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Location and orientation of mitotic figures in the developing mouse olfactory epithelium

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INTRODUCTION

The olfactory epithelium is a simple type of neural epithelium derived, like the epithelium of the central nervous system, from the surface ectoderm of the embryo. Unlike the latter, however, its internal structure remains undisrupted by the development of fibre tracts or an invaginated capillary network, and the nuclei of neurons and neuroglia remain spatially separate. According to standard accounts (e.g. Bloom & Fawcett, 1968) it is composed of three cell types, as depicted in Fig. 2(*f*). The nuclei of the *sustentacular cells* form the most superficial of the nuclear layers and their cytoplasm extends from the apical to the basal surface where it may show some branching. Morphologically at least, they resemble primitive ependymal glia cells. The *basal cells*, also of uncertain function, resemble neuroglia of the peripheral type, in that their cell bodies lie against the basement membrane and their cytoplasm ramifies among the superficial cells. The *olfactory receptor cells* are the most numerous. The six to ten layers of closely packed nuclei forming the bulk of the epithelium belong to these bipolar nerve cells, which have a peripheral process reaching the epithelial surface and a central process passing out of the epithelium to the olfactory bulb. This simple organization seemed to offer a good model for studying the origin and sequential laying down of neuron layers, using the ^{3}H-thymidine labelling technique. It was hoped that the distribution of mitotic figures would be similar to that found in the early stages of development of the neural tube, where dividing nuclei migrate to the apical pole of the cell (Sauer, 1937) and mitotic figures are consequently restricted to the apical or central canal surface of the epithelium, even though the ependymal layer may be as much as ten nuclei deep. This separation of the proliferative compartment is of particular advantage in following the migration of labelled cells.

To check this feature a survey of the location of mitotic figures in the developing olfactory epithelium was made, from the time of the appearance of the olfactory pit in fetal life until maturity. The results, however, showed that while mitotic figures are initially located at the cell apices, early in development they undergo a progressive shift away from the apical to the basal surface where the eventual major proliferation site is found. The olfactory epithelium thus begins its development like the neural epithelium but ends by having its germinal layer at the basal surface, as in other stratified epithelia. The orientation of the mitotic figures was also recorded, in order to find out what role the plane of cleavage played in the origin of deep cells. This too

followed a pattern similar to that found in stratified epithelia such as the epidermis or the oesophageal lining (Smart, 1970*a*, *b*).

The early dispersal of mitotic figures makes the olfactory epithelium less useful for tracing labelled cells, but this unexpected feature, which sets the olfactory epithelium in a position intermediate between the neural and other stratified epithelia, does give a possible insight into the evolution of more complex epithelia.

MATERIALS AND METHODS

The heads of mouse embryos of 11, 13, 15 and 18 days post-conceptional age were fixed in Carnoy's solution, embedded in paraffin wax, serially sectioned at 6 μm in a transverse plane, and stained with haematoxylin and eosin. The heads of 1-, 7-, 10-, 36-, 46- and 58-day-old mice were similarly treated after being decalcified in EDTA. The olfactory epithelium was then searched for mitotic figures and their location and orientation recorded. Counts were continued at each age period until a minimum of 100 figures whose orientation could be determined had been accumulated. The figures given in the results represent the pooled counts from two animals of each age group. The animals were all killed at the same time of day, between 10 a.m. and noon. The criteria for determining the location and orientation of mitotic figures were those used in a similar study on the oesophageal epithelium already reported (Smart, 1970*a*).

Location

A mitotic figure was designated 'apical' if it lay within or above the most superficial layer of nuclei; 'basal' if it lay within, or even partly within, the layer of nuclei adjacent to the basement membrane, and 'intermediate' if it lay wholly within any of the several intervening layers.

Orientation

The orientation of the plane of division may be judged from the orientation of the chromatin in metaphase, anaphase and telophase, as the partition between the daughter cells forms at the mid-point of the spindle at right angles to its long axis. The counts were confined to figures in which the long axis of the spindle lay approximately in the same plane as the section, that is to metaphase plates seen 'edge on', and anaphases and telophases in which the separating chromatin masses could be distinguished without superimposition. Metaphase plates seen edge on were divided into three groups, termed respectively 'vertical' if the angle made with the basal surface of the epithelium was obviously more than 45°, 'horizontal' if the angle was obviously less than 45°, and 'oblique' if the angle was so near 45° that it could not be assigned with any confidence to the other two groups. Anaphases and telophases were similarly classified according to the angle made by the right bisector of a line joining the centre of the two chromatin masses which constitute these figures. The terms 'vertical', 'oblique' and 'horizontal' thus refer to the orientation of the plane of cleavage of the cells, and not to the orientation of the long axis of the spindle. The percentage of obliquely orientated figures can be expected to be much smaller than the other two categories, as they form a boundary class occurring in an arc of only 5° or so on each side of the 45° mark. Cells cleaving vertically in the plane of the

section and in an oblique plane projecting out of the section (see illustrations in Smart, 1970*a*) were not included in the orientation counts, as their orientation was difficult to recognize.

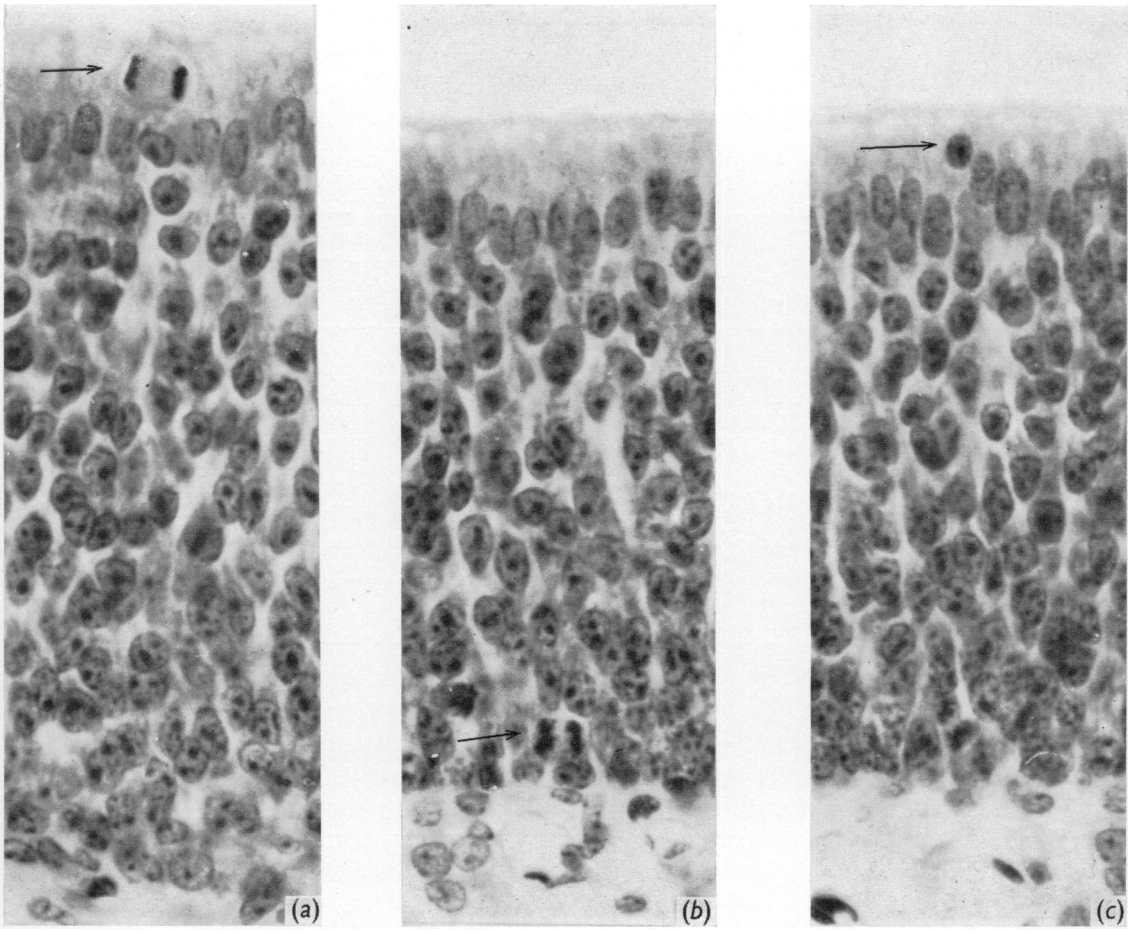

Fig. 1. Photomicrographs of H. & E. sections of olfactory epithelium of 56 day-old mouse. Features mentioned are marked by arrows. (*a*) anaphase at apical surface. (*b*) anaphase at basal surface. (*c*) small dark nucleus in apical cytoplasmic zone. All × 1100.

RESULTS

Histology

At 11 days post-conceptional age the olfactory pit was lined with an epithelium about 50 μm high and 5–6 nuclei deep. By 13 days it had increased to 60 μm and 6–8 layers of nuclei. At 15 days the first signs of differentiation were noticed; the nuclei of the most superficial layer became elongated and paler-staining, as in the mature sustentacular cells (Fig. 1), and the nuclei of the remaining layers became rounder, resembling the nuclei of the neuronal layer of the mature epithelium (Fig. 1). Between

15 and 18 days the epithelium reached a height of 70–90 μm with 8–10 layers of nuclei. By 1 day after birth it had reached its maximum height of 90–100 μm and 10–15 nuclei in depth. Thereafter the only change detected was an increase in depth of the cytoplasmic layer superficial to the sustentacular cell nuclei, from 1–2 μm at birth to about 10 μm at 10 days after birth. Also at 10 days the presence of small, round, dark nuclei lying in the apical cytoplasmic zone was first noticed.

Table 1. *Location of mitotic figures in developing mouse olfactory epithelium*

(The figures in parentheses in the second column give the numbers on which the percentages in the last three columns are based.)

Age (days)	No. of figures counted	Location		
		Apical (%)	Intermediate (%)	Basal (%)
Post-conception				
11	110 (105, 4, 1)	96	3	1
13	207 (108, 28, 71)	52	14	34
15	234 (102, 34, 98)	44	14	42
18	237 (119, 33, 85)	50	14	36
Postnatal				
1	118 (41, 24, 53)	35	20	45
7	109 (18, 31, 60)	17	28	55
10	107 (27, 21, 59)	25	20	55
36	102 (8, 24, 70)	8	24	70
46	104 (14, 4, 86)	13	4	83
58	104 (8, 7, 89)	8	7	85

Location of figures

At 11 days post-conceptional age over 95 % of mitotic figures lay at the apical surface (Table 1). From 13 days onwards mitotic figures became increasingly frequent in the other layers until, in the postnatal period, basally located figures were the most numerous, eventually making up about 80 % of the total. In the intermediate layers, the incidence of mitotic figures rose to between 20 and 30 % of the total by the early postnatal period and then declined, but never completely disappeared even at 58 days.

Orientation of figures

The orientation of mitotic figures had different patterns in the three locations (Table 2). Apical figures were always predominantly vertically orientated, but at all times there was a small percentage of horizontal cleavages. The intermediate figures were orientated with about equal numbers partitioning in each plane at all ages. The basal figures had at all times a slight preponderance of vertically dividing figures.

There appeared to be a progressive decrease in the frequency of mitosis with age, as judged by the increased time taken to perform the counts at successive ages.

Table 2. *Orientation of mitotic figures in the various layers of the mouse olfactory epithelium at different stages of development*

(The figures in parentheses in the third column represent the total count broken down into the constituent vertical, oblique and horizontal orientations in that order.)

Age (days)	Location	No. of figures in each location	Orientation of figures: Vertical (%)	Oblique (%)	Horizontal (%)
Post-conception					
11	Apical	105 (91, 2, 12)	87	2	11
	Intermediate	4 (2, 0, 2)	50	—	50
	Basal	1 (0, 0, 1)	—	—	100
13	Apical	108 (103, 1, 4)	95	1	4
	Intermediate	28 (19, 1, 8)	68	3	29
	Basal	71 (41, 6, 24)	58	8	34
15	Apical	102 (91, 2, 9)	89	2	9
	Intermediate	34 (16, 5, 13)	47	15	38
	Basal	98 (53, 8, 37)	54	8	38
18	Apical	119 (98, 6, 15)	82	5	13
	Intermediate	32 (14, 3, 15)	44	9	47
	Basal	85 (33, 9, 43)	39	10	51
Postnatal					
1	Apical	41 (40, 0, 1)	98	—	2
	Intermediate	24 (11, 1, 12)	46	4	50
	Basal	53 (29, 5, 19)	55	9	36
7	Apical	18 (14, 1, 3)	78	5	17
	Intermediate	31 (16, 3, 12)	52	9	39
	Basal	60 (33, 6, 21)	55	10	35
10	Apical	27 (23, 0, 4)	85	—	15
	Intermediate	21 (6, 4, 11)	29	19	52
	Basal	59 (33, 4, 22)	56	7	37
36	Apical	8 (7, 0, 1)	88	—	12
	Intermediate	24 (8, 3, 13)	33	13	54
	Basal	69 (40, 6, 23)	58	9	33
46	Apical	14 (7, 2, 5)	50	14	36
	Intermediate	4 (1, 0, 3)	25	—	75
	Basal	86 (52, 5, 29)	60	6	34
58	Apical	8 (4, 1, 3)	50	12	38
	Intermediate	7 (0, 1, 6)	—	14	86
	Basal	89 (55, 3, 31)	62	3	35

DISCUSSION

Although mitosis is primarily concerned with the duplication and equitable division of the genetic material between the daughter cells, it is also associated with certain interesting subsidiary mechanisms. According to Sauer (1937) the process of cell division in a columnar epithelium is as shown in Fig. 2 (*a–d*). It includes migration of the nucleus to the cell apex brought about by the 'rounding up' of the cytoplasm towards the more fixed pole of the cell, orientation of the mitotic figure to give a plane of cleavage at right angles to the plane of the epithelium, and the fashioning of a new segment of terminal bar at the cell apex from the equatorial plate of the

spindle. The cells of the olfactory placode in the early stages of development behave similarly. However, as the olfactory epithelium becomes more densely nucleated, modifications occur. Increasing numbers of nuclei no longer migrate to the apical pole of the cell to undergo mitosis, and the planes of cleavage assume a different but consistent pattern in each layer. As discussed elsewhere (Smart, 1970*a*, *b*), changes in the location and orientation of mitotic figures play an important part in the generation of tissue form, but the factors involved in the control of these 'paramitotic' events are virtually unknown.

It is possible to visualise several different ways in which the cells of a pseudostratified tissue such as the olfactory epithelium could vary their behaviour to achieve mitotic dispersal. The origin of apically unattached cells such as the basal cells can be attributed most simply to horizontal cleavages which leave one deep daughter cell. Once established, this 'released' population could proliferate without making the nuclear excursion to the apical surface which seems to be imposed by the apical tether. Similarly, sustentacular and olfactory receptor cells can be visualized as originating from the 'normal' vertical type of apical mitosis, which leaves both daughter cells attached to the terminal bar network. The layers of neuron nuclei increase during development from about 6 to 15, while the basal and sustentacular cells are throughout represented by a single layer of nuclei each. As the number of mitotic figures in the basal and apical layers during the period of maximum neurogenesis (15 days post-conception to birth) is considerably greater than in the neuronal layer itself (Table 1), it follows that both apical and basal mitoses are contributing daughter cells to the neuronal layers. The neurons recruited from basal mitoses could most simply arise from the more superficial daughter cells of horizontal cleavages occurring at the bases of cells which do not retract their cytoplasm from either surface, but remain columnar throughout the mitotic sequence. On the other hand, the peripheral process of neurons originating from cells which are not in contact with the apical surface would be required to re-establish contact with the exterior by separating the terminal bar network, which is noted for its adhesive properties. There is no direct evidence particularly favouring either method.

An additional feature is the continuation of mitotic activity in all layers, but predominantly in the basal layer, into postnatal life, after the maximum number of neuron layers has been built up. This suggests that some turnover of cells is taking place, and it is possible that the small dark nuclei (Fig. 1*c*) visible in the peripheral cytoplasmic layer from 10 days after birth onwards belong to degenerating cells. Neurogenesis in the olfactory epithelium thus has its own complexities and is far from providing the simple model hoped for at the beginning of the investigation.

In the more general context, the changing pattern of mitotic distribution in the olfactory epithelium resembles that found in the development of stratified epithelia such as the oesophagus and epidermis (Smart, 1970*a*). In these tissues mitotic figures are found in all layers in the initial stages of stratification. They then gradually become restricted to the basal layer, which is the definitive germinal layer of stratified epithelia in general. The orientation of the cleavage planes is also similar in all three epithelia; apical figures are at least 90% vertically orientated; intermediate figures tend to random orientation; and basal figures in the early stages cleave predominantly horizontally and in later stages vertically.

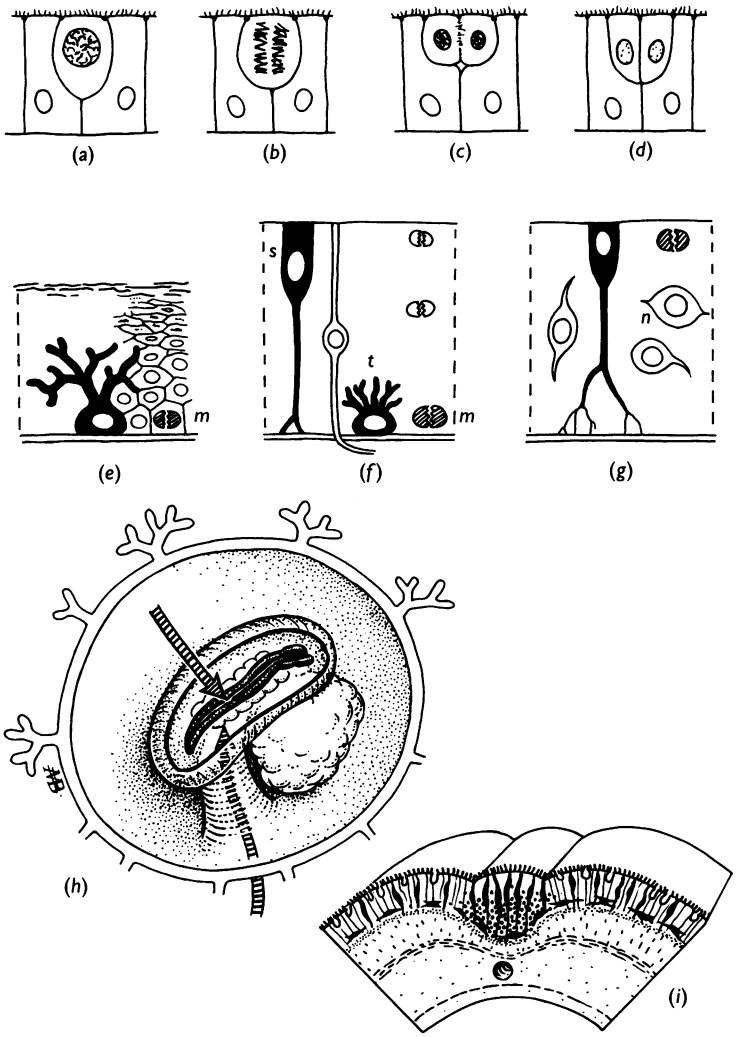

Fig. 2. (*a–d*) Diagrams showing changes occurring in a columnar epithelium during mitosis. (*a*) retraction of cytoplasm to terminal bars during prophase. (*b*) orientation of mitotic figure at right angles to plane of epithelium. (*c*) participation of equatorial plate in formation of terminal bars (after Sauer, 1937). (*d*) daughter cells re-establishing contact with basement membrane. (*e–g*) diagrams comparing structure of various epithelia. (*e*) epidermis with melanocytes (in black) and mitotic figures, *m*, located at basement membrane. (*f*) olfactory epithelium with sustentacular cell bodies (*s*) at apical surface, and mitotic figures, *m*, and basal cells, *t*, at basal surface. The outlined mitotic figures in the apical and intermediate zones are meant to indicate that figures are found in these areas but are infrequent compared to the basal layer. (*g*) primitive neural epithelium with ependymal cell bodies (in black) and mitotic figures both located at apical surface. Neurons (*n*) accumulate in basal part of epithelium. (*h*) diagram of early somite embryo (human) in chorionic sac before initiation of cardiac beat. The two arrows indicate the alternative routes available for oxygen and other metabolites to reach the neural plate. (*i*) diagram of segment of body wall of a hemichordate (after Hyman, 1959) showing nerve cells in black.

The most interesting comparison, however, is with the epithelium of the central nervous system, which is proliferatively and structurally a 'reversed' epithelium. This reversal is clearly noticeable in the early stages of neurogenesis in mammals and in the relatively avascular neural epithelium of lower vertebrates (Fig. 2*g*). Proliferative activity in these cases is restricted to the apical or central canal surface of the ependymal layer, and mature post-mitotic nerve cells accumulate in the mantle layer which is equivalent to the basal aspect of the tissue; the primitive ependymal neuroglia cells are also orientated with their cell bodies at the apical surface and their processes ramify among the underlying cells (Fig. 2*g*). In other stratified epithelia, e.g. epidermis and seminiferous epithelium, the proliferative layer is at the basal surface of the tissue and cells such as the melanocytes of the epidermis (Fig. 2*e*); and the Sertoli cells of the seminiferous epithelium, which are morphologically similar to the primitive ependymal cells, are orientated with their cell bodies at the basement membrane and their processes ramifying in the cells above. The olfactory epithelium occupies an intermediate position (Fig. 2*f*). Proliferatively it tends towards the epidermal pattern (cf. Fig. 2*e* and 2*f*) and structurally it has much in common with a primitive avascular neural epithelium (cf. Fig. 2*f* and 2*g*).

A possible explanation for the variation in the organization of these different types of epithelium may be found in the times at which they develop relative to the efficient functioning of the cardiovascular system. The epithelium of the central nervous system begins its development well in advance of the start of the embryonic heart beat, and thus evolves in a radically different environment from epithelia which thicken and stratify after an adequate capillary circulation has appeared. The best-documented information available for examining the development of the various epithelia of the body relative to other body systems comes from the chick embryo (Romanoff, 1960). Here the heart beat commences at the 9–10-somite stage, 36–40 h after the beginning of incubation, whereas 20 h previously, at the 2-somite stage, the epithelium of the neural fold is already several nuclei deep. The olfactory placode, by contrast, does not appear until the 23- or 24-somite stage, well after the embryonic circulation has been established, and epidermal stratification begins much later still. During the avascular stage the neural epithelium is presumably respiring by diffusion over the shortest route to the exterior. In the chick this is through the apical surface of the neural plate cells, which are at this stage in direct contact with the albumen (in birds the amnion is not present at these early stages as a complete membrane, and does not form a closed cavity until the 39-somite stage). In mammals the situation appears to be similar. In the rat, for example, the heart primordium starts to beat at $9\frac{1}{2}$ days (Goss, 1940), at a time when the epithelium of the neural fold is already several nuclei deep. The shortest diffusion route from the maternal circulation at this time is directly from the surface of the neural groove through the cavities of the amnion and extra-embryonic coelom, rather than through the cellular spongework of the body stalk (Fig. 2*h*).

In the phylogenetic sequence a similar pattern is observed. One of the most primitive vertebrate ancestors is the hemichordate *Balanoglossus*. The anterior segment of its nervous system is a tube with open neuropores (Hyman, 1959). Posteriorly it continues as a strip of cells and fibres lying among the basal portions of the columnar epidermal cells (Fig. 2*i*). The form is basically similar to that found in the embryonic

development of higher vertebrates when the neural groove is partially fused. These primitive vertebrates respire through the skin, as the gills are primarily concerned with filtering out food, and the pigmentless blood is said to leave them less well oxygenated than when it entered (Young, 1950). The central nervous system in early phylogeny and ontogeny may therefore conduct its respiratory exchanges directly with the exterior of the organism, and this could account for the structural reversal of the initial stages of this tissue.

The mammalian olfactory epithelium develops after the establishment of a circulation, but never loses contact with the external environment, and it may be that its retention of the primitive ependymal type of glia cell plays a role in connecting the neurons in this thick, densely nucleated, but nevertheless avascular, tissue with the atmospheric oxygen, which is at a higher partial pressure than that of the blood. On the other hand cell proliferation, which requires the raw materials for the synthesis of new cells, would ease these supply problems by locating itself at the capillary surface.

SUMMARY

The location and orientation of mitotic figures in the developing mouse olfactory epithelium were recorded at various time intervals between 11 days after conception and 58 days of postnatal life. Mitotic figures were restricted initially to the apical surface of the tissue, but spread later to all layers and after birth were most numerous in the basal layer. Olfactory neurons thus appear to be derived from apical and basal mitosis as well as from proliferation *in situ*. The persistence of mitotic figures in the young adult suggests that some cell renewal is occurring in the mature epithelium. Structurally and proliferatively the olfactory epithelium occupies an intermediate position between the neural epithelium and stratified epithelia generally. Possible reasons for this are discussed.

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