A cytological and cytochemical study of the 'epithelial body' on the carotid artery of the lizards, *Trachysaurus rugosus* and *Tiliqua occipitalis*

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With 4 plates (figs. 1 to 4)

Summary

Epithelial body III of *Tiliqua occipitalis* and *Trachysaurus rugosus* has been the subject of a histological and cytochemical investigation with the object of making a comparison with the mammalian parathyroid gland. The cytochemical reactions of the reptilian chief cell are similar to those of the mammalian 'pale principal' cell. On the basis of a series of cytological and cytochemical changes a secretory cycle commencing with the chief cell and culminating in the water-clear cell is postulated. Two further cell types distinguished as the dark cell and the 'epithelial' cell have been described in the reptilian epithelial body. The significance of these two cell types is discussed. There is some evidence suggesting that true sinusoids invest the cellular cords. There is no evidence, with the technique used, that the gland cells are innervated. It is suggested that the general histology is comparable to that of the mammalian parathyroid gland.

Introduction

During the course of a cytological and histochemical investigation of the carotid trifurcation (the junction of the external and internal carotid arteries and ductus caroticus) in the lizards *Trachysaurus rugosus* (Gray) and *Tiliqua occipitalis* (Peters), a number of observations were made on the associated epithelial body III. Generally, in lizards, this is a small oval body with a distinctive yellowish appearance, situated laterally in the concavity of the carotid arch (Trinci, 1912; Pischinger, 1937) at the origin of the internal carotid artery (Adams, 1952). In certain Reptilia a similar epithelial body may be situated in the concavity of the aortic arch (Wettstein, 1932; Adams, 1939, 1953; Peters, 1940).

Epithelial body III has been considered as the homologue of the mammalian parathyroid gland (Doyen and Kareff, 1904; Weber, 1909; Thompson, 1911) or its histology has been compared with that of this organ (Adams, 1952). According to Doyen and Kareff (1904) and Peters (1940), total extirpation of these bodies results, as in mammals, in paralysis and death. The results of the present investigation indicate that there are a number of cytological and histochemical similarities which support an homology of the reptilian epithelial body with the mammalian parathyroid gland.

Materials and methods

Mature specimens of *T. occipitalis* and *Tr. rugosus* (approximately 300 to 350 mm in length) were killed by transecting the spinal cord in the cervical
region and then pithing the brain and nerve-cord. The carotid trifurcation with the associated epithelial body III was rapidly dissected and fixed. Fixatives used were Bouin, Carnoy, Rossman, Aoyama, 4% formaldehyde, Regaud, and mercuric chloride/formaldehyde (Carleton and Drury, 1957). Material was cleared in benzene and embedded in paraffin.

One immature specimen (T. occipitalis, approximately 130 mm in length) was given intraperitoneal injections of Indian ink (0.5%) for 3 days before being killed. The carotid arch was perfused with 0.6% saline solution before dissection and fixation of the carotid trifurcation and epithelial body.

The following stains and histochemical methods were used: Masson's trichrome, Ehrlich's haematoxylin and aqueous eosin, azocarmine/aniline blue (Gomori, 1946), Holmes's silver method (Carleton and Drury, 1957), periodic acid/Schiff reaction (PAS) (Pearse, 1960), Sudan black B (modified from Thomas, 1948), polychrome methylene blue (Carleton and Drury, 1957), methyl green/pyronin (Pearse, 1960), bromphenol blue (Mazia, Brewer, and Alfert, 1953), and Cain's mitochondrial method (Cain, 1948).

**Observations**

**General features**

The epithelial body is embedded in dense, collagenous connective tissue on the lateral surface of the carotid arch at the base of the internal carotid artery (fig. 1, A). A thin lamellated connective-tissue capsule (11 μ thick) invests the organ over most of its surface. Towards the margin facing the carotid arteries the capsule is lost in the adventitia. There is no thinning of the arterial walls in this region in *Tr. rugosus*, although this was a feature of some specimens of *T. occipitalis*. Some thinning of the capsule may occur on the arterial margin of the organ. The adventitia continues into the body, forming a well-defined hilus. From this core of connective tissue stout, separate trabeculae penetrate into the main cellular mass of the gland. In *Tr. rugosus* there is almost invariably an apparently isolated column of epithelial tissue wedged in between the main mass of the gland and the carotid arch. On following this column by means of serial sections it can be seen to be merely an offshoot of the main mass (fig. 1, A).

The above-mentioned trabeculae, which contain arterioles, venules, and irregular, thin-walled vessels (possibly sinusoids), plunge into the cellular mass of the gland where they rapidly thin out to form a delicate interstitial

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*Fig. 1 (plate). A, section through carotid arch (ca) and epithelial body III (eb). Note peripheral arterioles (ar), irregular vascular channels (vc), and cellular cords (cc). Tr. rugosus (Bouin; PAS).

B, transverse section through a cellular cord showing the extremely granular cytoplasm of the chief cell (c) and the elongate nucleus of the 'epithelial' cell (e). Tr. rugosus (Rossman; Masson).

C, section through various cellular cords, the central one showing a superficial 'epithelial' arrangement of bipolar cells (e). Tr. rugosus (Rossman; Masson).

D, transverse section of a cellular cord closely invested by vascular channels (vc) with erythrocytes. Vacuolated and non-vacuolated chief cells; scattered vacuolation (sv), peripheral vacuolation (pv), and non-vacuolated (nv). Tr. rugosus (Aoyama; azocarmine/aniline blue).*
Fig. 1.

D. C. Rogers
tissue supporting and enclosing the cords and follicles of parenchymal cells. That the parenchyma consists mainly of solid cords of cells is clearly revealed by their appearance in numerous sections varying from transverse to longitudinal (fig. 2, b). The cords themselves may be further subdivided by extensions of extremely delicate collagen fibrils; the possible occurrence of reticulin was not investigated. Frequent anastomoses occur between adjacent cords which are, for the most part, separated by thin-walled (possibly sinusoidal) vessels, in which the diameter of the lumina varies considerably (fig. 2, b).

**Cell types**

Three categories of cells have been distinguished in the parenchymal cords. These will be referred to as (1) the *chief cell*, which has varying cytological appearances suggesting transition to the *wasserhelle* (Getzowa, 1907) or ‘water-clear’ cells (Castleman and Mallory, 1935) and to follicular cells; (2) the *dark cell*; and (3) the ‘*epithelial*’ cell.

**Chief cells.** The cords are composed of several layers of tightly packed cells. These, constituting the commonest cell type within these cords, have a large ovoid to round nucleus, about 6 or 7 μ in diameter, with dispersed, floccular chromatin, and a prominent spheroidal, usually excentric nucleolus (fig. 1, d); very rarely a chief cell has an indented nucleus, the concavity facing a large cytoplasmic vacuole. The disposition and microscopic appearance of the cytoplasm varies not only according to the fixative and stain used, but also presumably with the physiological state of the particular cell. Certain fixatives (Rossman, Regaud) produce a stellate, vacuolated appearance in the great majority of parenchymal cells; the appearance is clearly due to exaggeration of the extracellular spaces as a result of shrinkage during preparation. However, after other fixatives (4% formaldehyde, Carnoy, Aoyama) the cytoplasm of these cells can be seen to show various appearances suggesting intracytoplasmic vacuolation (fig. 1, d).

Many chief cells (figs. 1, b; 2, a) have a finely granular cytoplasm within which separate, minute, peri-nuclear vacuoles are evident. Towards the periphery of the cytoplasm but just beneath the cell membrane, irregularly shaped and spheroidal vacuoles of various dimensions (approximately 1 to 3 μ in diameter) occur (fig. 2, a). Other chief cells fixed and prepared in the same way or even within the same cord do not exhibit cytoplasmic vacuolation at all; instead the globular cytoplasm has a granular appearance. In certain localized groups, within various cellular cords, the cytoplasm of the chief cells appears to assume a peripheral position beneath the cell membrane, leaving what seems to be a ‘clear’ peri-nuclear region. Occasionally a cell with a similar nuclear form to the chief cell, but with very little peri-nuclear non-granular cytoplasm embedded in an apparently ‘clear’, unstained area, is present within the cell cords. The volume of the cell is obviously greater than that of the chief cell-types described above. These cells appear morphologically comparable to the water-clear cells of the mammalian parathyroid gland. Degeneration and mitosis (fig. 4, c) of the chief cells have been observed only rarely.
Dark cell. In the epithelial bodies of a young specimen of *T. occipitalis* (length 130 mm) there were distinct patches of 20 to 30 cells (fig. 4, D), which were morphologically and tinctorially different from the chief cells. The nucleus is smaller with a denser, floccular chromatin content and a spheroidal, centrally disposed nucleolus. The cytoplasm, although apparently similar to that of the homogeneous, granular type of the chief cell, can be distinguished on histochemical grounds (see below).

‘Epithelial’ cell. The ‘epithelial’ cell is characterized by its fusiform nucleus (with the chromatin dispersed in the form of short rods and granules), the prominent, spheroidal, centrally disposed nucleolus (occasionally there are two), and the non-granular cytoplasm prolonged in the direction of the long axis of the nucleus, giving the cell a bipolar appearance (fig. 3, A). Usually, but not always, this cell type can be found bordering the parenchymal cords, where it rests on the sub-endothelial connective tissue (figs. 1, c; 3, A) and is thus separate from the endothelial cells. Occasionally cells of similar tinctorial properties but usually with somewhat more irregular nuclear and cytoplasmic form are present between the chief cells of the cords.

Cytochemistry

*Periodic acid / Schiff reaction.* The non-vacuolated cytoplasm of many chief cells was only faintly positive (fig. 3, A), but others showed a dense, strongly positive, homogeneous substance. In those chief cell-types in which peripheral and scattered vacuolation of the cytoplasm had occurred, the PAS-positive material decreased in overall quantity until in what seemed to be the water-clear cell only a very few positive granules remained. In contrast to this variable distribution of PAS-positive material in the chief cells was the consistent, intense, homogeneous reaction in both the ‘epithelial’ (fig. 3, A) and the dark cell-type.

*Cytoplasmic basophilia.* In general the chief cell-type contains numerous irregularly shaped basiphil rods and granules scattered on a fine-grained, basiphil cytoplasmic background. In those cells in which peri-nuclear vacuolation has taken place a few basiphil granules may be apparent in the cytoplasmic ring. Other chief cells, however, show, besides the smaller inclusions described above, larger irregularly shaped lumps of basiphil material, usually situated in a peripheral position beneath the cytoplasmic membrane (fig. 2, B). This location is similar to that of the peripheral vacuoles and in some instances the basiphil material appears to be situated within these vacuoles. The water-clear cell, the dark cell, and the ‘epithelial’ cell contain no such inclusions. The cytoplasm of the ‘epithelial’ cell, however, has a more intensely basiphil fine-grained appearance than the chief cell. After treatment for 15 min at
Fig. 2.

D. C. R O G E R S
FIG. 3.

D. C. ROGERS
60° C in 1 N HCl (Carnoy fixation) no basophilia was observed after staining with pyronin and polychrome methylene blue.

Sudan black B and Cain's mitochondrial technique. A most distinctive distribution of lipid substances is revealed in the parenchymal cords. Both the 'epithelial' cells and the peripheral chief cells of the cords are devoid of material positive to Sudan black. The chief cells congregated within the inner layers of the cellular cords are strongly positive (fig. 3, c). The sudanophilia appears to be localized in granules and spheroids scattered through the cytoplasm. In some cases there may be a segregation of such material near the nucleus.

In general, numerous fuchsinophil granules pack the cytoplasm of the chief cells. The number of granules is variable from cell to cell, and in some cases most of the cells within a cord may be completely free of such bodies. Occasionally the cells containing the fuchsinophil granules occupy the centre of the cords, where they are surrounded by peripheral, unstained chief cells. There is thus a striking correlation between the distribution of fuchsinophilia and sudanophilia. The water-clear cell appears to contain no fuchsinophil bodies, but in those chief cells in which peri-nuclear vacuolation has occurred, the outer cytoplasmic ring is loaded with granules.

Bromphenol blue. With the exception of the water-clear cells, all the chief cells contain, after staining with bromphenol blue, numerous evenly distributed, separate granules both in the cytoplasm and nucleus (fig. 4, a, b). Also after this technique well-defined membranes can be seen clearly outlining the peripheral vacuoles (fig. 4, b). The vacuoles contain granules similar to those of the general cytoplasm but they have a less intensely stained background.

Other cells

These are cells which are not strictly specific to the parenchymal cords. Mast cells within the connective-tissue trabeculae and very occasionally within the parenchyma are common elements. Typical endothelial cells lining the irregular, tortuous vascular channels which invest the parenchymal cords are clearly evident. The possibility that phagocytic endothelial cells may also be present cannot be excluded, since after injections of ink into the body-cavity of a young lizard (*T. occipitalis*) localized accumulations of ink particles were visible along the walls of these vascular channels.

Follicle formation

Various stages suggestive of the formation of follicles from the cords have been observed. The sequence of events appears to be as follows. A group of chief cells (8 to 10 in a transverse section) rounds off (fig. 4, a) and the

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Fig. 3 (plate). a, section showing strongly PAS-positive cytoplasm of 'epithelial' cells (e). *Tr. rugosus* (Rossman; PAS).

b, transverse section through a follicle containing a PAS-positive, homogeneous substance (s). *Tr. rugosus* (Rossman; PAS).

c, sharply defined distribution of sudanophil substances in cellular cords. Note that the peripheral chief cells (c) have not stained. *Tr. rugosus* (Aoyama; Sudan black B).
individual cells begin to lose their globular shape and assume a pyramidal form. The apices of the cells are directed towards the incipient follicular channel. At the same time the nuclei (always rounded) retreat towards the bases of the pyramidal cell-bodies.

Vacuolation is not observed in follicle cells, although the cytoplasm may be drawn into strands directed towards the centre of the follicle. Within or on these cytoplasmic strands is a fine, faintly PAS-positive granulation (fig. 3, B); numerous fuchsinophil granules also occur. The basiphil substance of the chief cells appears to retreat with the nucleus and in the follicle cell lies around the nucleus. The formerly sharp outlines of these inclusions are no longer visible. Colloid-like material within the follicle channel stains blue with aniline blue, and light green after polychrome methylene blue; it is strongly PAS-positive (fig. 3, B).

**Vascularization**

The main arterial supply to the epithelial body arises from the medial wall of the internal carotid artery (at its origin) as a stout arteriole with a well-defined, regular medial layer. This arteriole of supply passes into the body through the connective-tissue core, where it diverges into a number of branches which pass along the trabeculae either deep within the gland or about its periphery. The peripheral branches connect not only with vessels from the deeper regions of the body but also with numerous sub-capsular arterioles (fig. 1, A) that supply the marginal regions. Arterioles within the trabeculae rapidly lose their well-defined medial layer and abruptly pass into connexion with the irregular, tortuous channels coursing between the parenchymal cords. From these irregular vessels, with their single, endothelial lining, small channels of capillary size pass off to ramify between the cells of the parenchymal cords (apparently along the fine intraparenchymal collagenous extensions). The large, irregular channels unite with peripheral venules; no attempt was made to investigate the main venous drainage.

**Innervation**

A prominent fascicle of nerve-fibres passes into the body along the connective-tissue core, but the destination of the individual axons could not be ascertained.

**Discussion**

The epithelial bodies of the lizards studied in the present work are similar in general form and anatomical position to those described by Doyen and Kareff.
Fig. 4.

D. C. ROGERS
Rogers—'Epithelial body' of lizards

(1904), Weber (1909), Peters (1940), and Adams (1939, 1952). They are not so closely related to the wall of the carotid arch or internal carotid artery as appears the case in Varanus varius (Adams, 1952), although the adventitia of the carotid arch is continuous with the core of connective tissue which passes into the body. The general internal structural arrangement of cellular cords invested by capillary-like vessels (Doyen and Kareff, 1904; Weber, 1911) or 'sinusoids' (Adams, 1952) is not dissimilar to descriptions of the epithelial bodies in the African tortoise (Doyen and Kareff, 1904), the gecko (Weber, 1911), Lacerta agilis, L. muralis, L. vivipara (Peters, 1940), and V. varius (Adams, 1952). There is also a general resemblance in these respects to the mammalian parathyroid gland as described by Morgan (1936), Gilmore (1929), and Ham (1957).

In both T. occipitalis and Tr. rugosus the extensive vascularization of the cords by large, peripheral, irregular vessels of a somewhat problematical nature and by thin-walled capillaries closely related to the chief cells is most evident. The full extent of the minute vascular channels is not perceived without injection; hence the apparently sparse vascularization in some lizards (Krause, 1922; Peters, 1940) may be due to the collapse of these thin-walled channels during preparation. Peculiar duct-like tubes have been described (Thompson, 1911) on the periphery of the epithelial body of certain Chelonia (Chrysemys picta, Pseudemys scripta, Kinosternon pennsylvanicum). Some of these were said to represent a radial arrangement of gland cells around a blood-vessel and others to be ducts of true 'parathyroid alveoli' (Thompson). Similar structures were present in preparations of T. occipitalis and Tr. rugosus, but on carefully reconstructing serial sections these apparent 'ducts' proved to be the major branches of the artery of supply. Apparently a similar arterial supply showing abrupt transition into the capillary-like vessels of the body is present in V. varius (Adams, 1952).

Cells with very elongated nuclei, forming the major portion of the epithelial body, as observed and figured by Peters (1940), were seen in a few specimens of T. occipitalis and Tr. rugosus. A similar feature is figured by Thompson (1911). It has been suggested that this (in other vertebrates) may be due to seasonal variations or age (Nonidez and Goodale, 1927; Benoit and Clavert, 1947; Benoit, 1950). In general the nuclei of the chief cells appeared to be ovoid to round (as in mammals, Morgan, 1936; Gilmore, 1939; Bensley, 1947). Occasionally cells with elongated nuclei were interspersed between the chief cells. These cells appeared to be identical with those situated in a superficial 'epithelial' position. Morphologically similar superficial 'epithelial' cells have been described by Adams (1952) in V. varius. Comparable cells do not appear to be present in the mammalian parathyroid gland.

Mitosis has been seen in one preparation only (young T. occipitalis) during the course of the present investigation. It is not a prominent feature of the mammalian parathyroid gland (Bensley, 1947). In view of the rarity with which degeneration of the chief cells is observed, it seems unlikely that the 'epithelial' cell divides to give rise to transitional forms which migrate into
the cords as replacements. The possibility that they are compressed and distorted chief cells (Adams, 1952) appears unlikely in view of their distinctive cytological and cytochemical characters. Cells comparable in general form and tinctorial properties to water-clear cells have been observed only as isolated elements in the parenchymal cords of *T. occipitalis* and *Tr. rugosus*, although it would seem that they may be more numerous in other reptilian species (Peters, 1940). Vacuolated cells with indented nuclei have been described in *V. varius* (Adams, 1952); comparable cells are present in *T. occipitalis* and *Tr. rugosus*, but they do not resemble the water-clear cell of the mammalian parathyroid gland. It has been suggested that the water-clear cell, in the mammal, may 'represent a functional phase of cell activity' (Bensley, 1947). In the epithelial body of *T. occipitalis* and *Tr. rugosus* a series of stages can be depicted ranging from the typical chief cell with a non-vacuolated globular cytoplasm through forms exhibiting various types of vacuolation (peripheral, random, and peri-nuclear) to culmination in the bloated water-clear cell. Morgan (1936) postulates a somewhat similar sequence for the mammalian parathyroid gland. Apart from the presence of intravacuolar basiphil material in a few cells of the epithelial body, no secretion has been localized within these vacuoles. This may be due, as in the mammalian parathyroid gland, to inadequate fixation (Bensley, 1947).

Cytochemical changes associated with the morphological transition to the water-clear cell are represented by a decrease in both PAS-positive substance and cytoplasmic basiphilia. This is also a feature of follicle cells. If the material contained within the core of the follicle represents stored secretion (Ham, 1957) comparable to the hormone of the gland, then the only observed feature common to both the follicular material and the cytoplasm of the chief cell is the PAS-positive reaction. There are no grounds for postulating that any of the separate inclusions of the chief cell are secretory precursors, though they may be involved in the elaboration of the secretion. The nature of the mammalian parathyroid hormone is very imperfectly known but the evidence suggests a protein component (Greep, 1948; Davies and Gordon, 1955). The remarkably granular nature of the chief-cell cytoplasm after staining with bromphenol blue or Masson may be significant in the search for secretory precursors; however, neither bromphenol blue (Baker, 1958a) nor the acid fuchsine (Baker, 1958b) of Masson's stain can be considered as specific for proteins.

Histochemical studies of the mammalian parathyroid gland have demonstrated the presence of pyronin-positive, finely granular material (Morgan, 1936; Gilmore, 1939), glycogen (Morgan, 1936), mitochondria (Morgan), and lipid (Morgan), mainly in the principal or chief cells. Gilmore (1939) believes that the appearance of the water-clear cell is due to the removal of glycogen during preparation. Cytoplasmic inclusions with similar reactions to histochemical tests have been reported, in the present work, in the chief cells of *T. occipitalis* and *Tr. rugosus*. The significance of the dark cell cannot be ascertained from the information available. It may represent the mammalian
dark principal cell. It would seem to be a modification of the chief cell (which on this basis appears comparable to the mammalian pale principal cell), but apparently it is not a stage in the secretory cycle. Possibly it represents a stage in the differentiation of the chief cell-type.

An unmyelinated fascicle of nerve-fibres entering at the hilus of the mammalian parathyroid gland is said to innervate the blood vessels (see Ham, 1957; Maximow and Bloom, 1960). A similar innervation for the reptilian epithelial body is suggested by the present work (see also Adams, 1952).

This work was carried out during the tenure of a C.S.I.R.O. Junior Postgraduate Award and a General Motors / Holden’s Post-graduate Research Fellowship. The advice and criticism of Dr. G. Burnstock and Mr. A. G. Willis is gratefully acknowledged.

References