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The development of the olfactory mucosa in the mouse: electron microscopy

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INTRODUCTION

In a previous paper we outlined the chief events occurring in the development of the olfactory organ in mice as seen with the light microscope (Cuschieri & Bannister, 1974); although these observations provide a broad description of cytological changes, many details of cell differentiation require the use of the electron microscope for their study.

The olfactory epithelium possesses many of the structural characteristics of the central nervous system, since it contains the cell bodies of neurons which develop *in situ* from the ectodermal cells of the olfactory placode in a manner reminiscent of the origin of neuroblasts in the neural tube. Light microscope studies indicate that the differentiation of the placode is a complex matter, for during fetal development a number of distinct types of non-neural cell are formed in addition to the receptors. The development of the olfactory epithelium is also related to the ontogeny of the neighbouring olfactory bulb, into which the central ends of the olfactory axons grow to synapse with second-order neurons at about the 12th day of gestation in mice (Hinds, 1972*a*, *b*; Hinds & Hinds, 1972).

In view of the interesting problems associated with the development of the olfactory epithelium it is surprising that the ultrastructural changes have been so little studied. A few short accounts of certain aspects of olfactory epithelial development have been published recently (Breiphol, 1972; Breiphol, Laugwitz & Bornfeld, 1974; Breiphol, Mestres & Meller, 1973) and early postnatal changes have also been mentioned by Frisch (1967) and by Seifert & Ule (1967), but no overall account of the various stages of fetal olfactory organ development has so far been given. The present observations concern the development of the olfactory epithelium and olfactory axons from the 10th day of gestation until the postnatal period, and deal with some of the important cytological changes occurring during this time.

MATERIALS AND METHODS

Albino mice of strain S.A.S. (I.C.I.) were used in this study. Fetuses of various ages were obtained in the manner outlined in a previous paper (Cuschieri & Bannister, 1974), including gestational ages of 10, 11, 12, 13, 15, 17 and 19 days. Young mice of 1, 3, 7 and 15 days postnatal age were also used.

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Fetuses were transferred to cold fixative and, in the later ages, their heads were cut into approximately 5 mm cubes. After 15 minutes of preliminary fixation the nasal region was cut into smaller blocks with a razor blade and placed for a further 2 hours in fresh fixative at 0 to 5 °C. The heads of postnatal animals were divided sagittally and immersed in cold fixative. Parts of the olfactory mucosa were dissected out immediately and fixed for 2 hours in fresh, ice-cold fixative.

The particular fixative used varied with the age of the animal and was selected by trial and error. For fetuses of 10 to 17 days' gestation the best results were obtained with a mixture of 5% glutaraldehyde, 4% paraformaldehyde and 0·55% $CaCl_2$ in 0·1 M cacodylate buffer at pH 7·2 (Karnovsky, 1965). Older fetuses and postnatal animals were fixed with 3% glutaraldehyde in 0·1 M cacodylate buffer (pH 7·2). After fixation the tissues were washed in 0·1 M cacodylate buffer (pH 7·2) containing 0·44 M sucrose, at 4 °C for at least 30 minutes and were then treated with cacodylate-buffered osmium tetroxide for 1 hour. Specimens were dehydrated in graded alcohols, passed through propylene oxide and embedded in 'TAAB' epoxy resin. Ultrathin sections were stained in an alcoholic solution of uranyl acetate followed by lead citrate. The sections were viewed with an RCA EMU 3E electron microscope.

RESULTS

The olfactory epithelium at 10 *days of gestation*

Although this was the first stage at which it was found feasible to obtain electron microscope sections through the developing olfactory organ, cellular differentiation had already begun, as shown by the variation in electron density of the cells and the presence of axon-like processes at the base of the epithelium (Figs. 1–5). Most cells appeared either uniformly dark or pale, although a small proportion of cells were of intermediate density (Fig. 1). In addition, there were small numbers of abnormal cells with a swollen and disrupted cytoplasm containing dilated cisternae and few organelles (Fig. 5). Since these were surrounded by well-fixed cells, it is probable that they represent stages in cell death rather than preparative artefacts caused by inadequate fixation.

Of the normal dark and pale cells which constituted most of the epithelium, both types were similar in their fusiform shape, the presence of simple or branched microvilli where they reached the apical surface of the epithelium, the occurrence of junctional complexes, numerous microfilaments, unattached ribosomes, sparse mitochondria, small Golgi complexes and large nuclei containing one or two conspicuous nucleoli (Fig. 1), all features of typical embryonic cells. The difference in density between the dark and pale cells could be attributed to the presence, in much larger concentrations in the former than in the latter, of fine fibrils and granules of about 3 nm diameter which pervaded the whole cytoplasm and nucleus.

At 10 days' gestation the dark cells were much more numerous than the pale ones

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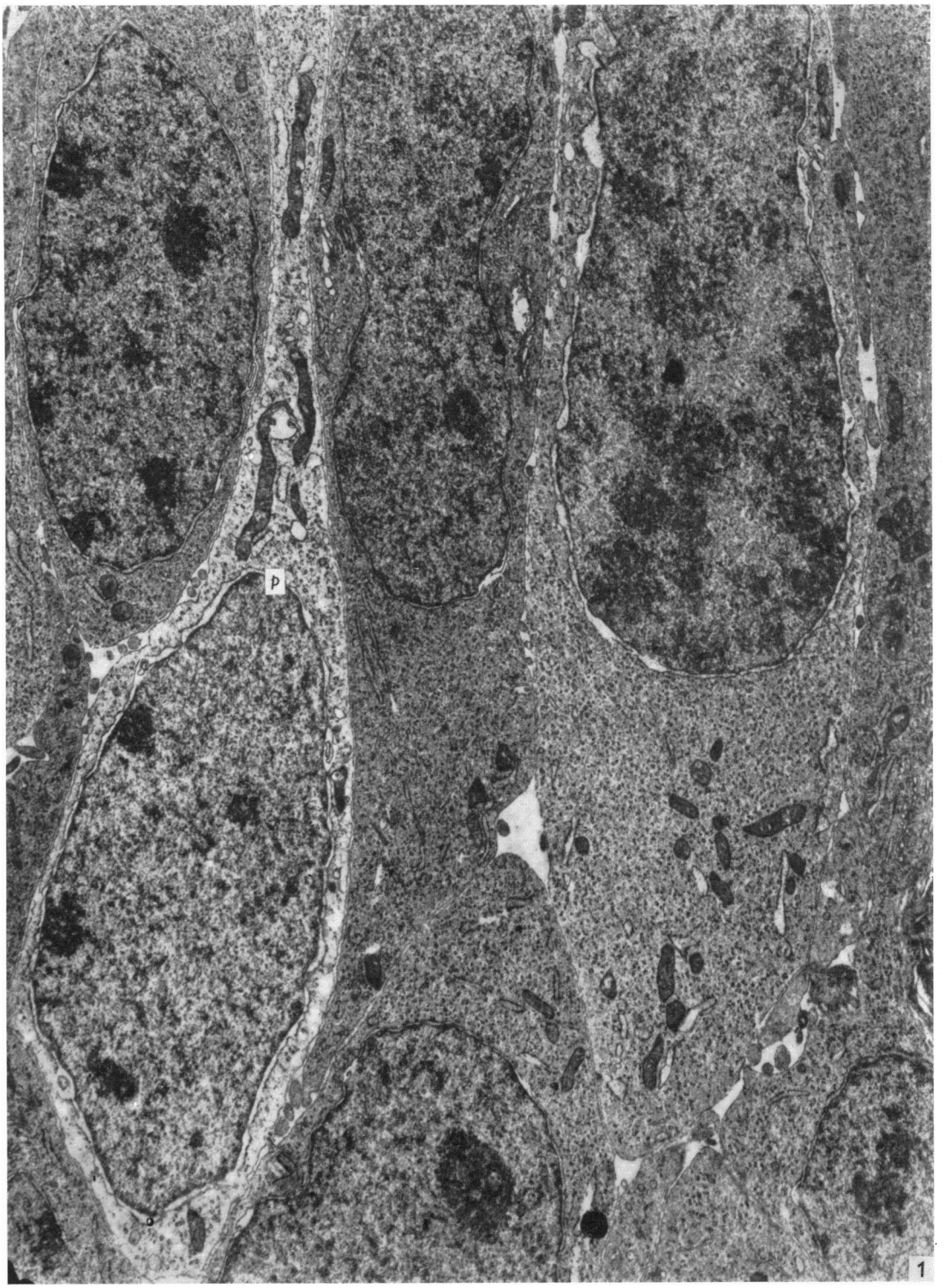
Fig. 1. Vertical section through the epithelium lining the olfactory pit at 10 days of gestation. Note the predominance of cells with an electron-dense cytoplasm; a single pale cell is also visible (*p*). ×9800.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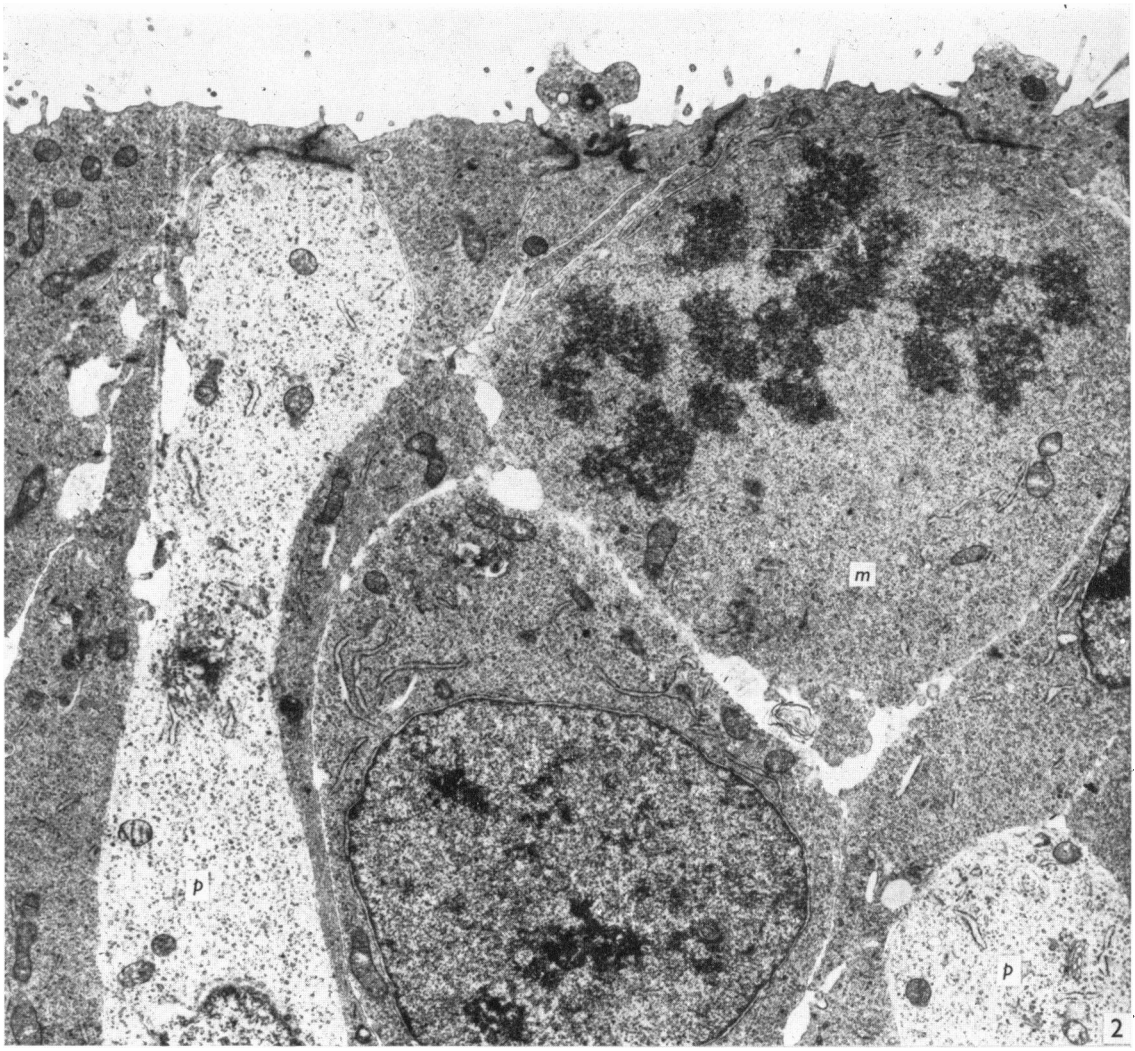

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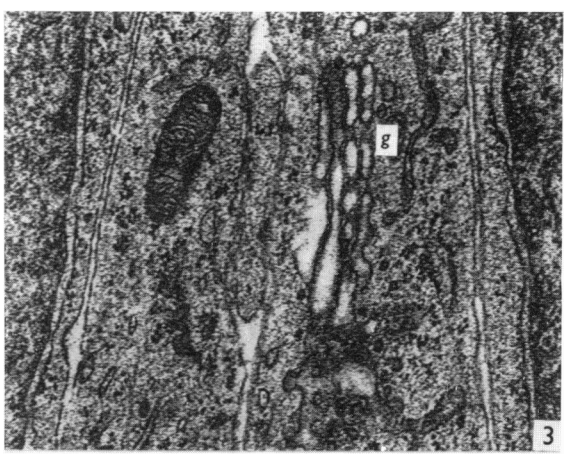

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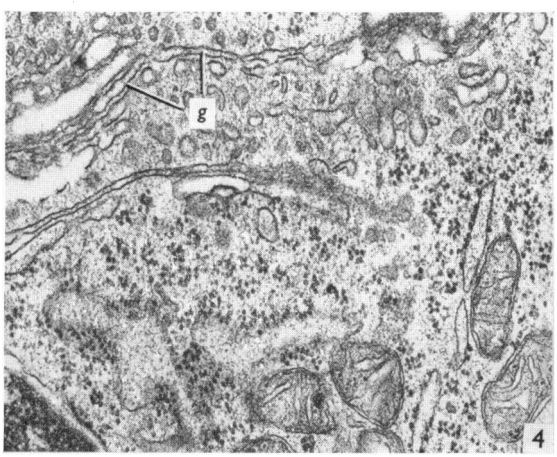

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although the actual ratio varied considerably from one region to another. A few rounded dark cells in various stages of mitotic division were also seen at the apical surface (Fig. 2). At the base of the epithelium a thin but distinct basal lamina was present, periodically interrupted by elongate processes arising from the bases of either pale cells or cells of intermediate density (Fig. 5). Such cellular processes showed many of the structural features of developing axons, and will be described in detail in a separate section of this paper.

The olfactory epithelium at 11 *days of gestation*

Dark and pale cells were still present, but some new features of cell structure were recognizable. The supranuclear region in some of the pale cells contained microtubules and microfilaments orientated vertically in the epithelium, and there were often groups of centrioles here as well. The apices of such cells usually protruded slightly above the surface of the epithelium as incipient terminal swellings.

The dark cells were similar in all respects to those at the previous stage of gestation. In the mesenchyme subjacent to the epithelium bundles of axonal processes grouped in Schwann cell sheaths were also visible. These will be described in more detail later.

The olfactory epithelium between 12 *and* 16 *days of gestation*

During this period the dark cells were still numerous, but gradually decreased relative to the pale cells; their nuclei appeared to be situated increasingly apical to those of the pale cells, although the cytoplasm of the two formed a mosaic of dark and light areas throughout the epithelium (Figs. 6, 9). Mitotic figures were found in dark cells in all layers of the epithelium. The swollen degenerating cells noticed at 10 days' gestation were also present during the later period, and concentric membranous whorls were also observed in the intercellular spaces and, less often, within all types of cell, usually in contact with the nuclear envelope.

Olfactory dendrites with terminal swellings were clearly recognizable at 12 days gestation (Figs. 6–9). At this stage they occurred most frequently in the epithelium of the recesses in the most caudal parts of the nasal cavity where differentiation of olfactory neurons appeared to be most advanced. Typically, olfactory receptor dendrites contained pale cytoplasm with numerous longitudinally orientated microtubules, mitochondria and small coated vesicles, and centrioles were often found either in groups or scattered singly. Ribosomes were sparse or absent.

At this stage (12 days) the terminal swellings of dendrites were often spherical, measuring 1·5 to 2·0 μm across; they contained numerous microtubules, some arranged concentrically around the periphery of the swelling (Fig. 8) while others

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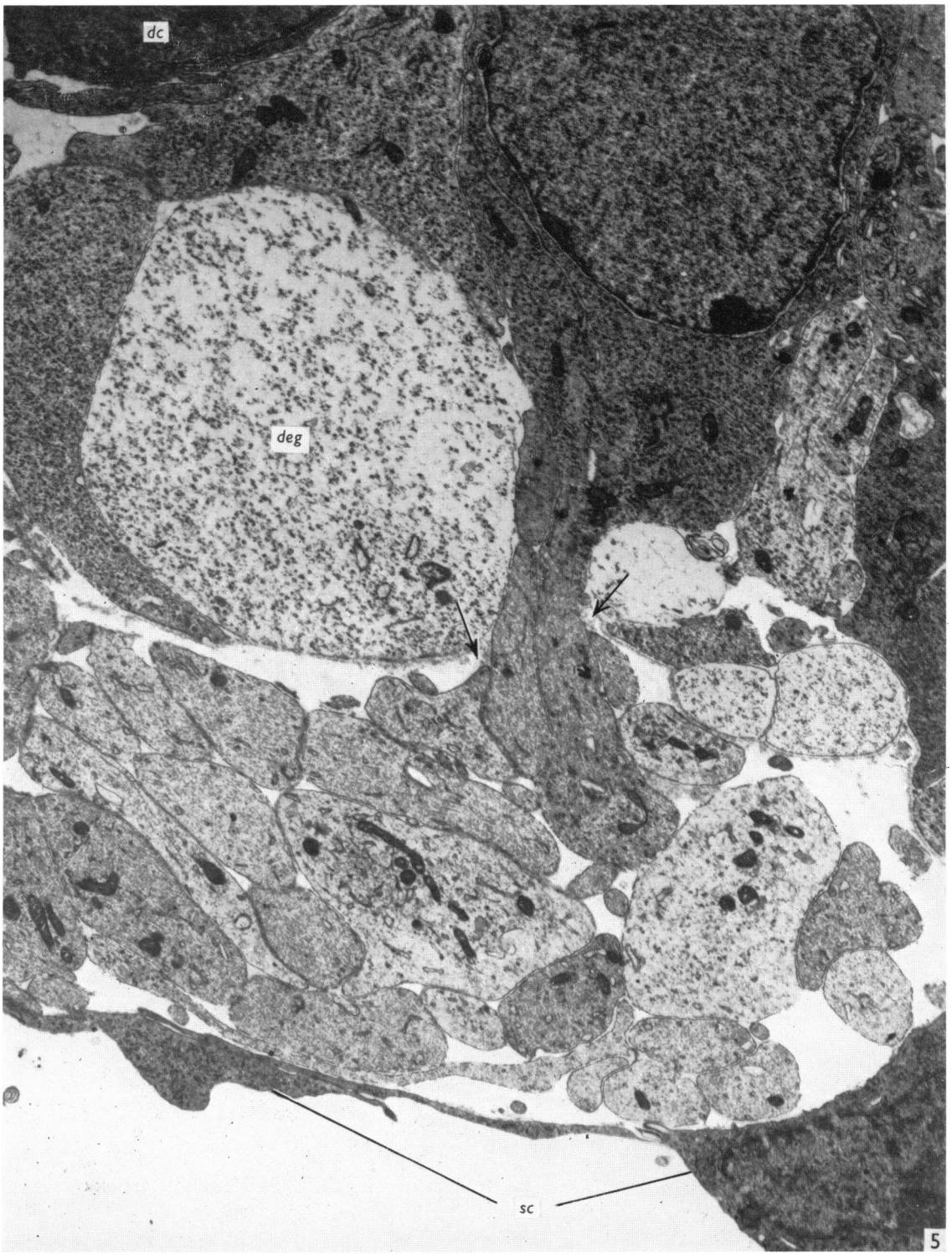
Fig. 2. Vertical section through the surface of the epithelium lining the olfactory pit at 10 days of gestation. The cytoplasm of the cell in mitosis (*m*) is similar to that of non-dividing dark cells. Note the apical regions of pale cells (*p*). × 6300.

Fig. 3. Portion of the supranuclear cytoplasm of a dark cell at 10 days of gestation, in vertical section. Note the dense fibrillar cytoplasmic matrix, Golgi complex (*g*) and clusters of unattached ribosomes. × 31 000.

Fig. 4. Portion of the cytoplasm of a pale cell at 10 days of gestation, showing a Golgi complex (*g*) and other organelles. × 31 000.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extended longitudinally into the deeper regions of the cell (Fig. 7). These swellings also contained numerous mitochondria and centrioles, the latter sometimes occurring in rosette-like clusters (Fig. 8). Cilia were infrequent at 12 days, but a few short microvilli were often present on the surface of the terminal swellings. At the level of the epithelial surface, junctional complexes were present between dendrites and neighbouring cells.

The cell bodies of the receptor cells (Fig. 9) were still similar to the surrounding dark cells except for their lesser density, and the microtubules of the receptor dendrites did not extend into the perikaryal cytoplasm. Centrioles were often seen in the supranuclear region of the cell bodies, most often arranged in rosette-like arrays.

After the 12th day of gestation the numbers of olfactory dendrites and pale receptor cell bodies increased rapidly. The numbers of cilia at the surface also increased and the terminal swellings bearing them became more cylindrical in shape. Centriole clusters increased in frequency both in the cell bodies and in the dendrites, and in one cell 14 centrioles were counted in a single rosette-like cluster in the supranuclear region. Examples of centriole groups are depicted in Figs. 10–12.

Horizontal sections of the epithelium showed that the receptor cells were formed in clusters of 2–6, each group surrounded by the dark cells (Fig. 14). Juxtaposition of receptors was also found at the level of their dendrites (Fig. 15) and of their terminal swellings (Fig. 13). At most levels in the epithelium receptors were separated by the usual 20 nm intercellular space, but between adjacent terminal swellings they approached each other closer to form specialized junctions; it could not be determined, using the methods available in this study, whether these were of the 'tight' or 'gap' variety.

At 16 days' gestation, occasional capillaries were found within the confines of the epithelium (see also Cuschieri & Bannister, 1974). Each consisted of the usual endothelial lining surrounded by a thin basal lamina, no mesenchyme being present between the latter and the surrounding epithelial cells.

The olfactory epithelium at and after the 17th day of gestation

After the 16th day marked structural changes were evident in the olfactory receptors, progressively leading to their final maturation during the postnatal period. Such alterations were most obvious in the cell bodies, in which the amounts of granular and agranular endoplasmic reticulum increased greatly, particularly in the supranuclear region, and dense membrane-bound bodies similar to lysosomes appeared in conspicuous numbers. Golgi complexes also increased in size, but unattached ribosomes became increasingly rare (Fig. 17). The terminal swellings of dendrites assumed the appearance of fully formed olfactory knobs, with typical cilia, and finely microfilamentous cytoplasm containing endocytic or exocytic

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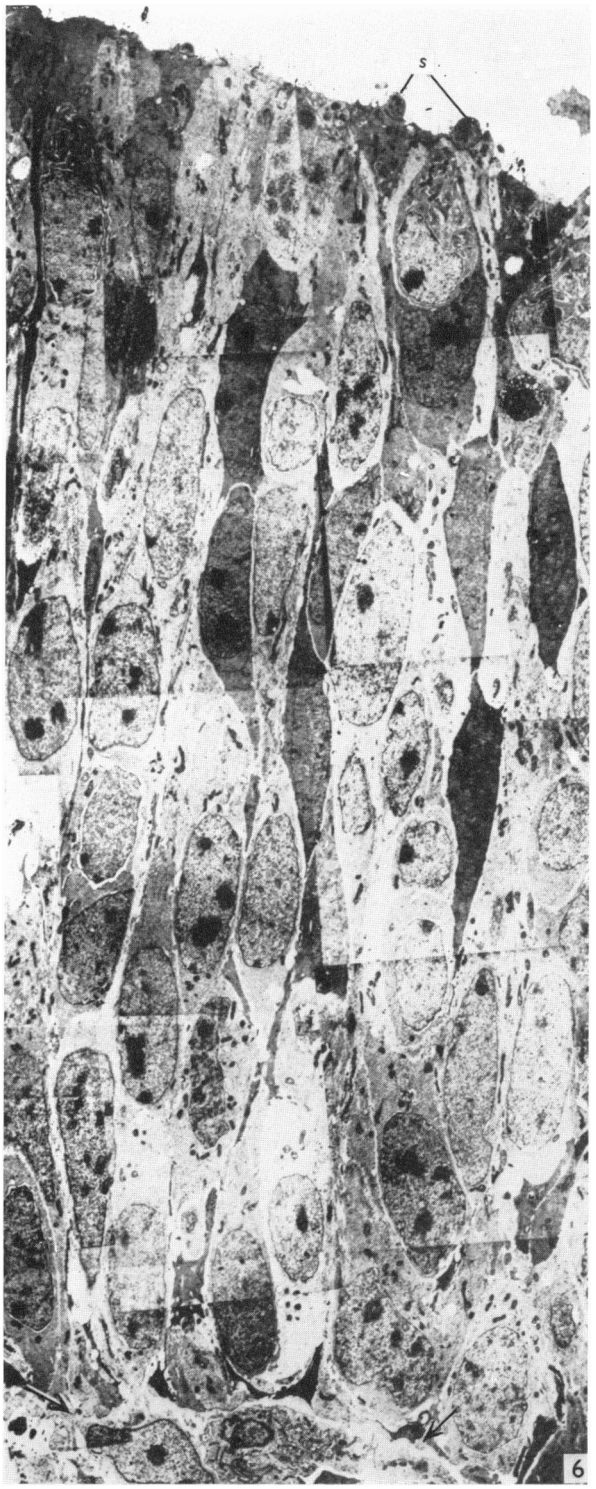
Fig. 5. Vertical section through the base of the olfactory epithelium at 10 days of gestation showing two adjacent axonal processes emerging through the basal lamina (arrows) into the mesenchyme where they lie alongside other similar processes and associated Schwann cells (*sc.*) One axonal process can be traced to an epithelial cell of intermediate density. Note a portion of an apparently degenerating cell (*deg*), and a dark cell (*dc*). × 7200.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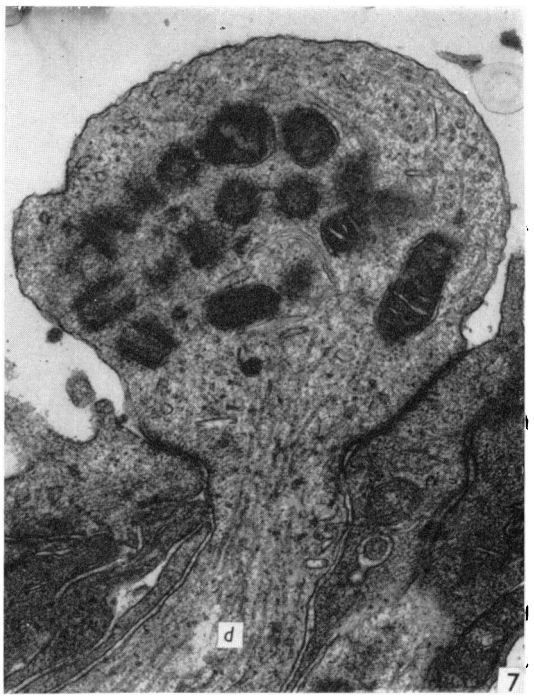
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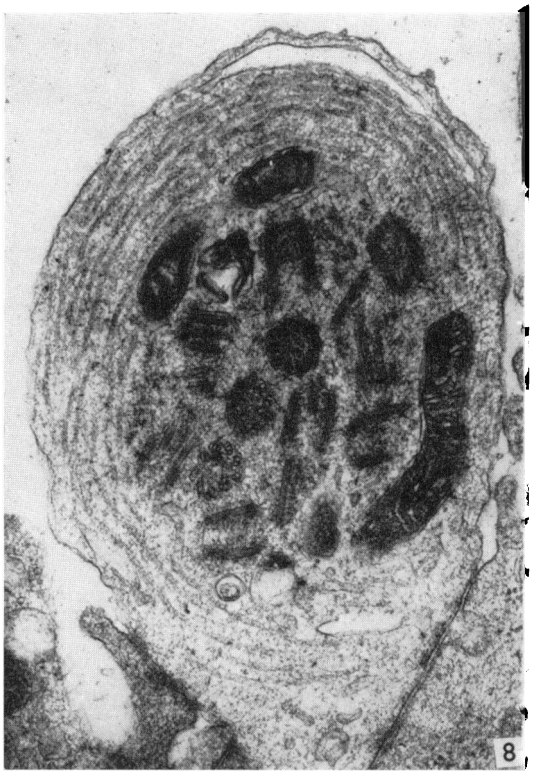
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vesicles. Initially (up to the time of birth) dense granular bodies were present in the vicinity of the bases of cilia (Fig. 16). Mitochondria became numerous in the dendrites, although absent from the terminal swellings.

In addition to such maturing cells, receptors showing no signs of final maturation were also present at 17 days of gestation, and after birth. Some of these cells contained numerous centrioles in their cell bodies and along their dendrites, as reported by Heist & Mulvaney (1968) in postnatal rabbits. By the third week of postnatal life immature receptors were confined to the basal layers of the epithelium.

Juxtaposition of olfactory dendrites, a common feature of earlier stages of development, became progressively less evident, and from 2 months' postnatal age onwards was rarely encountered.

During the late stages of gestation and the early period of postnatal life, the other types of cells in the epithelium showed the final steps in their structural maturation. From the 17th day onwards the remaining dark cells, the nuclei of which were situated near the surface of the epithelium, transformed into supporting cells (Fig. 16), characterized by conspicuous amounts of agranular and granular endoplasmic reticulum, mitochondria, pinocytotic and other vesicles near the surface, and numerous straight and branched microvilli. Immediately before birth such cells were still quite dark in appearance and contained many unattached ribosomes, but in the early postnatal period they became much paler and the amounts of endoplasmic reticulum in the supranuclear zone increased greatly. The basal processes of the supporting cells were at first indistinguishable from those of the surrounding cells, but after the first 2 weeks of postnatal life they contained their characteristic dense membrane-bound inclusions (see Frisch, 1967).

In the basal layer of the epithelium after the 17th day of gestation differentiated basal cells were identifiable by their indented nuclei (Fig. 18), and later by their numerous microfilaments. Their cytoplasm also contained clusters of unattached ribosomes and small amounts of granular endoplasmic reticulum.

Development of Bowman's glands

The glands of Bowman were first visible on the 17th day of gestation as small outgrowths at the base of the olfactory epithelium (Fig. 19). Two principal types of cell were distinguishable in the secretory portions, one pale in appearance and the other dark. The pale cells contained numerous large vacuoles, expanded cisternae filled with a granular material, and large Golgi complexes (Figs. 19, 21). Granular endoplasmic reticulum, unattached ribosome clusters and mitochondria were also present in conspicuous amounts. The dark cells, however, had no secretion vacuoles

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Fig. 6. Montage showing the appearance of the olfactory epithelium at 12 days of gestation, in vertical section, showing the distribution of pale and dark cells. A few terminal swellings (*s*) of dendrites are visible at the surface, and at the base of the epithelium (arrows) mesenchyme cells are present. × 3000.

Fig. 7. Terminal swelling of an olfactory dendrite at 12 days of gestation, showing centrioles, mitochondria and longitudinally orientated microtubules extending into a dendrite (*d*). × 20 000.

Fig. 8. Olfactory terminal swelling at 12 days of gestation, showing the circumferential arrangement of microtubules around a central cluster of mitochondria and centrioles. × 33 000.

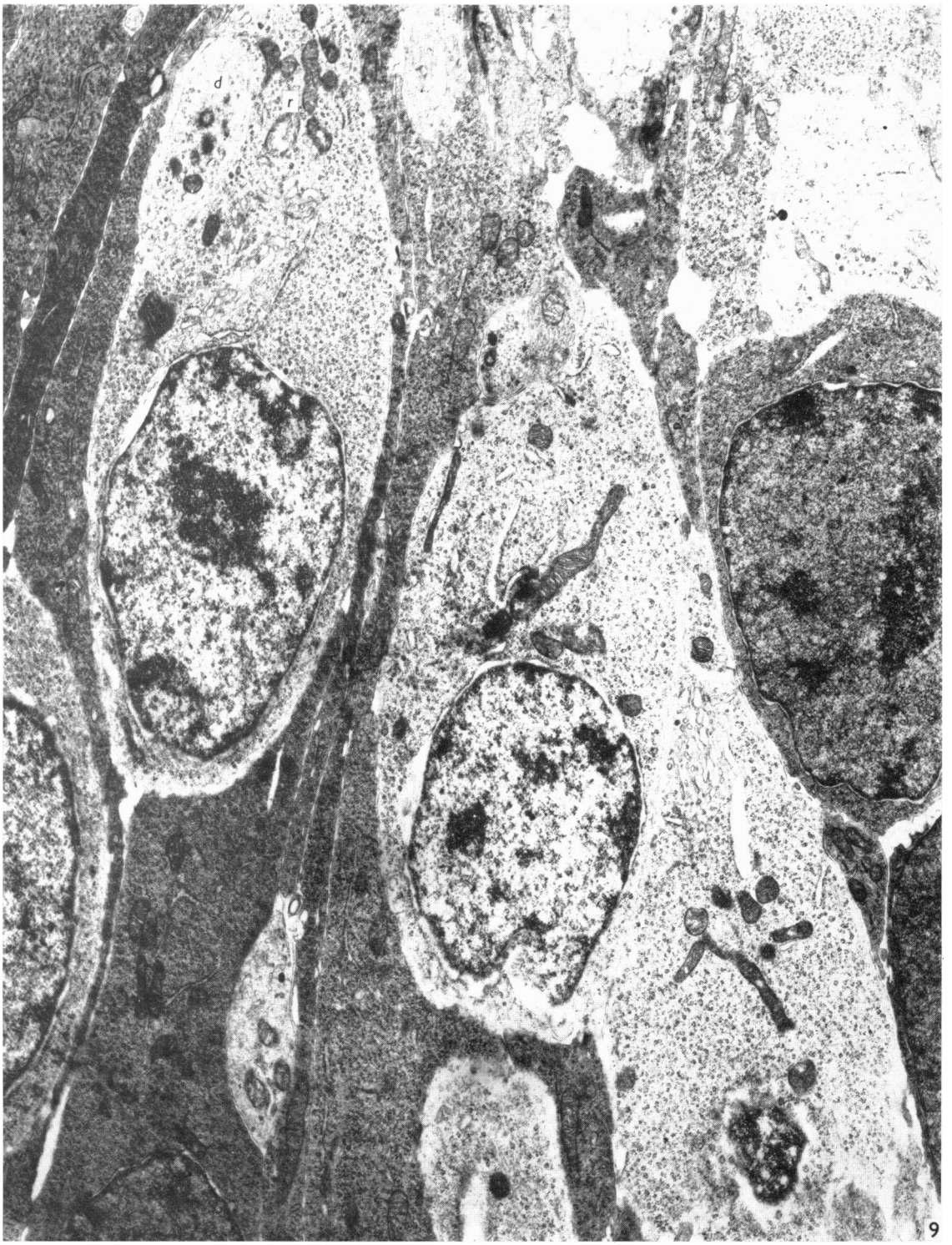
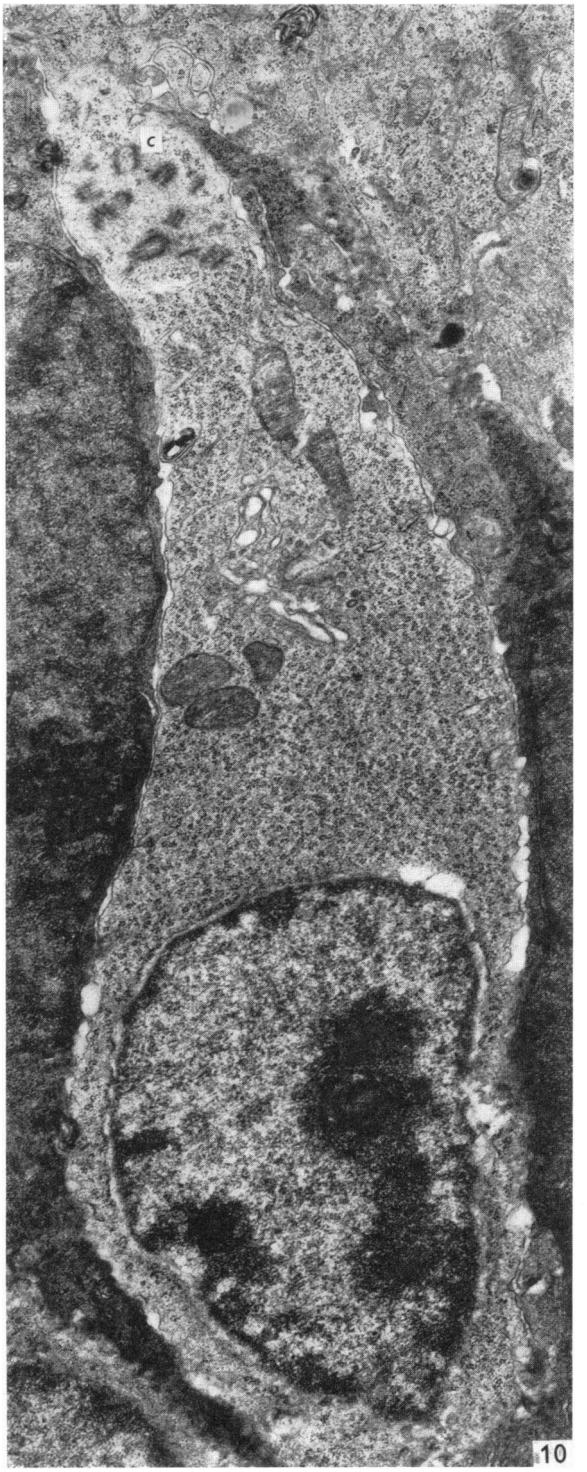
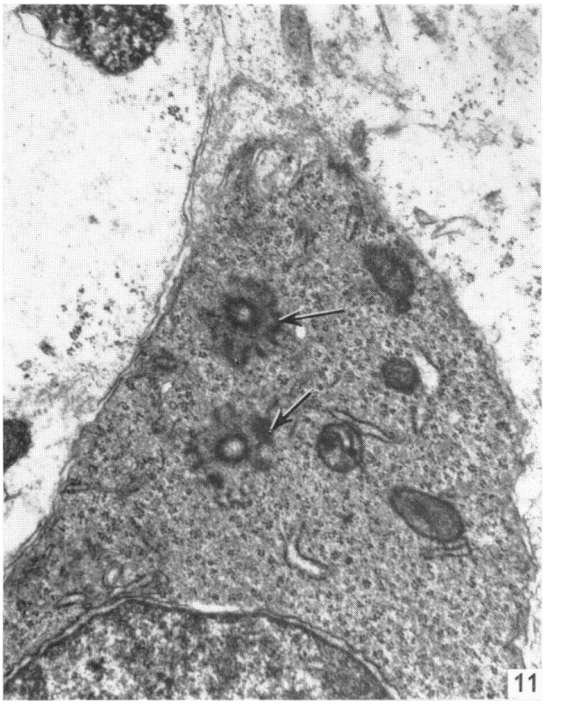


Fig. 9. Pale, developing receptor cells (*r*) and dark cells in olfactory epithelium at 12 days of gestation, in vertical section. The pale cells are more numerous than at 10 days (see Fig. 1). Note the close proximity between adjacent receptor cell body and dendrite (*d*). ×11000.





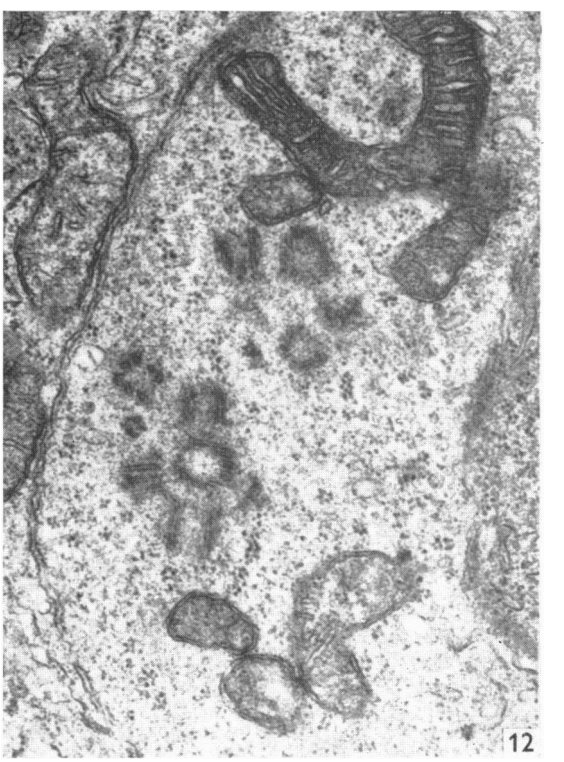


Fig. 10. A developing receptor at 13 days of gestation, showing the abundance of ribosome clusters in the cell body, and the presence of a group of centrioles (*c*) in the base of the dendrite. ×12000.

Fig. 11. Two rosettes of centrioles (arrows) situated in the supranuclear cytoplasm of a receptor at 15 days of gestation. ×12000.

Fig. 12. Detail of an irregular group of centrioles in the cell body of a receptor at 17 days of gestation. Some of the centrioles are incomplete and may be in the process of formation. ×23000.

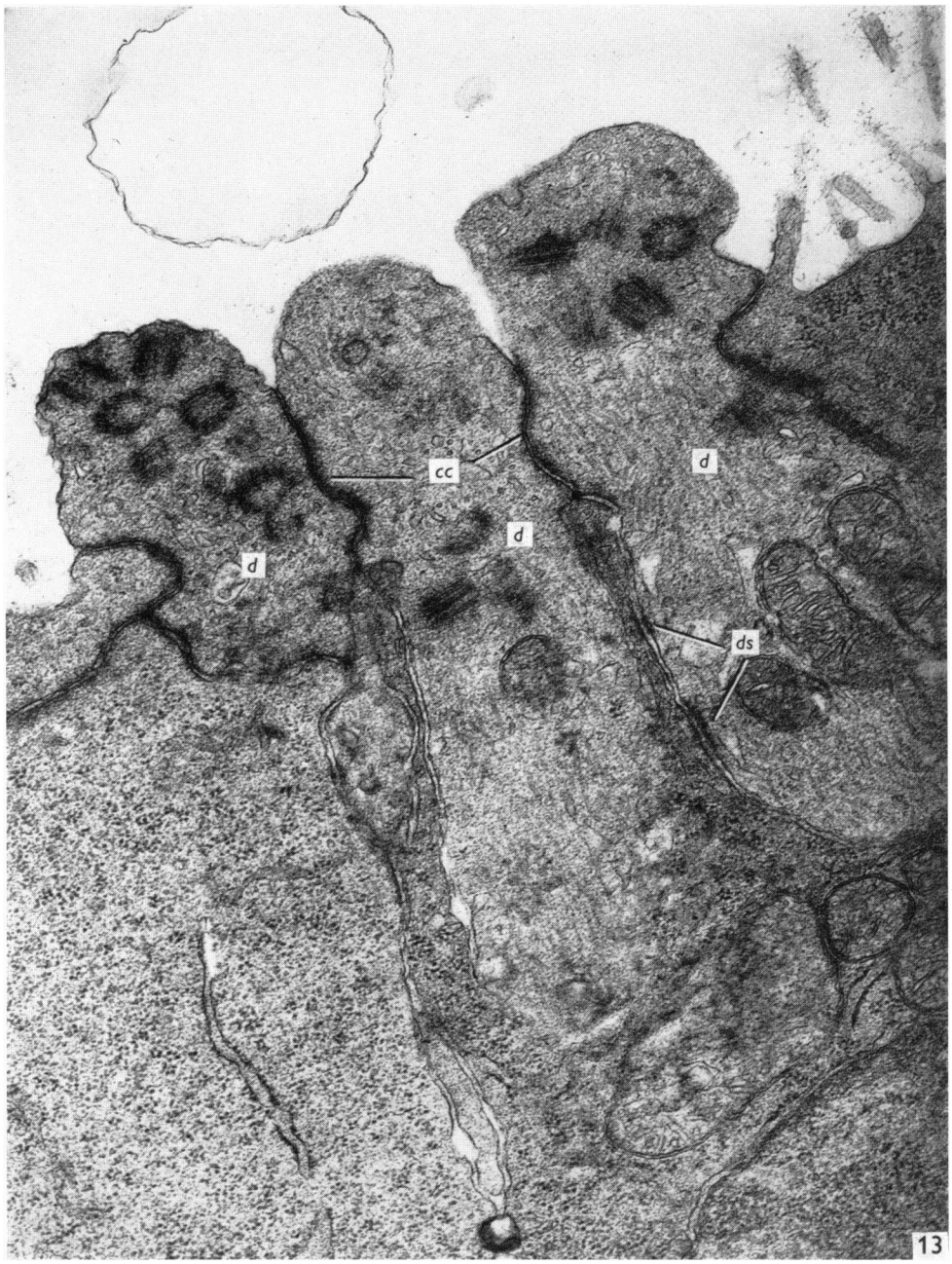
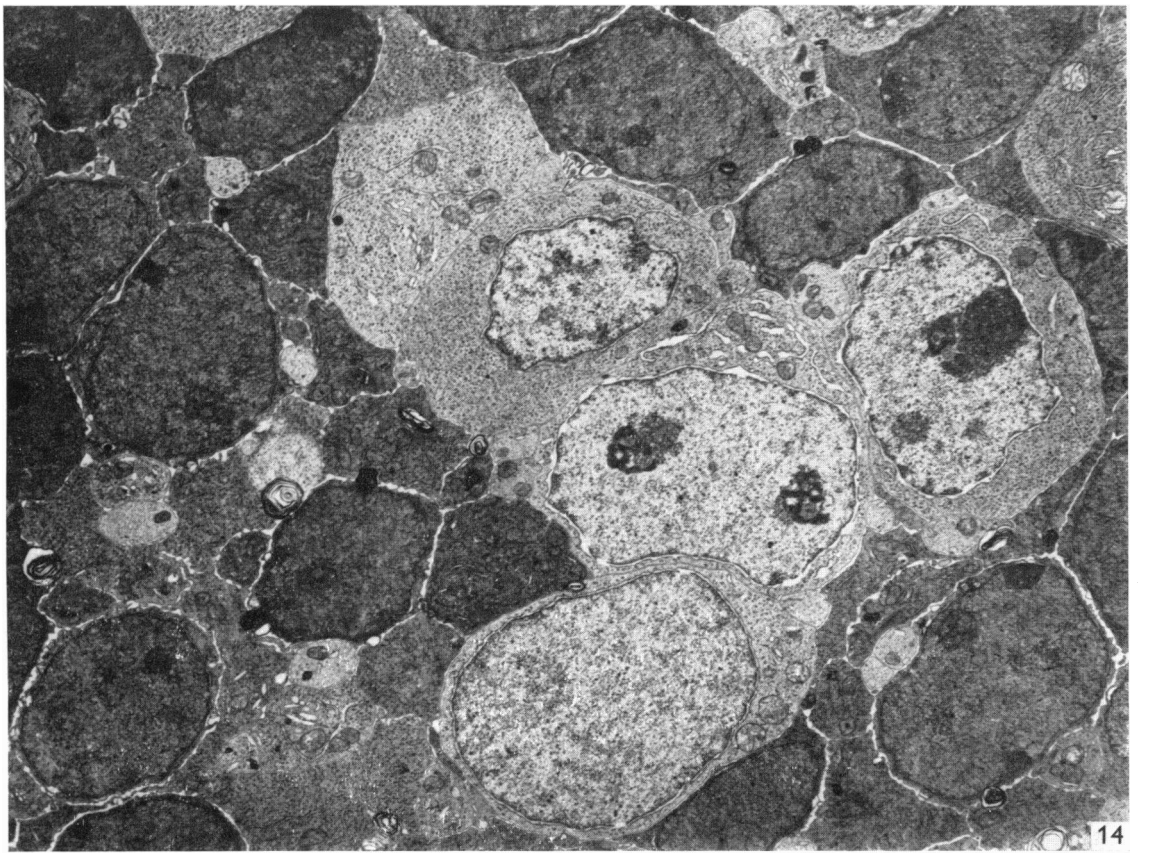


Fig. 13. Three adjacent receptor terminals, seen in vertical section at 13 days of gestation. Note the presence of junctional complexes between neighbouring dendrites (*d*), including close contacts (*cc*) near the surface of the epithelium, and desmosomes (*ds*) at a deeper level. Centrioles and microtubules are also visible in the dendrites. × 18000.



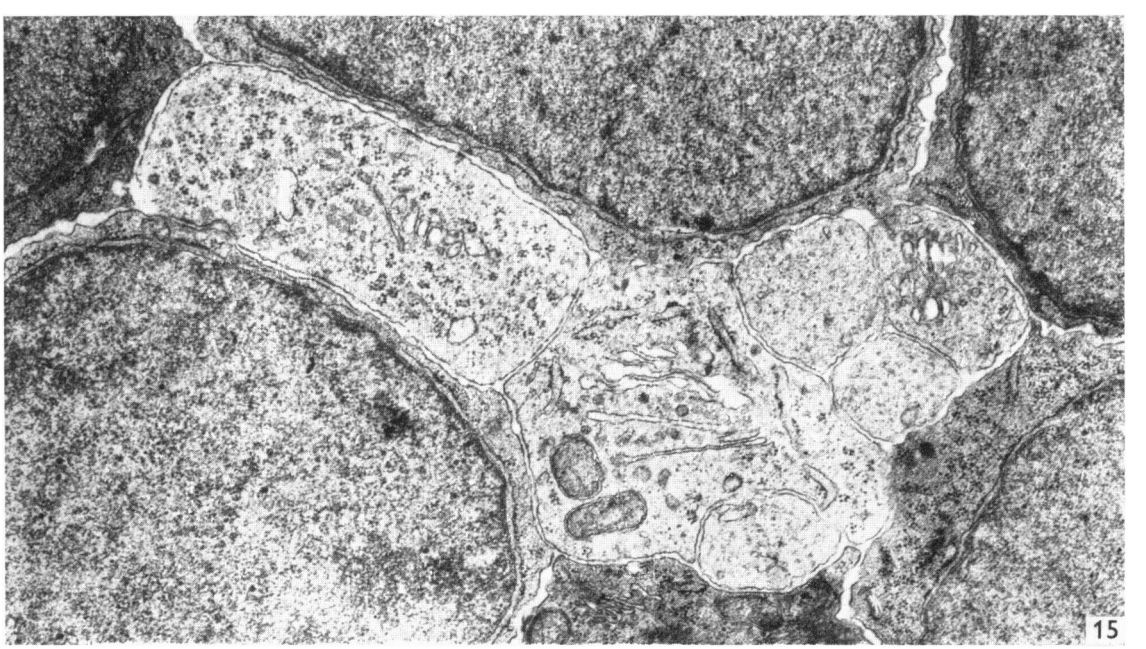
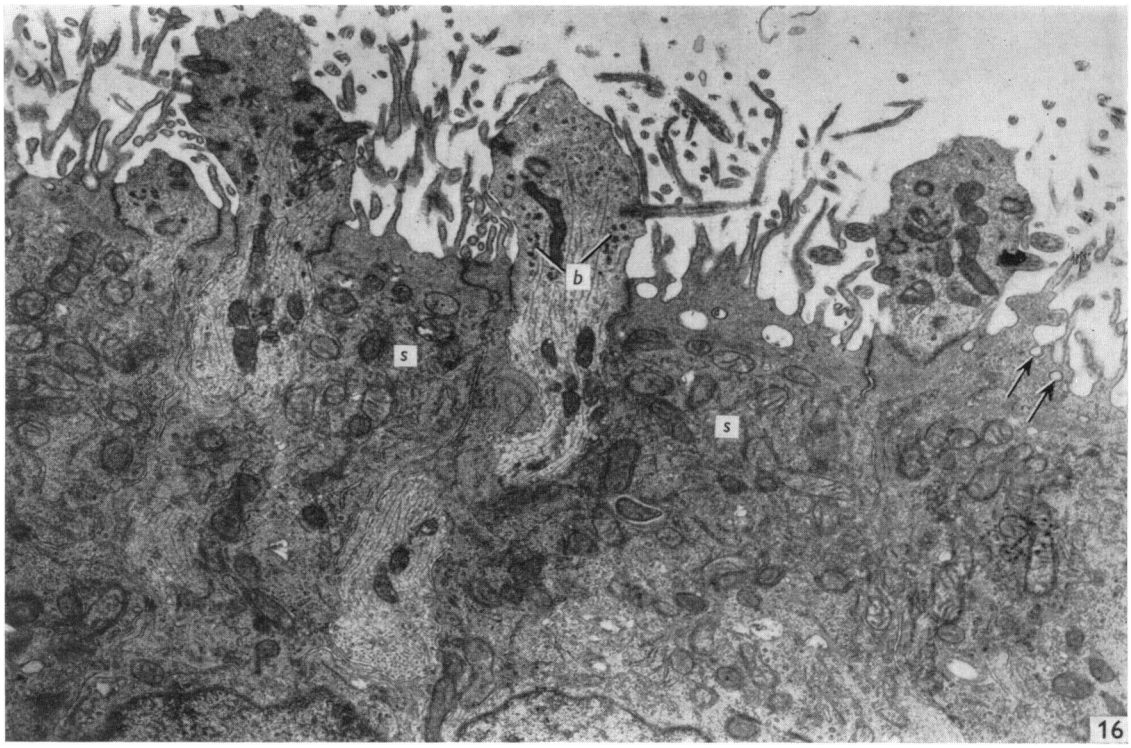


Fig. 14. Section through the epithelium, cut in the horizontal plane, to show arrangement of pale receptor cell bodies in clusters, surrounded by dark cells, at 13 days of gestation. ×11000.

Fig. 15. Section cut as in Fig. 14 but at a more superficial level in the olfactory epithelium, at 13 days of gestation, showing six pale receptor dendrites in a single group, surrounded by dark cells. ×24000.



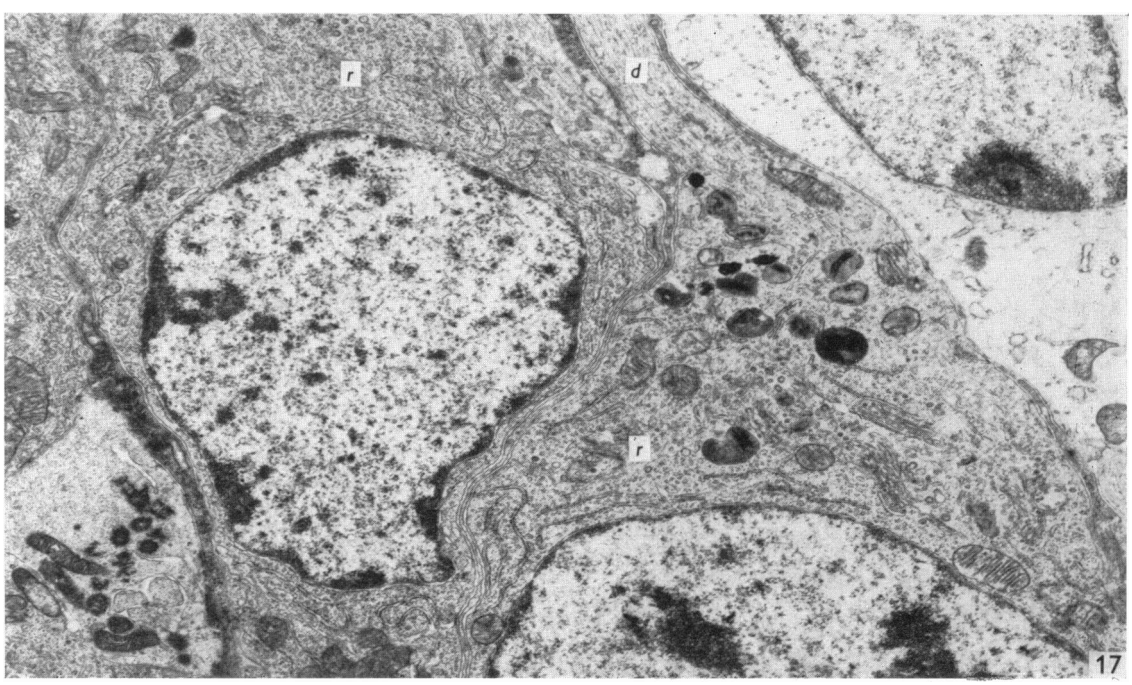
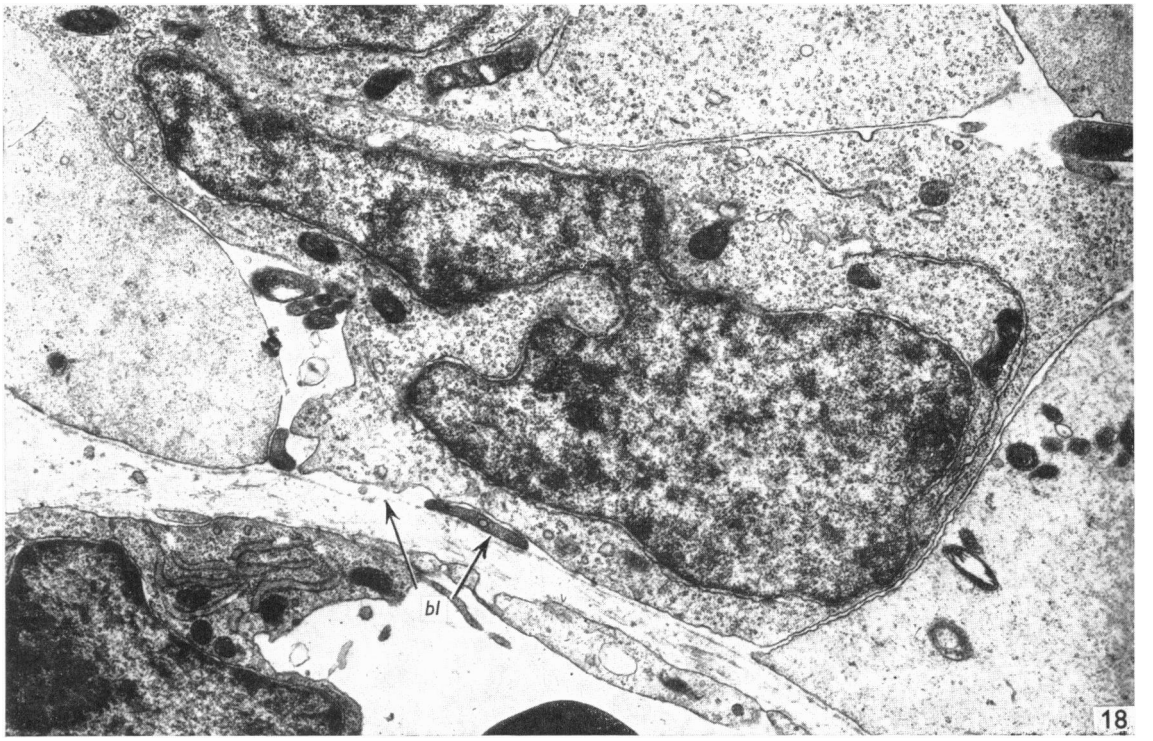


Fig. 16. Surface of olfactory epithelium at 18 days of gestation in vertical section. Cilia are present on the olfactory knobs, in which clusters of dense bodies (*b*) are also visible. The supporting cells (*s*) contain numerous mitochondria and membranes of endoplasmic reticulum; pinocytotic vesicles (arrows) and microvilli are present at the surface. × 7000.

Fig. 17. Vertical section of the olfactory epithelium from a 2 day old mouse showing receptor cell bodies (*r*) and a dendrite (*d*). Note the presence, in the receptor cell body, of lysosomes, Golgi apparatus, smooth and granular endoplasmic reticulum. × 7000.



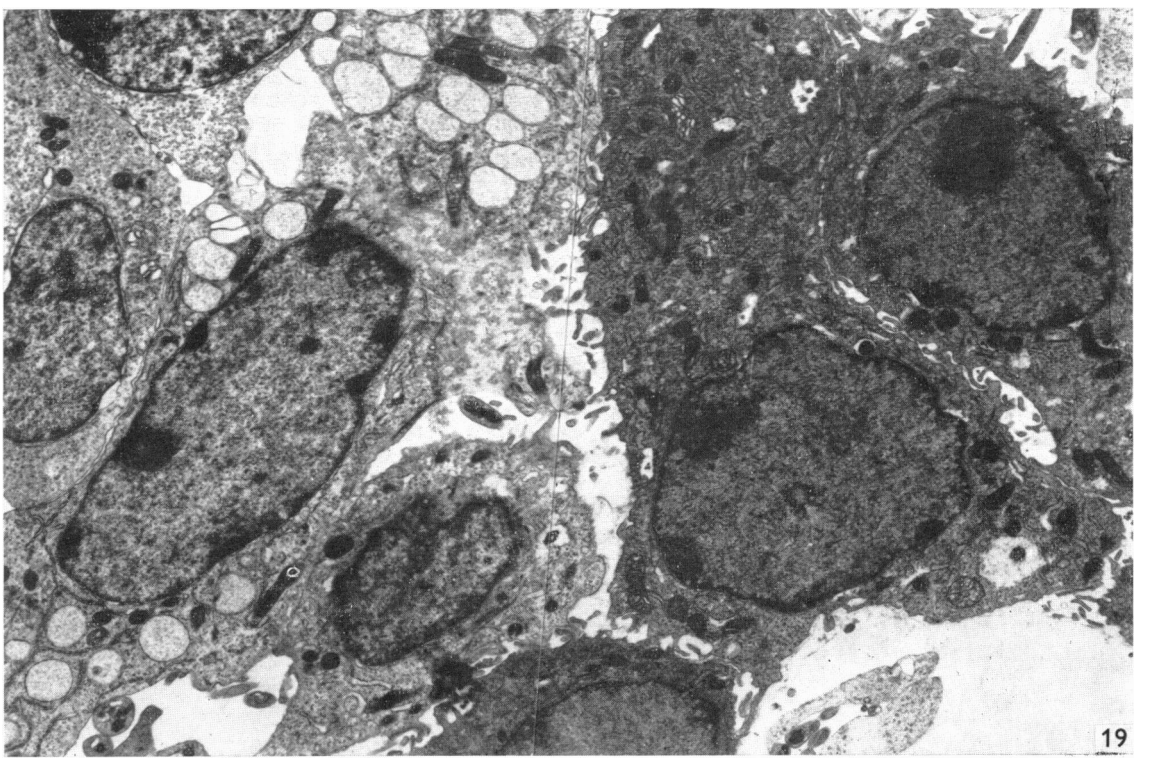
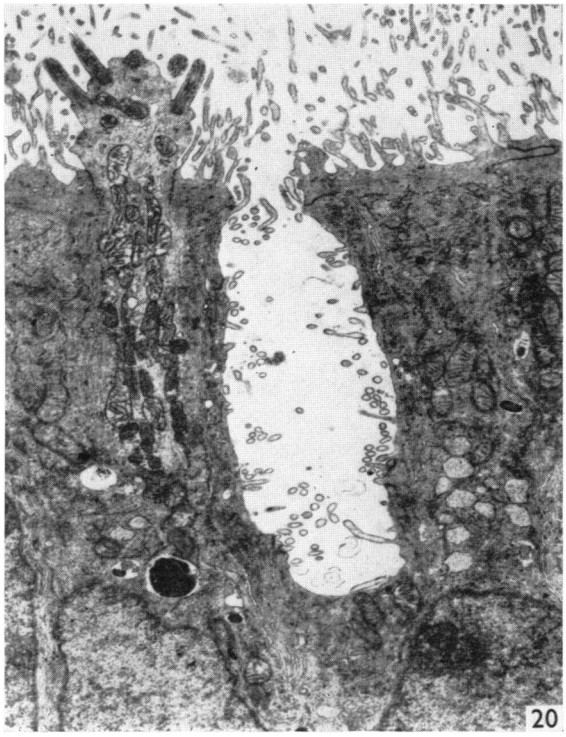


Fig. 18. A differentiated basal cell with an indented nucleus in the olfactory epithelium at 18 days of gestation. Note basal lamina (*bl*). × 6600.

Fig. 19. Section through a Bowman's gland at 18 days of gestation showing a group of dark cells on the right and a group of pale cells on the left, the latter containing secretion vacuoles. × 4500.



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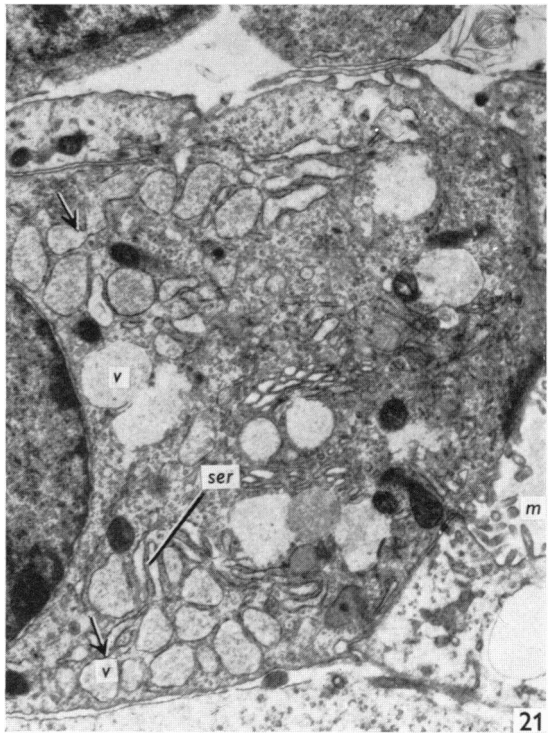

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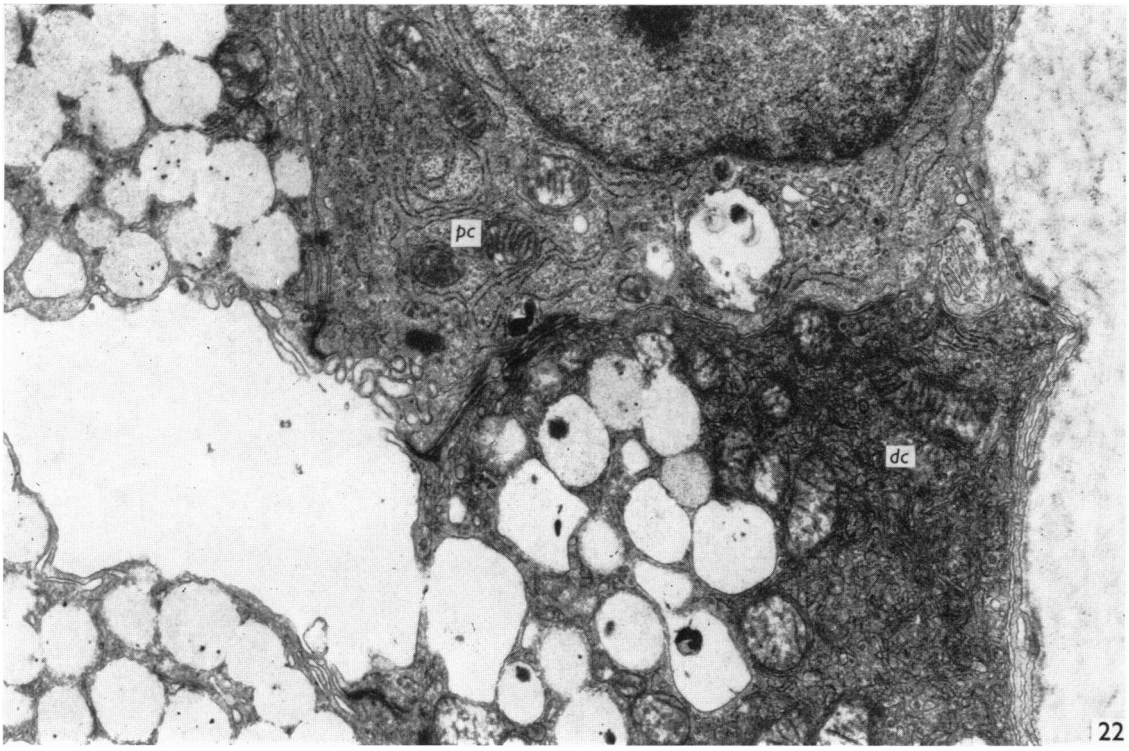

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and very few membrane-bound organelles such as endoplasmic reticulum and mitochondria. Like the dark cells of the early olfactory epithelium their dense appearance was related to the presence of fine granules and fibrils, and such cells were sometimes seen to be in mitosis.

Microvilli were present on the luminal surfaces of both the dark and pale cells, and a number of irregular finger-like projections also occurred at their bases. Junctional complexes were situated close to the lumen, and numerous microfilaments extended across the cytoplasm in association with desmosomes at the cell membranes.

It should be emphasized that this division into pale and dark cells was not always clear-cut, and intermediates were sometimes seen. Furthermore, they did not correspond to the pale and dark cells present in the adult glands of Bowman, as described by Frisch (1967) and which were also observed in the present study (Fig. 21). In adults, the dark cells contained many secretion vacuoles, and their cytoplasmic density was caused by the presence of closely packed cisternae of the agranular endoplasmic reticulum. In contrast, the pale cells, in adults, contained little agranular endoplasmic reticulum and lacked secretion vacuoles; their cytoplasm was largely occupied by granular endoplasmic reticulum.

The ducts of the glands extending vertically through the epithelium (Fig. 20) could be recognized clearly after the 17th day of gestation. Where they passed through the base of the olfactory epithelium the cells lining the ducts were similar to the pale secretory cells of the early glands, but in the more superficial regions they resembled the neighbouring supporting cells in their cellular contents and in the presence of numerous microvilli (see also Breiphol, 1972).

Development of the olfactory nerve fasciculi

As already mentioned, at 10 days of gestation processes similar or identical to developing axons were visible in the mesenchyme adjacent to the olfactory epithelium (Figs. 5, 23, 24). These were present in small groups bounded at least in part by neighbouring cells with the characteristics of developing Schwann cells (Tennyson, 1965), including the presence of copious quantities of ribosomes, large indented nuclei, and an apparently high cytoplasm:nucleus ratio. The cytoplasmic density of such cells varied considerably, and in the earlier stages of development they appeared quite dark (see Figs. 5, 23, 24).

The axons, at 10 days' gestation, contained microfilaments, microtubules, mitochondria and small vesicles, and their diameters varied widely between 0·5 and

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Fig. 20. Duct of Bowman's gland opening at the surface of the olfactory epithelium; and a mature, ciliated olfactory terminal 2 days after birth. Microvilli project into the lumen of the duct which is sectioned obliquely. × 5200.

Fig. 21. Detail of a pale cell of Bowman's glands 2 days after birth. Note continuity (at arrows) between smooth endoplasmic reticulum (*ser*) and secretory vacuoles (*v*). Microvilli (*m*) project into the lumen of the gland. × 8000.

Fig. 22. Section through a Bowman's gland from an adult mouse showing two cell types. The dark cell (*dc*) contains numerous secretory vacuoles, smooth-surfaced endoplasmic reticulum and mitochondria. The pale cell (*pc*) lacks secretion vacuoles but contains profiles of granular endoplasmic reticulum. Note microvilli on the luminal surfaces of the cells. × 9000.

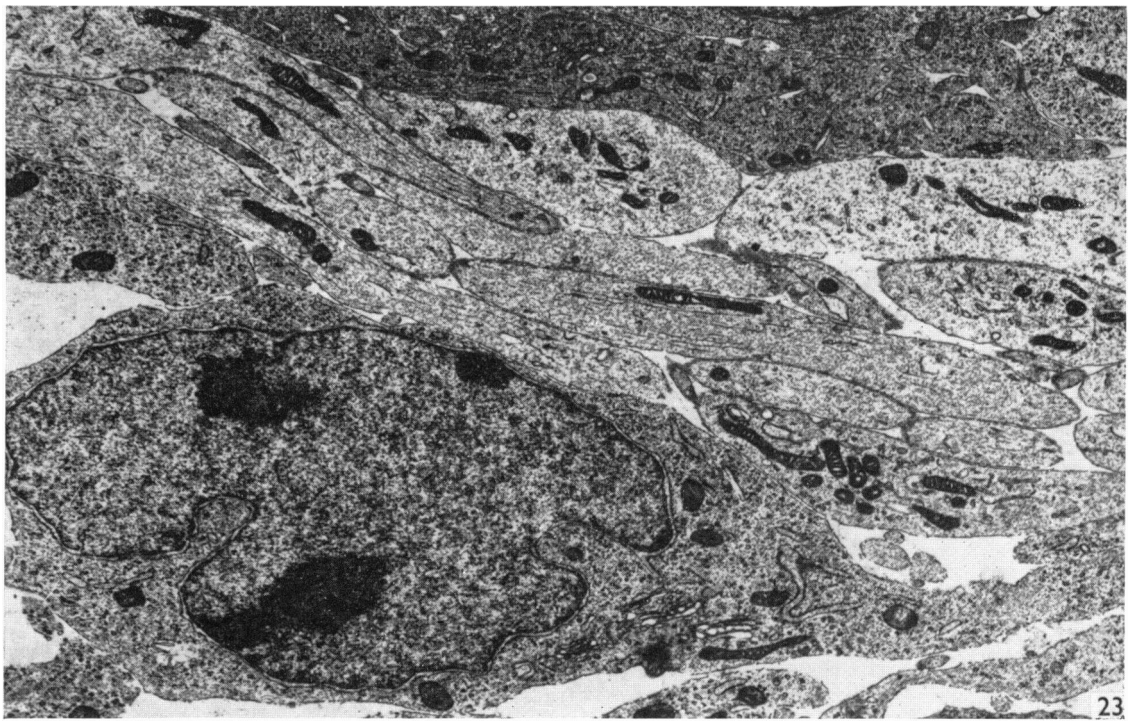
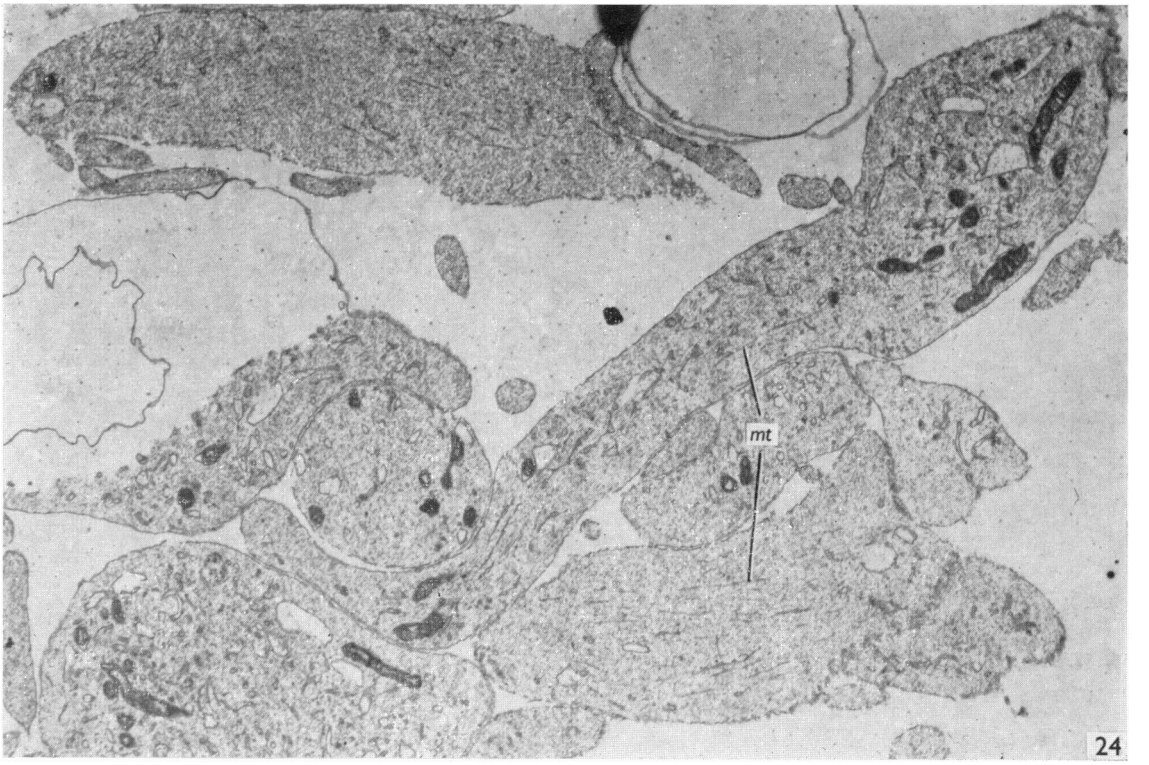


Fig. 23. Cellular processes in longitudinal section in the mesenchyme underlying the olfactory pit at 10 days of gestation. The narrower processes contain numerous microtubules. Mitochondria and vesicles are more abundant in the wider processes. The cytoplasm of the surrounding Schwann cells is relatively electron-dense and contains numerous free ribosomes. × 8000.

2·0 μm, a single axon often showing one or more dilatations in a single longitudinal section (Fig. 24).

From the 11th day of gestation onwards an increasing number of finer axons appeared, some with diameters as low as 0·05 μm. Identification of such axons was made on the basis of their proximity to other generally similar cellular processes within Schwann cell sheaths, and of their content of longitudinally orientated microtubules. A histogram showing the frequency distribution of axonal diameters at various stages of development is shown in Fig. 28. It can be seen that the sizes of axons at 11 days' gestation are markedly diverse, with only 22% lying within the range for adult axons reported by Gasser (1956) and Frisch (1967) and observed in the present study – that is, from 0·05 to 0·3 μm. With increasing fetal age a progressively greater proportion of axons lay within adult limits, and by the 18th day of gestation about 95% of the axons were within this range.

Changes also occurred in the arrangement of axons in nerve bundles. Well formed fasciculi of rounded cross section (Fig. 25) were first visible at 11 days' gestation, although, as already mentioned, less regular groups were already present at 10 days. Later, finger-like extensions of the Schwann sheath penetrated between the axons to separate them into smaller bundles. Mitotic figures were often observed in the Schwann cells, particularly those with a relatively dense cytoplasm (Fig. 27). Such



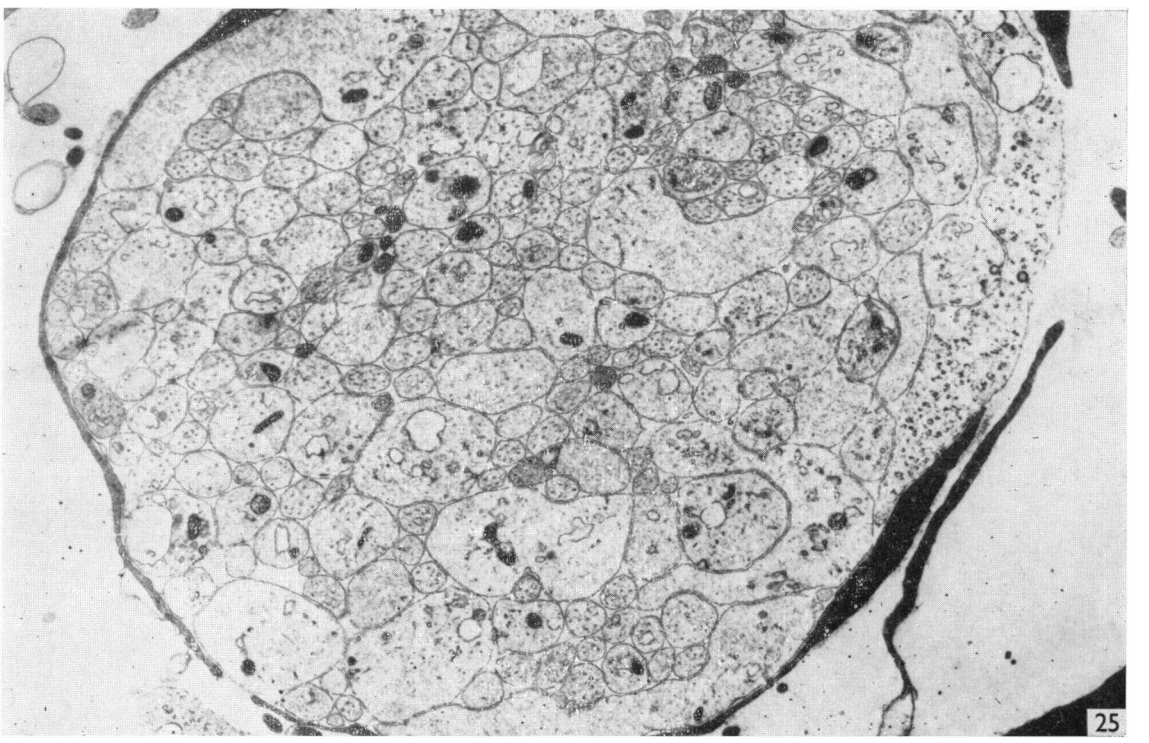
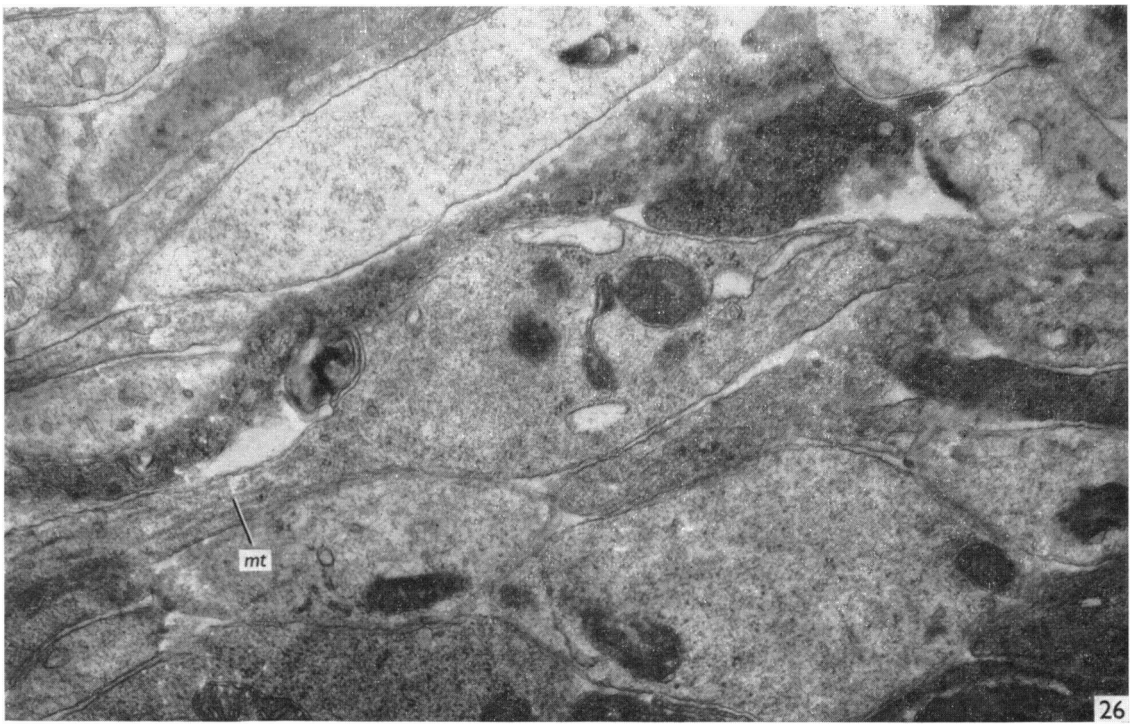


Fig. 24. Dilatation in a cellular process in the mesenchyme underlying the olfactory pit at 10 days of gestation. Mitochondria and smooth-surfaced vesicles are present in the dilated segment; microtubules (*mt*) are present in the narrow and wide segments. × 8000.

Fig. 25. Transverse section of an olfactory nerve bundle at 12 days of gestation. Note variation in the diameters of the axons. The cytoplasmic processes of Schwann cells are of both the dense and pale types. × 7000.



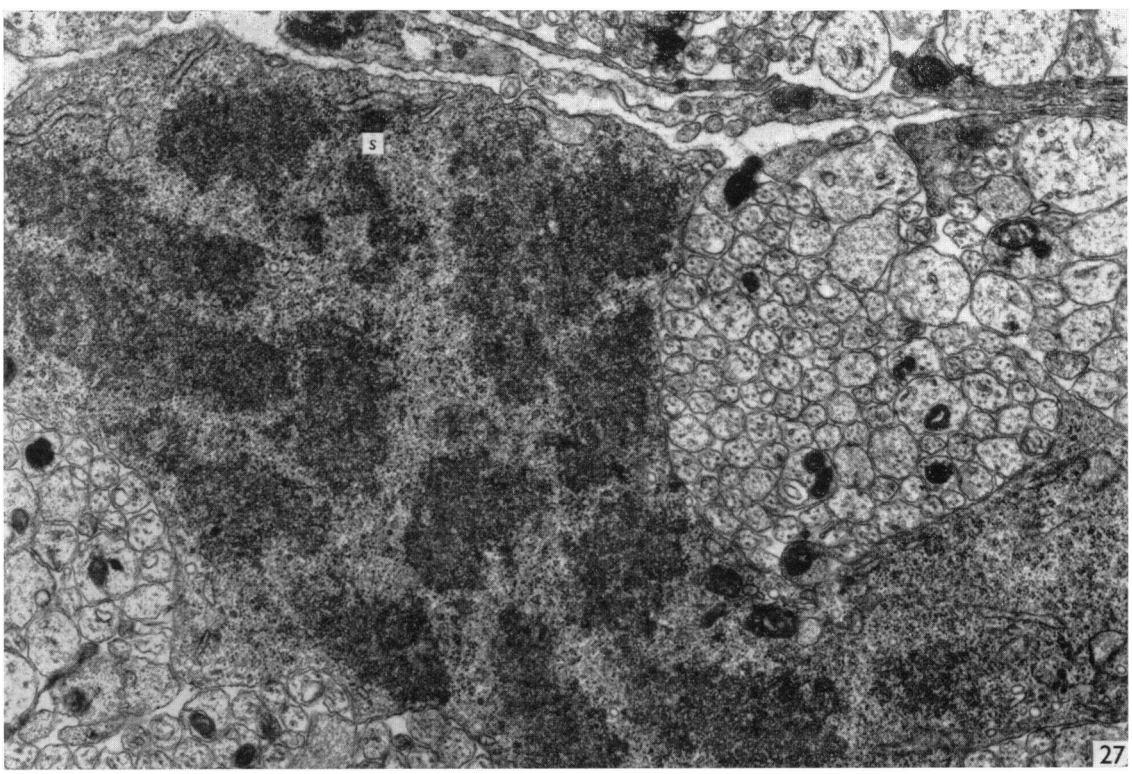


Fig. 26. Longitudinal section through an olfactory nerve bundle at 13 days of gestation showing a dilation in an axon containing microtubules (*mt*). × 36000.

Fig. 27. Transverse section of part of an olfactory nerve bundle at 15 days of gestation showing a Schwann cell (*s*) in mitosis, and two groups of axons. × 8000.

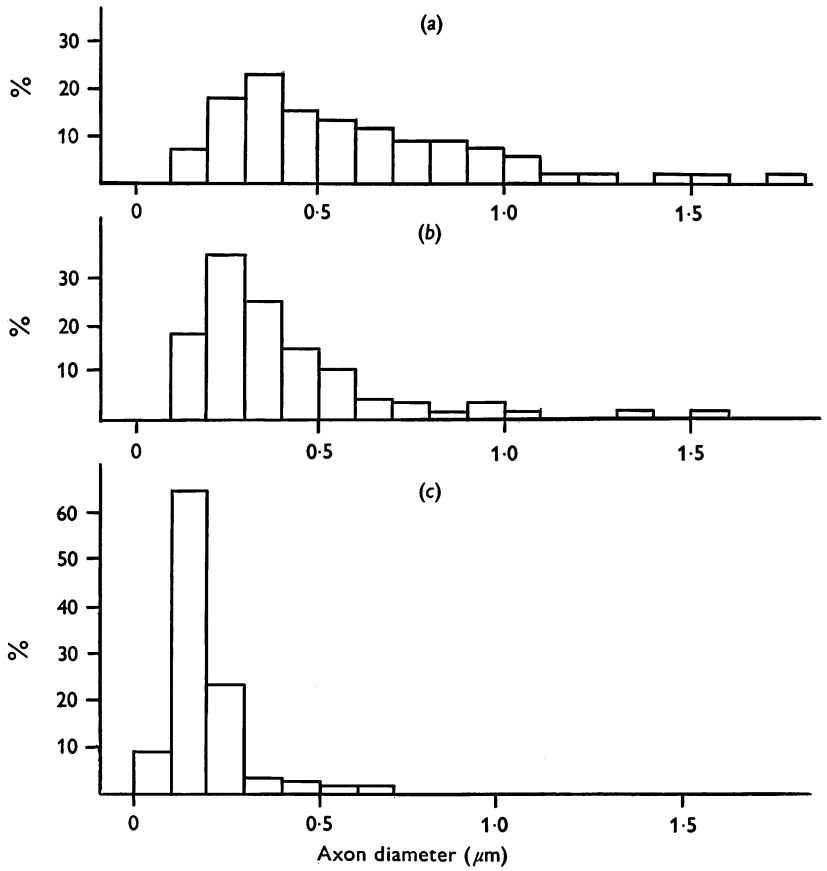


Fig. 28. Histograms showing the distribution of olfactory axon diameters, (*a*) at 11 days of gestation, (*b*) at 13 days' gestation and (*c*) at 18 days' gestation. Note the gradual decrease in range of diameters.

dense cells preponderated before the time of birth. After birth the cytoplasmic density of the Schwann cells decreased, and the amounts of granular endoplasmic reticulum increased, with a parallel reduction in the numbers of free ribosome clusters. At the same time, the number of axons in a single Schwann cell compartment increased greatly (Fig. 27).

DISCUSSION

During the development of the olfactory epithelium several distinct embryological processes appear to be occurring, some sequentially and others simultaneously (see Fig. 29). These may be divided, somewhat arbitrarily, into (1) an initial stage of stem cell proliferation, occurring chiefly during the formation of the olfactory placode, (2) the initial differentiation of receptor cells, marked by the outgrowth of their axons, (3) the formation of receptor dendrites and terminal swellings, (4) the final

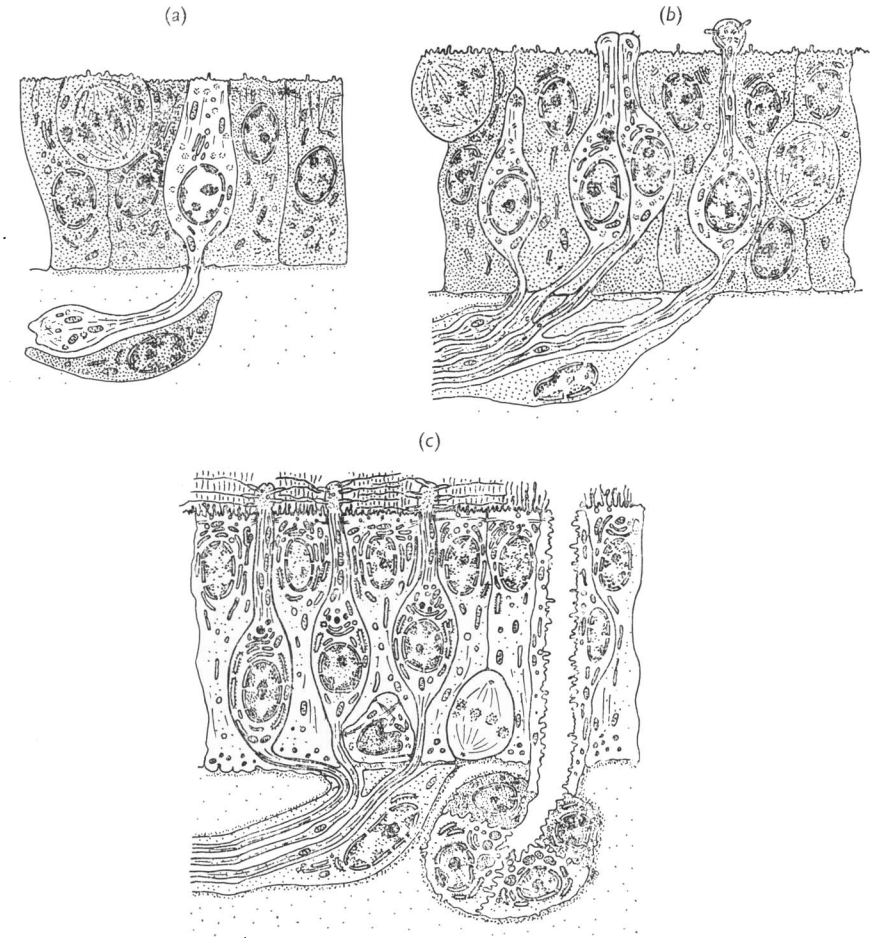


Fig. 29. Diagram summarizing the chief events occurring in the development of the olfactory epithelium from 10 days of gestation onwards. (*a*) Differentiation of pale receptor cell amongst dark stem cells at 10 days, with sprouting of olfactory axon. (*b*) Formation of receptor dendrites and grouping of receptors in clusters. (*c*) Final maturation of receptors, formation of supporting and basal cells, and outgrowth of Bowman's glands with differentiation into dark and pale cells.

steps in receptor maturation and (5) the final differentiation of non-nervous elements – that is, the supporting cells, basal cells and the glands of Bowman. It must, however, be emphasized that since olfactory receptors are formed also in postnatal life (Moulton, Çelebi & Fink, 1970; Graziadei, 1973) many of the events which occur in fetal development are probably occurring after birth, too, although the sequence of differentiation is less clear in the postnatal epithelium.

(1) *Multiplication of stem cells*

By the 10th day of gestation much of this stage of development is presumably already over, since the olfactory placode is fully formed and differentiation of receptor cells has commenced. Light microscope studies have shown that before

the 10th day of gestation, and indeed right up to the 13th day, the pattern of mitotic activity in the epithelium closely resembles that of the developing neural tube, with divisional stages being present only in the superficial marginal zone of that structure (Smart, 1971; Cuschieri & Bannister, 1974). In the present study dividing cells were visible in this position, and it is probable that these had rounded up prior to division in the same manner as the 'matrix cells' of the developing central nervous system (Fujita, 1967). The multiplication of stem cells is probably the chief factor in epithelial area expansion in the developing olfactory chamber of fetal mice, at least in its earlier stages.

(2) *Initial differentiation of receptor cells*

Since some axons are already visible at 10 days of gestation, it follows that receptor differentiation has begun by this time. Also at this stage the epithelial cells can be divided into pale, dark and (less frequent) intermediate cell types. Such axons as have been traced to an epithelial cell are found to connect to pale or intermediate cells, so it appears that at least some of the pale cells are differentiating receptors, and that the dark cells are the original stem cells of the early placode. Such a conclusion is supported by the observation that dividing cells are usually of the 'dark' type, and that, with increasing gestational age, more and more pale cells with the characteristics of receptors are found in the epithelium, with a corresponding decrease in the numbers of dark cells.

The identification of axonal processes at 10 days of gestation is supported by several observations which will be considered in some detail, because it is possible that developing Schwann cell processes, or elements of other non-neural cells could be mistaken for the sprouting olfactory axons (Sandborn *et al.* 1964; Vaughn & Peters, 1967; Grainger & James, 1970). The narrow basal processes of epithelial cells, seen in the early stages of development, show all the expected features of developing axons, including characteristic organelle content, shape, and arrangement with respect to other similar processes. The more expanded regions appear to be either the terminal growth cones or intermittent dilations, since in longitudinal section the thinner, more typically axonal portions can be seen to be in continuity with them. In fact their structure closely resembles that of growth cones, described at a slightly later stage in development by Hinds (1972*b*) and by Hinds & Hinds (1972) in the olfactory bulb. Similar appearances have also been described in other developing neural tissues, such as the spinal cord of fetal monkeys (Bodian, 1966), mouse cerebellum (Del Cerro & Snider, 1968), dorsal root ganglia in rabbits (Tennyson, 1965) regenerating nerves (Estable, Acosto-Ferreira & Sotelo, 1957; Wettstein & Sotelo, 1963; Lampert, 1967) and neuroblasts in tissue culture (Yamada, Spooner & Wessels, 1971). Grainger, James & Tresman (1968) have pointed out that expanded regions of developing axons occur intermittently along their length, as we observed in the present study, so caution must be exercised in identifying terminal growth cones. It is interesting that argyrophilic fibres were observed at the base of the epithelium at 10 days of gestation by light microscopy (Cuschieri & Bannister, 1974), indicating the presence of axons. It appears from this and other lines of evidence discussed above that the cellular processes seen at this stage are indeed axons and not some other type of cellular extension.

(3) *Formation of receptor dendrites*

The next step in receptor differentiation involves the formation of microtubules and clusters of centrioles in the apical parts of the pale cells and the development of an elongated pyriform shape. Such changes are first seen on the 11th day of gestation when silver stained apical processes of receptors can be seen by light microscopy (Cuschieri & Bannister, 1974). Microtubules are characteristic features of dendrites in developing and mature neurons (Lyser, 1964, 1968; Tennyson, 1965): it is interesting that their formation in the olfactory receptors coincides with the acquisition of their typical bipolar shape and the terminal expansion which is initially filled with microtubules (Figs. 7, 8). Other early signs of dendrite differentiation are reduction in ribosome numbers, increase in mitochondria, and the occurrence of centrioles which may either be formed in the dendrites or else migrate there from the cell bodies, where they are first seen to proliferate. Transport of centrioles to the dendrite terminals from the perikarya has also been suggested to occur in adult animals, such centrioles ultimately forming the basal bodies of olfactory cilia (Seifert & Ule, 1967; Heist & Mulvaney, 1968).

(4) *Final maturation of receptors*

Until the 17th day of gestation the general appearance of increasing numbers of developing receptors is basically similar to those formed first on the 11th day, apart from small details of structural change such as a gradual increase in the numbers of cilia, and a change in the shape of the terminal swellings. From the 17th day onwards, however, major changes begin to occur in the perikarya and dendrites. In the perikarya, granular and agranular endoplasmic reticula proliferate, the Golgi complexes enlarge greatly, and lysosomes become abundant, so that the cytoplasm comes to resemble that of mature postnatal cells (Frisch, 1967). Such changes have also been found in the developing neuroblasts of the central nervous system (Lyser, 1964; Meller, Eschner & Glees, 1966) and in dorsal root ganglia (Tennyson, 1965). In the dendrites there is a redistribution of organelles: mitochondria no longer occupy the terminal swellings, which come to be filled with a finely filamentous material, and few microtubules are present in these expansions, which adopt the final mace-like shape of adult receptor endings. Olfactory cilia proliferate greatly and the number of free centrioles in the dendrites is much reduced. These activities probably reflect the specialization of different regions of the dendrite for specific chemoreceptor functions. Around or near the bases of cilia, dense bodies similar to those described in adult vomeronasal endings by Kolnberger (1971) are, at first, numerous but disappear in postnatal life. Such structures are similar to microtubule organizing centres of other cells (see Pickett-Heaps, 1971) and may be concerned with ciliogenesis or the formation of microtubules in the dendrites. At the same time smooth and coated vesicles become quite numerous along the dendrites, and endocytic or exocytic vesicles appear more frequently at the surface of the terminal swellings, indicating an increased traffic between the distal ends of the dendrites and the cell bodies. Such a process has also been suggested in the vomeronasal sensory cells, and may be responsible for the continual renewal of the sensory surface in mature cells, the old, denatured membranes being autolysed in the numerous lysosomes which

appear during late development (Bannister & Cuschieri, 1972; see also Mulvaney, 1971). Whatever the biological significance of these features, it appears that after the 17th day of gestation the receptors have all the features of mature cells and are presumably capable of sensory activity (see also Cuschieri, 1972).

Another interesting aspect of receptor maturation is the way in which receptor cells are arranged with respect to the surrounding cells. Graziadei (1971, 1972) has shown that receptor cell bodies and dendrites are often not isolated from each other by supporting cells, but only by the usual 20 nm intercellular gap which, at the receptor endings, may lessen to a 'close apposition' as part of a junctional complex. In the present study it was found that such an arrangement was common in early development but that as gestation proceeded the dendrites became more and more separated by supporting cells, and contact between adjacent receptor endings was lost. The occurrence of early developmental features in adult animals is perhaps to be expected, since small numbers of receptors are continually being formed and presumably pass through similar stages of development. It would, however, be interesting to know if close contacts between receptor endings are 'gap' junctions, since such structures have been widely associated with developing cells, and may be of importance in coordinating tissue differentiation (see Bennett & Trinkaus, 1970).

The characteristic development of receptors in groups suggests an organized rather than a random pattern of receptor differentiation, and may indicate a mechanism for producing receptors with specific patterns of chemoceptory response. Although receptors appear structurally all alike in electron microscopic preparation, it has been shown electrophysiologically that they react differentially to odours (Gesteland, Lettvin & Pitts, 1965). Such specificity presumably arises during receptor development. It is possible that each receptor in a developmental group acquires a different range of odour specificities, ensuring that a wide variety of receptor types is formed in all parts of the epithelium. Alternatively all the receptors in a group may possess the same odour specificity, which is different from that of adjacent groups.

(5) *The final differentiation of non-nervous elements*

On the 17th day of gestation there is an abrupt onset of differentiation of supporting and basal cells and of the glands of Bowman. The supporting cells appear to be formed directly from the remaining stem cells, the nuclei of which are situated in the most superficial layers of the epithelium. In contrast, the classical basal cells and the Bowman's glands are formed from stem cells with basally situated nuclei. What determines the differentiation of a stem cell into any one of these structures is obscure, but it is possible that the position of the nucleus in the cell is an important factor.

The late differentiation of non-nervous elements is reminiscent of the central nervous system, where it has been suggested that there may be a predetermined 'programming' of differentiation related perhaps to the number of divisions of a particular stem cell (Taber Pierce, 1966; Fujita, 1967; Angevine, 1970). Alternatively, external influences may determine the pattern of cell production, and the practically simultaneous differentiation of all the non-nervous cells is suggestive of the latter rather than the former proposal.

In the developing glands of Bowman a sequence of cellular changes occurs before the mature structure is achieved. Initially, the dark cells are the stem cells of the glands (identified as such by their lack of secretory apparatus and their similarity to the stem cells of the main epithelium) while the pale cells are secretory. In postnatal life, the position is apparently reversed, since the secretory cells then become 'dark' because of the proliferation of agranular endoplasmic reticulum, whereas the seemingly non-secretory cells are now 'pale', as described by Frisch (1967) and others. The significance of the pale cells of the adult is uncertain, but they could represent either resting secretory cells or relatively undifferentiated elements capable of forming new secretory tissue.

The development of olfactory axons

During the development of the olfactory nerve bundles three distinct stages or processes are evident. Firstly, the numbers of axons within the confines of Schwann cell sheaths gradually increase; secondly, the axons decrease in diameter and reach a more uniform size; and thirdly, various changes take place in the arrangement of axons within the Schwann cells so that the axon bundles as a whole decrease in size. The Schwann cells, during these developments, are dividing and maturing in their organelle content.

The early wide variation in axon diameters appears to reflect the presence of numerous growth cones and other dilatations of the sprouting axons, and the gradual reduction in the range of diameters no doubt indicates the smaller proportion of sprouting axons during the later stages of development. The change in numbers of axons within a Schwann cell fasciculus may be correlated with changing mechanical, and also possibly nutritional, interactions between the Schwann cells and the axons. Further work of a quantitative nature is required to explore this aspect of development.

SUMMARY

The development of the olfactory epithelium from the 10th day of gestation to postnatal life has been examined electron microscopically in the mouse. At 10 days' gestation the epithelium is already differentiated into dark and pale cells, the former representing embryonic stem cells and the latter the developing receptors. Axons are also visible at this stage.

At 11 days the first signs of dendrite formation appear, and at 12 days spheroidal terminal swellings containing numerous microtubules are present at the apices of receptor dendrites. Centriole clusters also appear in the receptor cell bodies and dendrites. From the 12th to the 16th day of gestation a few cilia are formed on the receptor endings.

Final steps in the maturation of differentiating receptors begin on the 17th day of gestation, when membranous organelles and lysosomes increase greatly in numbers. However, immature receptors can still be found in the base of the epithelium in postnatal life.

Supporting cells are first recognizable on the 17th day of gestation, derived apparently from the remaining stem cells. At the same time differentiated basal cells and glands of Bowman begin to appear.

In the early development of the olfactory nerve bundles the axons have large and varying diameters, but later on axonal sizes are progressively reduced and the adult size range is achieved at about 18 days of gestation.

The significance of these findings is discussed.

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