J. Anat. (1978), **127**, pp. 505–513
With 16 *figures*
Printed in Great Britain

The development of the rods in the tapetal cells of the cat

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(*Accepted* 3 *January* 1978)

The tapetal cells in the cat are filled with electron-dense structures, uniform in diameter, usually termed rods. Their long axis is parallel to the retinal surface and they are packed in groups or domains, within which they are oriented in the same direction (Bernstein & Pease, 1959; Pedler, 1963; Bortolami, Callegari & Lucchi, 1974). No data are available as to their chemical nature. Owing to the coexistence of melanin granules and rods in some cells located at the periphery of the tapetum (Bernstein & Pease, 1959; Pedler, 1963), and to some developmental similarities between melanosomes and rods (Hamburg, 1975), it has been postulated that the tapetal rods are melaninic in nature.

The aim of the present study was to investigate the development of the rods in the tapetal cells of the cat by ultrastructural and cytochemical (DOPA reaction) research.

MATERIALS AND METHODS

For ultrastructural observations, two 51 days old fetuses, three newborns, four 3 weeks old and four 1 month old kittens were utilized. The eyes were enucleated and the posterior hemisphere was rapidly immersed in a cold mixture of 4 % formaldehyde and 2·5 % glutaraldehyde in 0·1 M phosphate buffer, pH 7·4. After 3 hours the specimen was washed overnight in 0·15 M phosphate buffer at 4 °C, the sclera detached, and the area corresponding to the tapetum trimmed in small slices. Specimens were then post-fixed in a cold 1 % OsO_4 solution buffered with veronal acetate at pH 7·4, rapidly washed in the same buffer, dehydrated in alcohol and embedded in Durcupan ACM. During dehydration the tissue was infiltrated with 0·2 % uranil acetate in absolute alcohol.

For electron microscopic demonstration of tyrosinase localization (DOPA reaction) in the tapetal cells, the eyes of three newborns and four 3 weeks old kittens were utilized. Fetal material was not used since our ultrastructural observations have shown that the initial developmental steps found in the fetal cells are also present in newborn animals. The posterior hemisphere was fixed in a mixture of 4 % formaldehyde and 1 % glutaraldehyde in 0·1 M phosphate buffer, pH 7·4, and washed for 5–6 hours in 0·15 M phosphate buffer, pH 7·4. After removing the sclera, the thin slices of the area corresponding to the tapetum were transferred to the incubation medium of Becker, Praver & Thatcher (1935) (0·1 % solution of L-DOPA, Sigma or BDH, in 0·1 M phosphate buffer, pH 7·4), to which 5 % sucrose was added. To ensure the penetration of the substrate into the tissue, the slices were kept in the incubation medium overnight, at 4 °C, prior to incubation for 1 hour 45 minutes at 37 °C. Control samples for the enzyme reaction were subjected to the same procedure, but the substrate was omitted from the medium. Following incubation the specimens were washed in two changes of 0·15 M phosphate buffer (pH 7·4) + 5 % sucrose, for

a total of 20 minutes, post-fixed, dehydrated and embedded as described above. Ultrathin sections, stained with lead acetate (Millonig, 1961), were examined with a JEM 100 B electron microscope. The sections of the tapetum lucidum were cut perpendicular to the retinal surface.

OBSERVATIONS

Ultrastructural results

In the fetuses, in the area of the developing tapetum lucidum, just below the retinal epithelium, a few layers of elongated cells with an elliptical nucleus and a voluminous nucleolus were seen (Fig. 1). In these cells the rough surfaced cisternae of the endoplasmic reticulum (RER) were elongated, numerous and contained fuzzy material. Many Golgi complexes, richly endowed with small smooth-surfaced vesicles and tubules (GERL), were scattered in the cytoplasm. Membrane-bound tubular structures, of uniform diameter (about 70 nm), were present in GERL as well as scattered throughout the cytoplasm (Fig. 2). They were filled with a slightly electron-dense material which presented a regular cross striation (Fig. 3). In deeper layers the cells had fibroblastic features, and contained glycogen particles, and elongated cisternae of RER located around the mitochondria (Figs. 1, 4). Often these cells were connected by desmosomes to one another and to tapetal cells (Fig. 2). Some melanoblasts were also present among the fibroblast-like cells (Figs. 1, 4).

In the newborn animals the layers of tapetal cells were augmented. In the cells nearer the retinal epithelium the cross striated structures were more elongated and larger in number (Fig. 5). Moreover, in many sites within the cells, grouped Golgi complexes (up to 5–6) and a very wide region with GERL features were present (Fig. 5). On the other hand, the melanoblasts displayed many melanin granules, and GERL was not as prominent as in the developing tapetal cells (Fig. 6).

In the 3 weeks old kittens the tapetal cells of the layers nearer the retinal epithelium presented many rods, which tended to form domains. Among these primitive domains, wide cytoplasmic areas containing mitochondria, RER, grouped Golgi complexes and cross striated tubular structures, were still present (Fig. 7).

In the 1 month old kittens the tapetal cells exhibited a definitive polyhedral form

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Fig. 1. Layers of developing tapetal cells of a 51 days old fetus. *RE*, retinal epithelium; *B*, blood vessel; *M*, melanoblasts; *FC*, fibroblast-like cells. × 4600.

Fig. 2. Developing tapetal cells of a 51 days old fetus. Many vesicles and tubules of GERL are associated with the Golgi complex (*G*). The arrows indicate the membrane-bound cross striated tubular structures. *RER*, rough endoplasmic reticulum; *FC*, fibroblast-like cell; *D*, desmosome. × 30000.

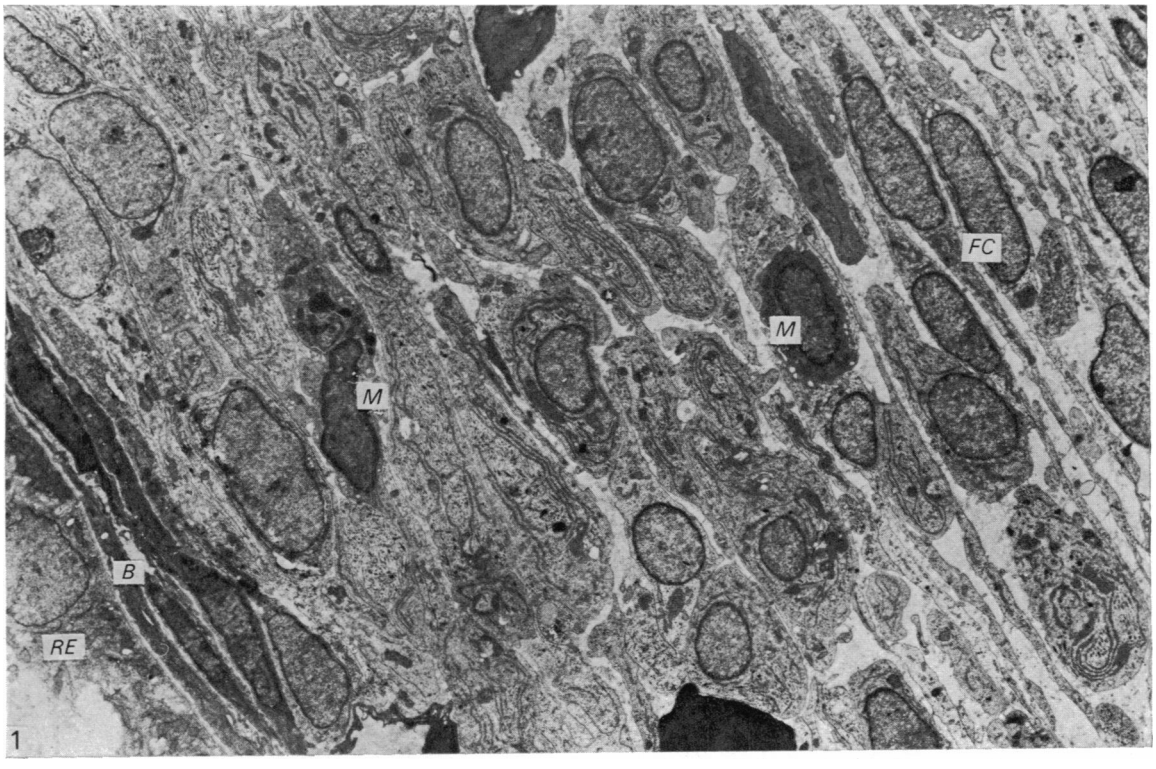
Fig. 3. High magnification of a membrane-bound cross striated tubular structure (arrow) in a fetal tapetal cell. *G*, Golgi complex. × 60000.

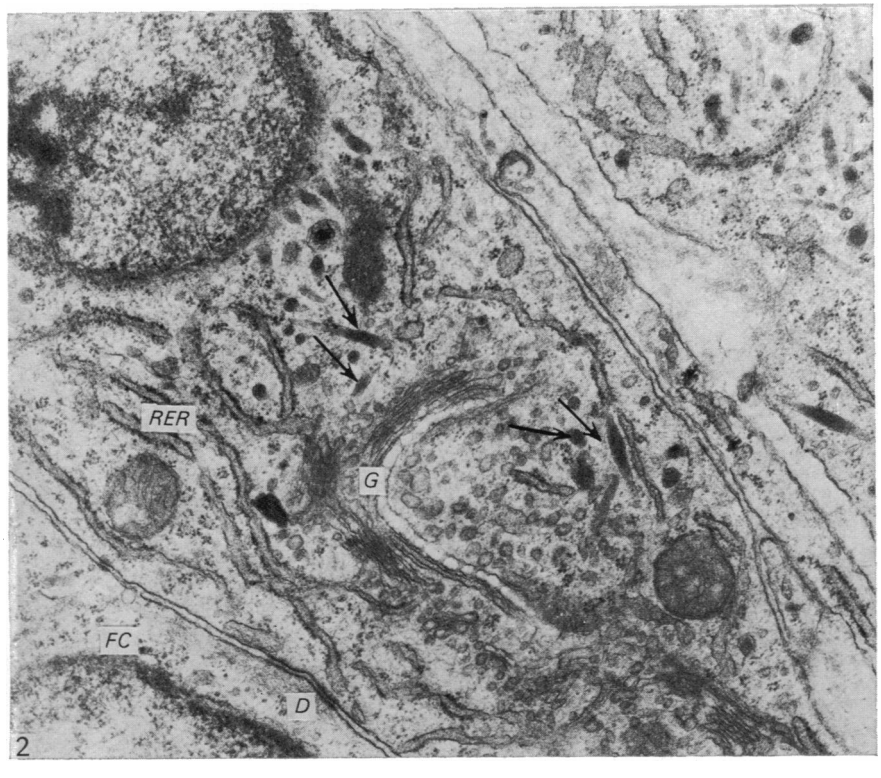
Fig. 4. Fibroblast-like cells and a melanoblast at the periphery of the developing tapetum of a 51 days old fetus. *M*, melanoblast; *FC*, fibroblast-like cells. × 6000.

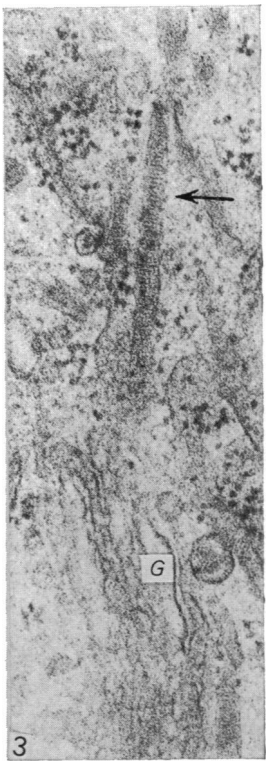
Fig. 5. Developing tapetal cells of a newborn kitten. *G*, grouped Golgi complexes. × 13200.

Fig. 6. Melanoblast at the periphery of the developing tapetum of a newborn kitten. *G*, Golgi complex; *FC*, fibroblast-like cell processes containing glycogen particles. × 10000.

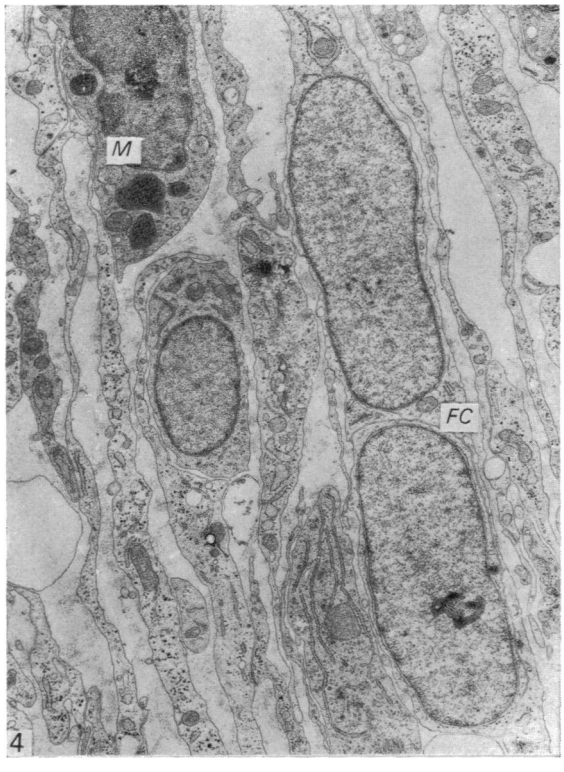
Fig. 7. Developing tapetal cells of a 3 weeks old kitten. *G*, grouped Golgi complexes; *D*, rods grouped to form primitive domains. × 8000.

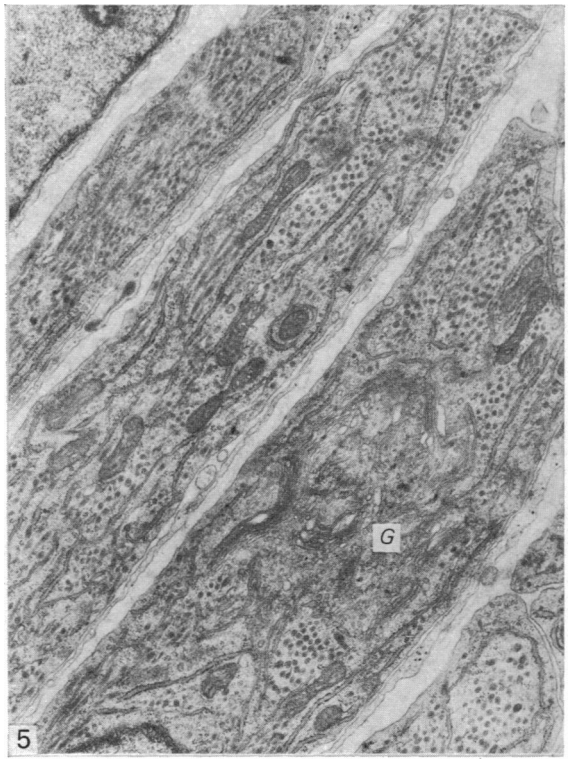


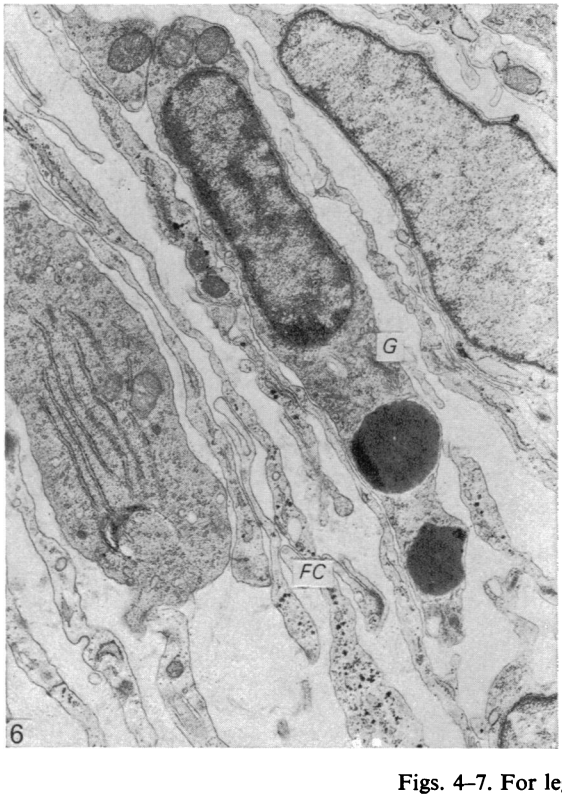


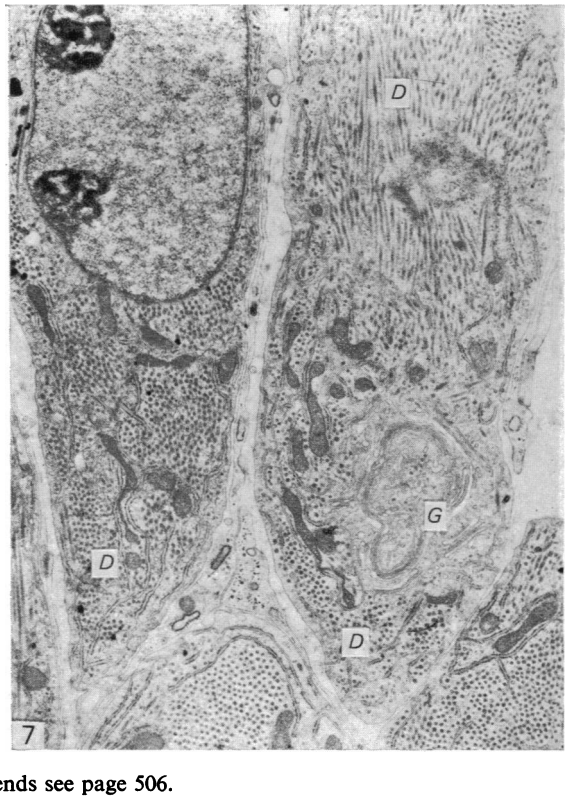


Figs. 1–2. For legend see facing page.

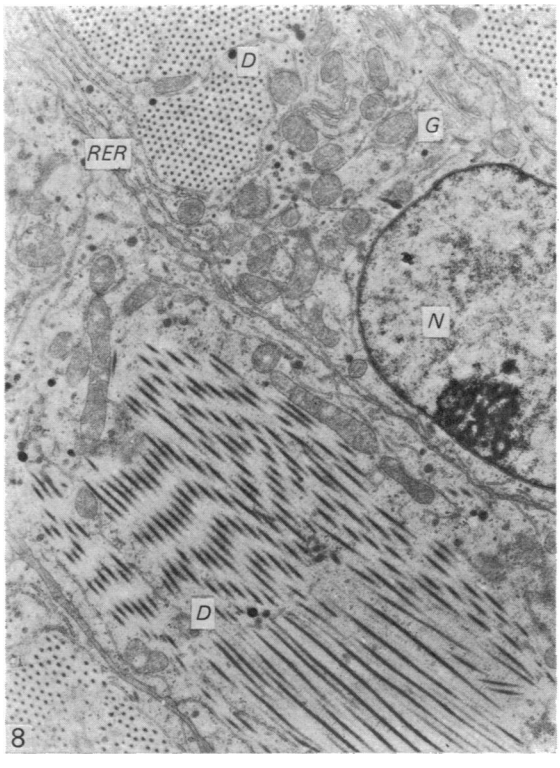


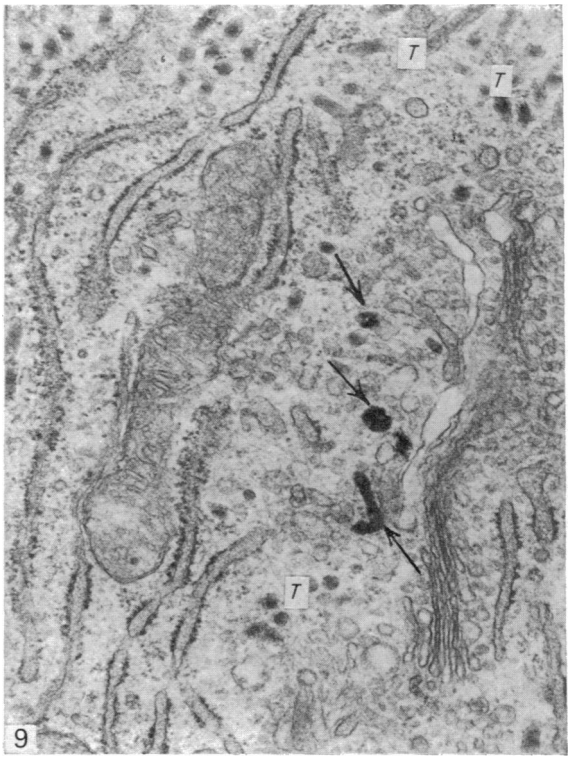


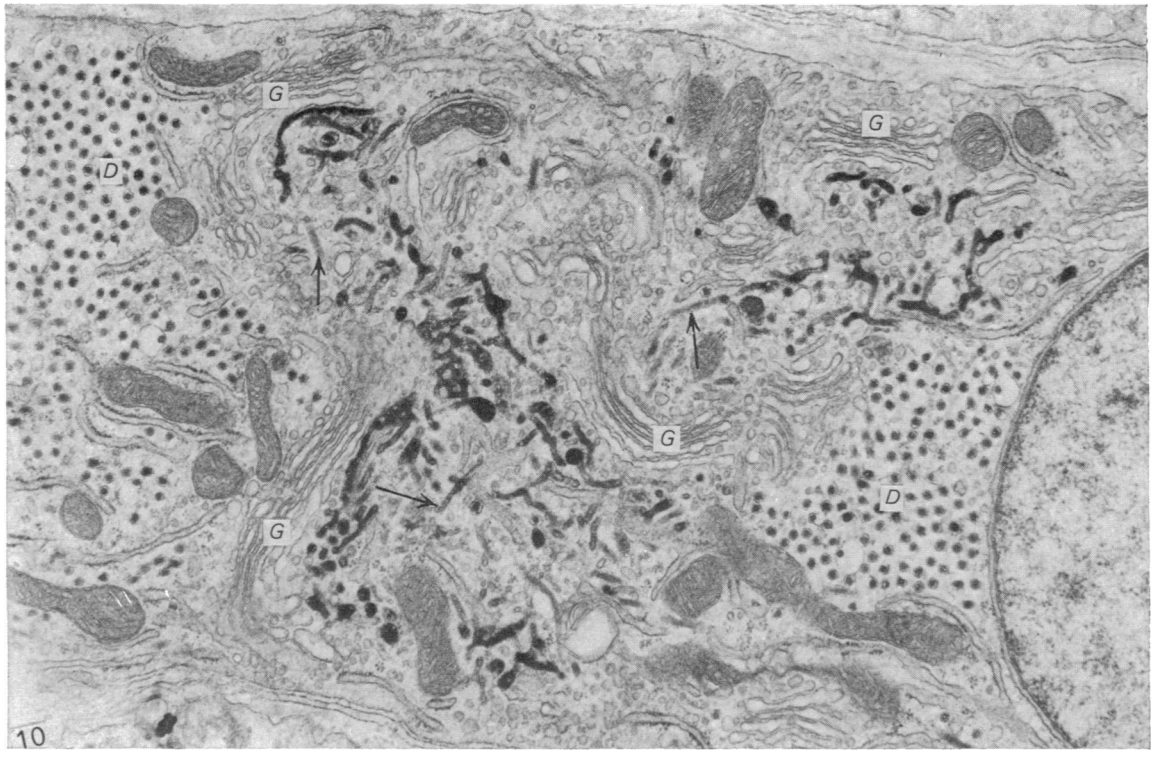




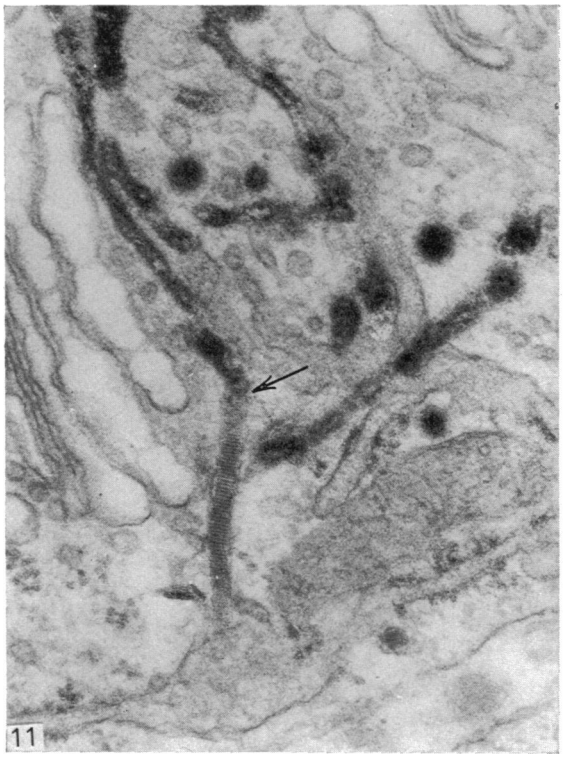
Figs. 4–7. For legends see page 506.

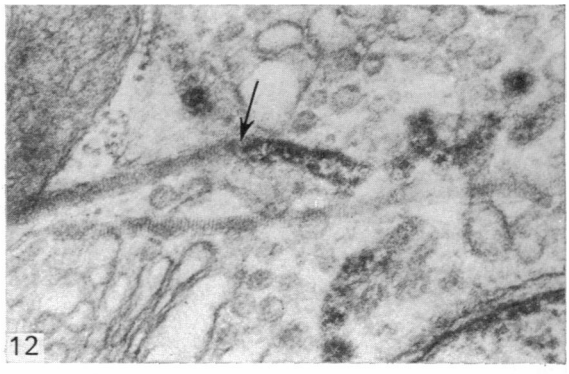


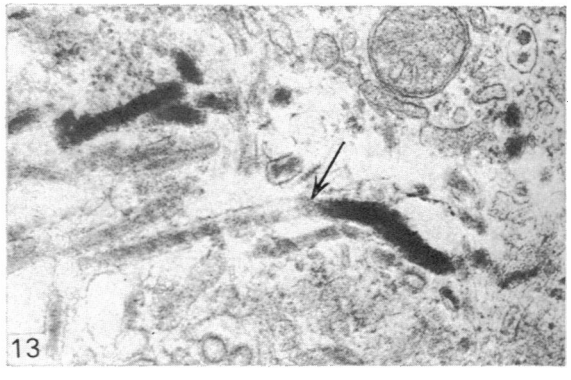


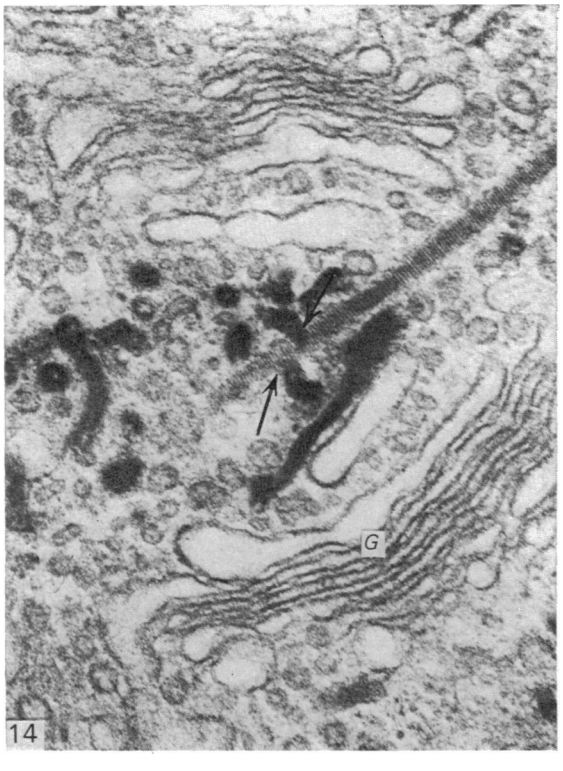


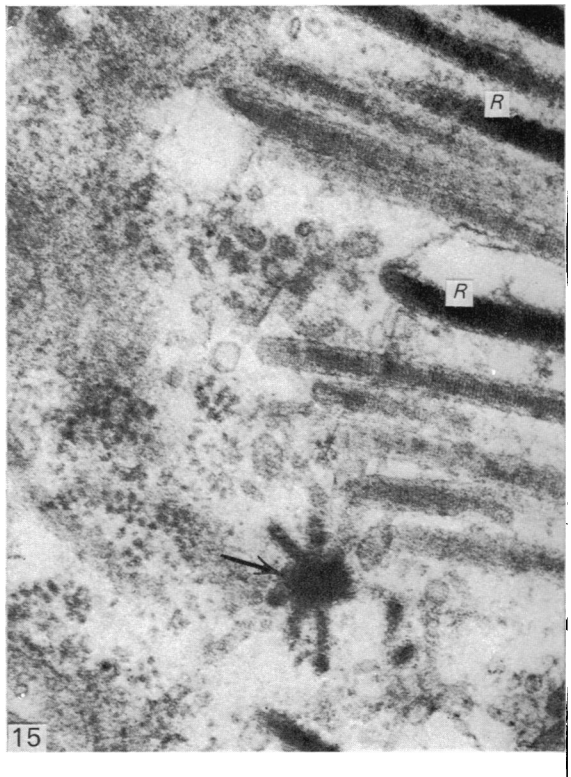
Figs. 8–10. For legends see p. 511.











Figs. 11–15. For legends see facing page.

(Fig. 8). The domains, made up of rods with a diameter of about 150 nm, occupied the greater part of the cell. The cross striated structures were no longer detectable. The rough endoplasmic cisternae, partially degranulated, and the mitochondria, were less numerous and were mostly confined to the peripheral cytoplasm, while the Golgi complexes, located near the nucleus, were less prominent, and GERL appeared less extensive (Fig. 8). However, in deeper layers, many tapetal cells were differentiating, and fibroblastic cells were no longer present. Many melanocytes were found behind the tapetal cell layers. In the transitional zones between the tapetum lucidum and the tapetum nigrum, both melanocytes containing cross striated structures and tapetal cells presenting melanosomes could be seen.

Cytochemical results

In the developing tapetal cells of the newborn kittens, scarce, coarse precipitates were present within some vesicles of GERL (Fig. 9).

In the 3 weeks old kittens the end product of the DOPA reaction was seen in the developing tapetal cells. This product was always detectable in numerous smooth-surfaced vesicles and tubules of GERL (Fig. 10). In these cells the structures containing the DOPA reaction product formed a very wide network between the Golgi complexes and the neighbouring cross striated tubular structures. DOPA-positive tubules were seen to merge with these latter structures; at the point of merging, the striation was masked by a homogeneous electron-dense material (Figs. 11–15). In the cells richly endowed with rods and displaying the primitive domains, the DOPA reaction was very weak. The grouped Golgi complexes were less extensive. The network of DOPA-positive channels differed in width from one GERL to another, or was no longer detectable (Fig. 16).

DISCUSSION

Our ultrastructural observations confirm the findings of Wolff (1968) concerning the features of developing tapetal cells. However, we have noted a close relationship between the formation of rods and the magnitude of Golgi complexes. In fact, our

Fig. 8. Tapetal cells of a 1 month old kitten. The rods are grouped in domains (*D*) which occupy almost all of the cytoplasm. The *RER* is confined to the periphery of the cell, while the Golgi complexes (*G*) can be observed near the nucleus (*N*). × 8000.

Fig. 9. DOPA reaction in a developing tapetal cell of a newborn kitten. Some vesicles of GERL (arrows) show the reaction product. *T*, cross striated tubular structures. × 30000.

Fig. 10. DOPA reaction in a tapetal cell of a 3 weeks old kitten. The reaction product is present in the wide interconnecting network of tubules of GERL. The arrows indicate some cross striated tubular structures. *G*, Golgi complexes; *D*, primitive domains. × 23200.

Figs. 11, 12, 13. DOPA reaction in a tapetal cell of a 3 weeks old kitten. The arrow indicates a DOPA-positive channel of GERL, which appears continuous with the cross striated tubular structure. Figs. 11, 12, × 60000; Fig. 13, × 40000.

Fig. 14. DOPA reaction in a tapetal cell of a 3 weeks old kitten. DOPA-positive channels are seen to merge with a cross tubular structure (arrows). *G*, Golgi complexes. × 60000.

Fig. 15. DOPA reaction in a tapetal cell of a 3 weeks old kitten. *R*, tubular structures, whose cross striation is masked by a homogeneous electron-dense material. The arrow indicates a cross sectioned tubular structure, filled with electron-dense material and connected to DOPA-positive channels. × 60000.

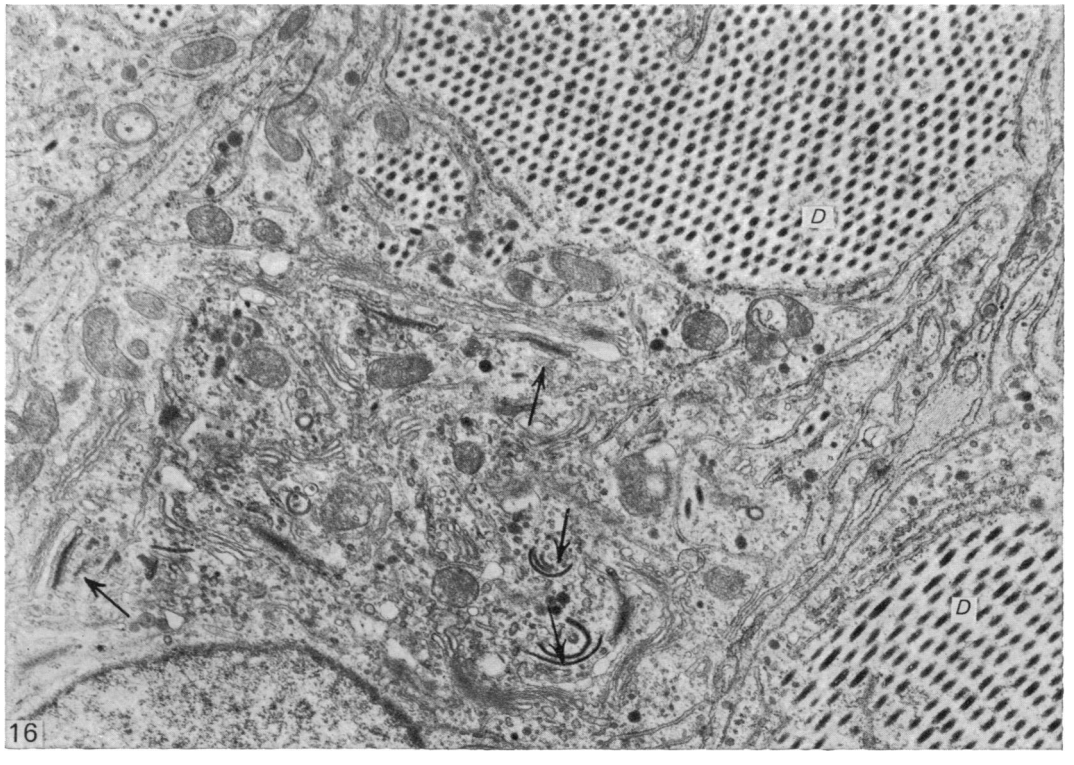


Fig. 16. DOPA reaction in a tapetal cell of a 3 weeks old kitten. The rods are grouped in domains (*D*). Some GERL shows the reaction product and appears less extensive (arrows). × 13200.

cytochemical data have shown that the rods are composed of melanin, and that their development follows a similar course to that described in other melanogenic systems (Novikoff, Albala & Biempica, 1968; Stanka, 1970; Maul & Brumbaugh, 1971; Eppig & Dumont, 1972; Ide, 1972; Eppig, 1974; Eppig & Dumont, 1974). Melanin precursor is 'packaged' by the Golgi complexes into the tubular cross striated structures, which correspond to the early pre-melanosomes of Eppig & Dumont (1972). Melanization occurs when tyrosinase is transferred to them from the Golgi complexes by the DOPA-positive channels of GERL. The large number of rods in the tapetal cells accounts for the size of the RER, Golgi complexes and GERL in the developing cells, i.e. of the organelles involved in melanogenesis. In particular, the large interconnecting DOPA-positive channels of GERL permit rapid melanization of the very large number of cross striated structures. When rod formation is achieved, and the cells present domains, GERL no longer displays tyrosinase activity, and is greatly reduced. In this latter step, according to the histochemical data of Wolff (1968), the Golgi complexes can provide enzymes for the massive regression of these organelles.

It is concluded that the tapetal cells of the cat exhibit a new melanogenic system in which the melaninic structures have a constant diameter and a regular pattern.

SUMMARY

The development of tapetal rods in the cat was studied ultrastructurally and cytochemically (DOPA reaction). The tapetum lucidum of fetuses, newborns, 3 weeks and 1 month old kittens was considered. The DOPA reaction showed that rod formation involved similar enzymic mechanisms to those concerned in the construction of melanosomes.

The authors wish to thank Mr L. Bell and Dr M. M. Falzone for reviewing the English manuscript, and Dr V. Stefani for her technical assistance. This investigation was supported by a grant from C.N.R.

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