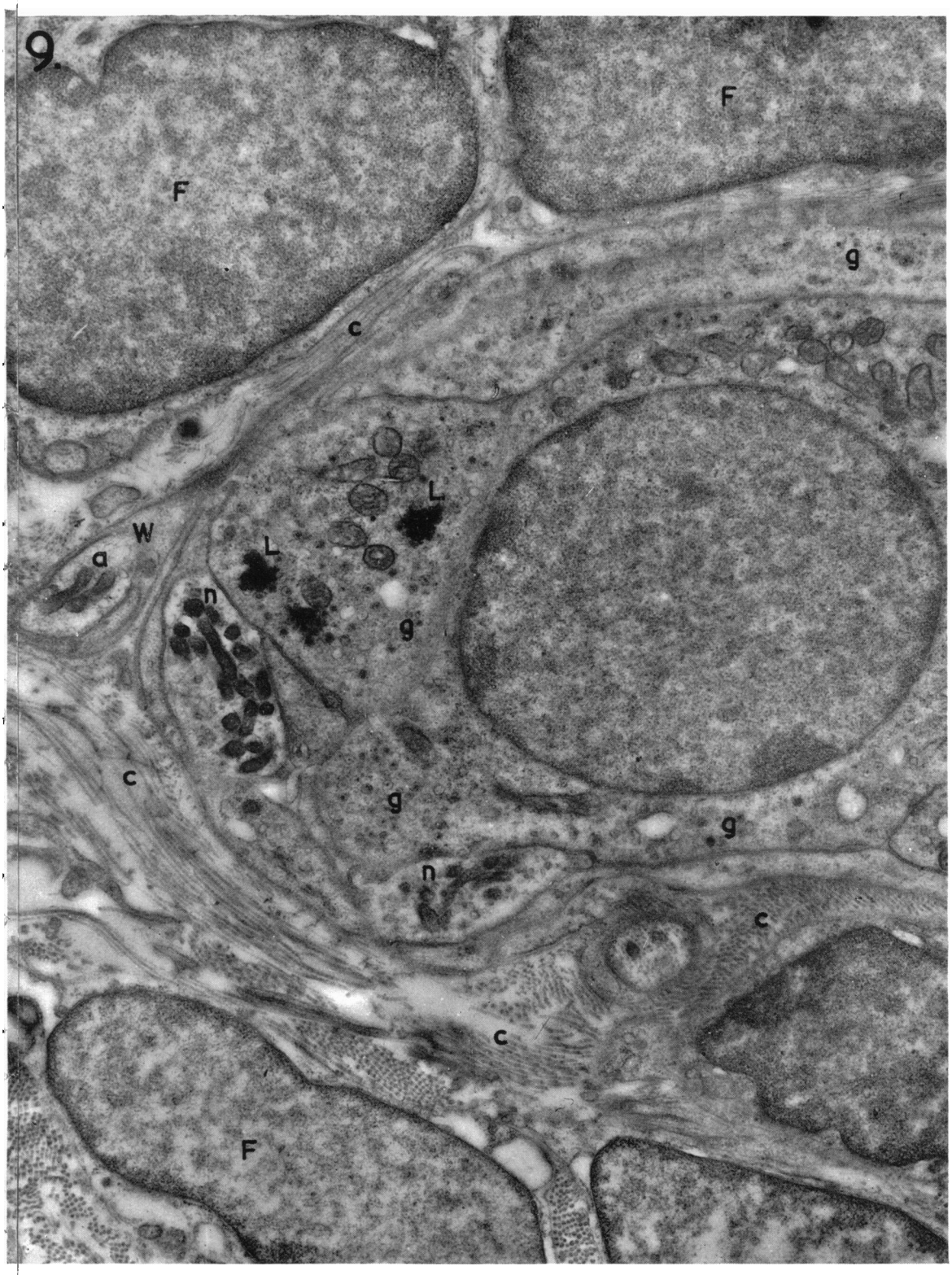


LEVER, LEWIS AND BOYD—FINE STRUCTURE AND HISTOCHEMISTRY OF CAROTID BODY

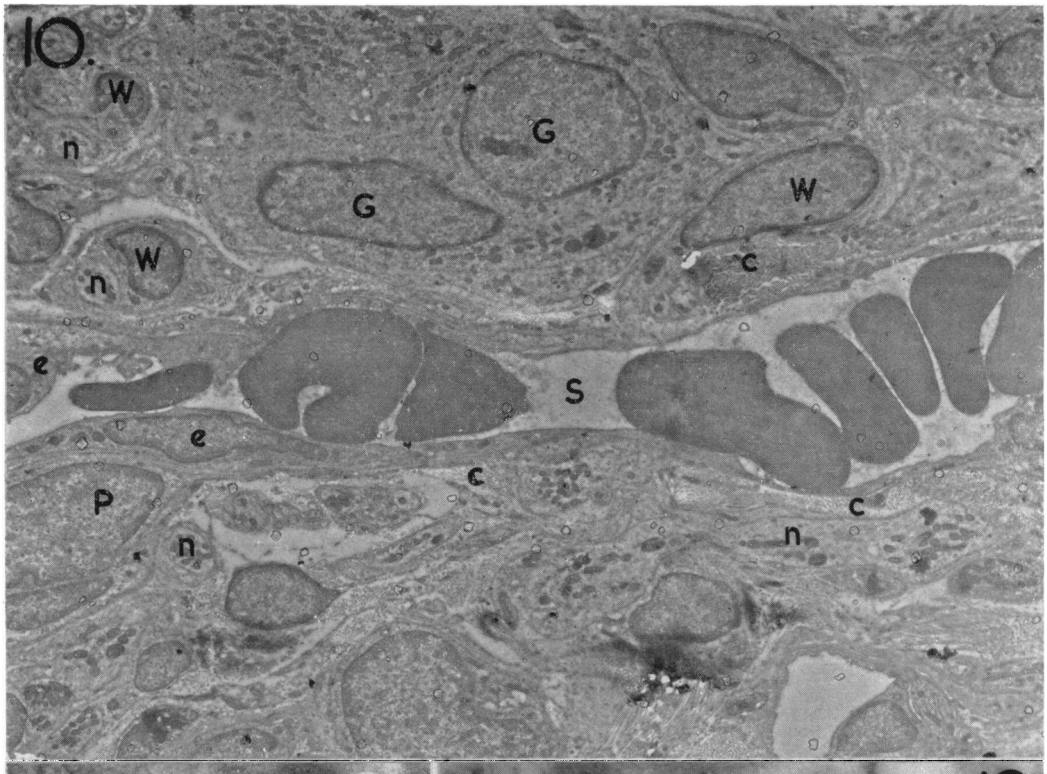
(*Facing p.* 490)



LEVER, LEWIS AND BOYD—FINE STRUCTURE AND HISTOCHEMISTRY OF CAROTID BODY



LEVER, LEWIS AND BOYD—FINE STRUCTURE AND HISTOCHEMISTRY OF CAROTID BODY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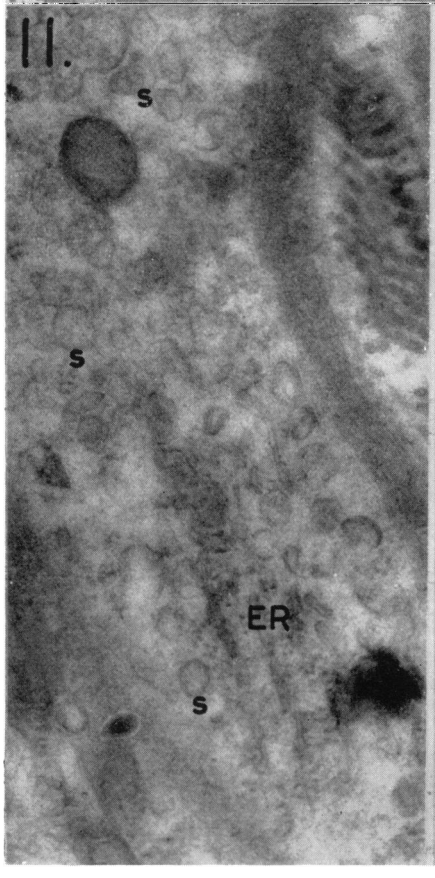
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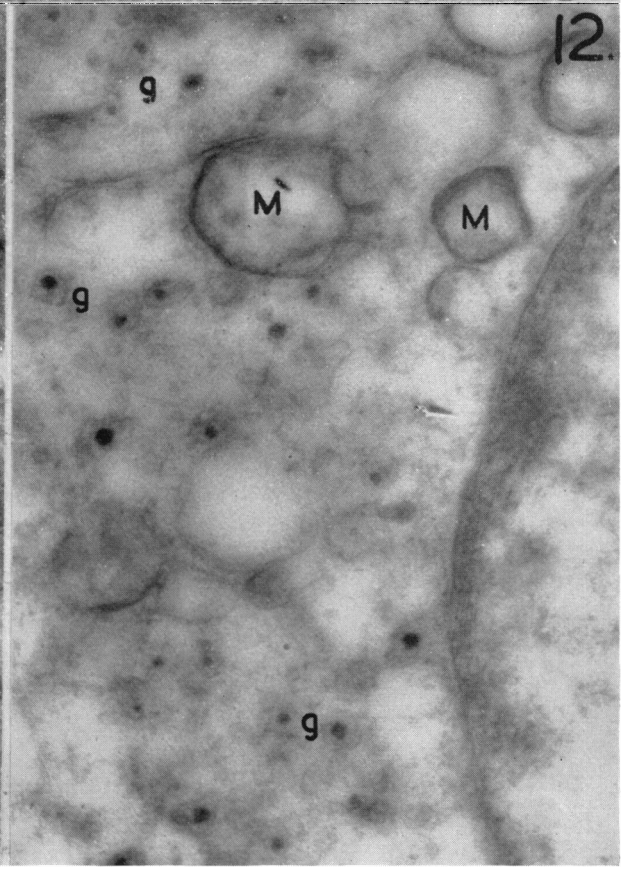
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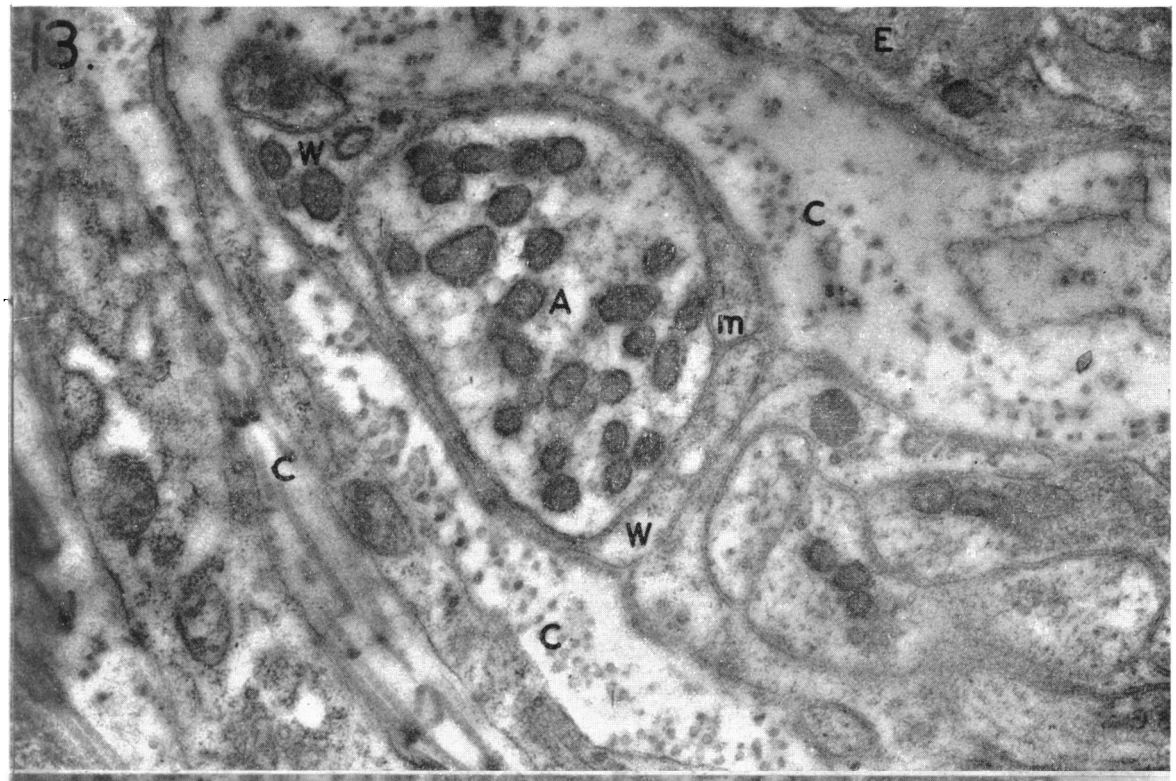
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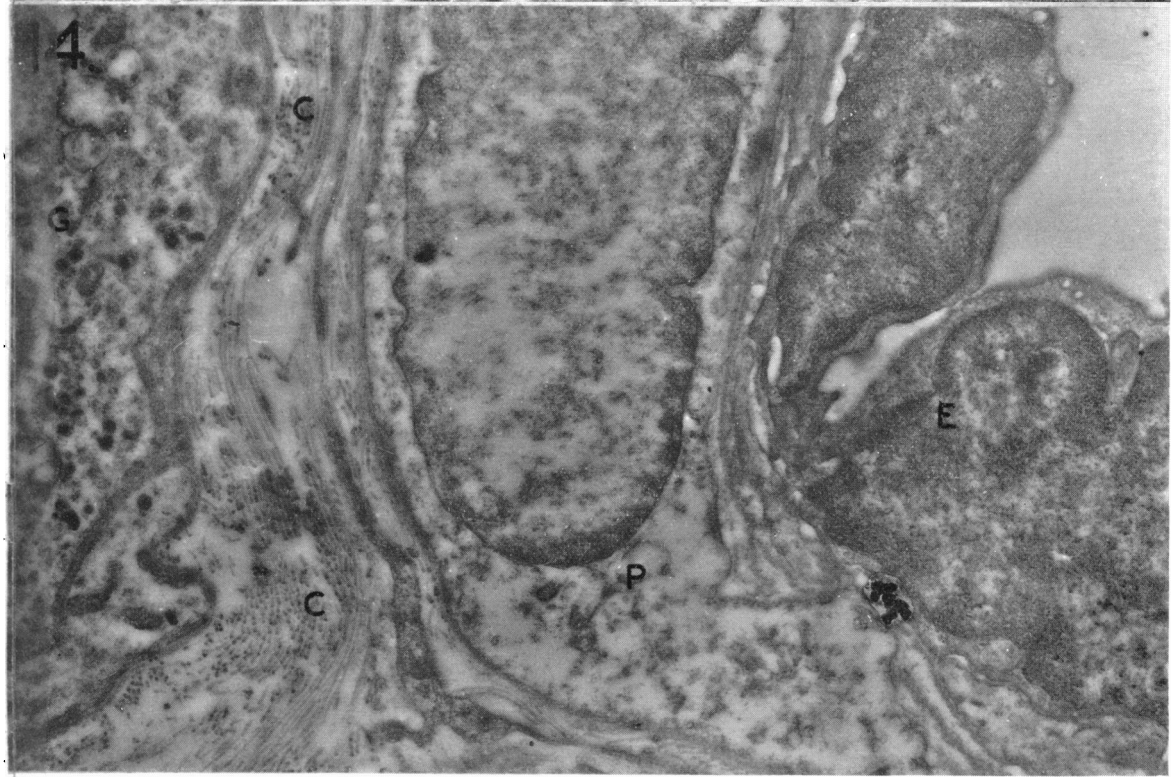
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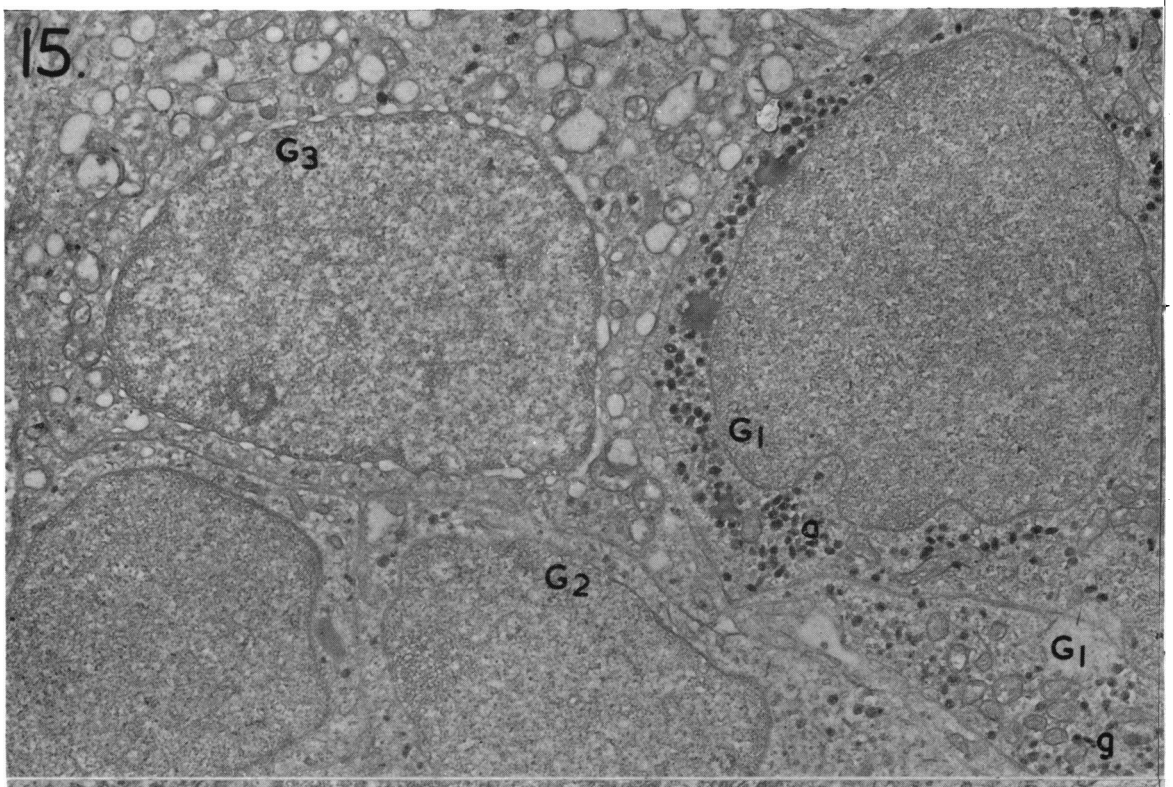
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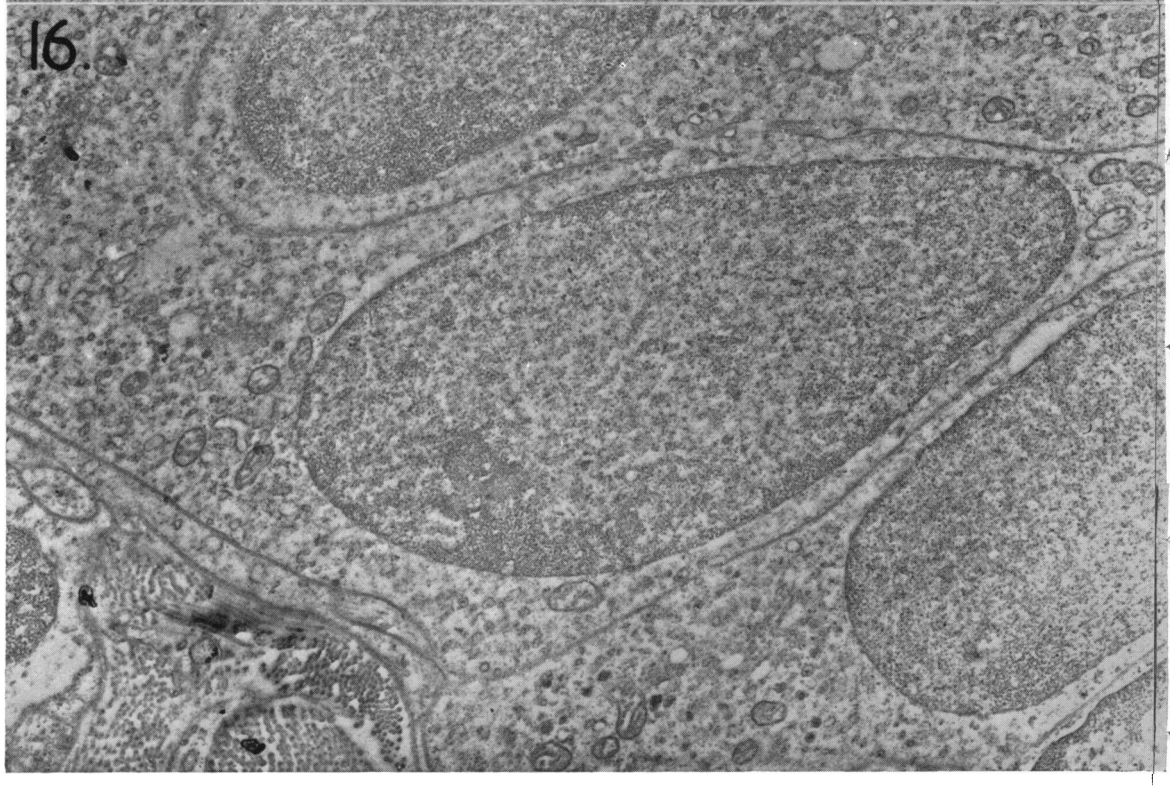
16.