Changes in location and orientation of mitotic figures in mouse oesophageal epithelium during the development of stratification

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INTRODUCTION

When a cell in a simple columnar epithelium undergoes mitosis the nucleus moves towards the apical surface of the epithelium and the plane of cleavage occurs more or less at right angles to the epithelial surface, so that the resulting daughter cells come to lie side by side in the plane of the epithelium. In stratified epithelia, on the other hand, mitotic figures are restricted to the basal surface of the tissue, and give rise to daughter cells which lie in different strata. The location and orientation of mitotic figures during this transition has been examined in the mouse oesophageal epithelium which changes during embryonic development from a layer of simple columnar cells to a stratified squamous cornified layer, and the results obtained indicate that the change over to basally located mitotic nuclei is associated with rotation of the plane of cleavage. The investigation is essentially a continuation of the work of Sauer (1935, 1936, 1937) on the morphology of epithelia which was terminated by his death. Sauer's studies in their turn had their antecedents in the work of several investigators in the latter half of the last century who were interested in the relation of the plane of cell division to the three-dimensional growth of epithelia, e.g. Rauber (1882), Flemming (1885), Merk (1885). More recently, Marques-Pereira & Leblond (1965) published a study of cell migration in the adult rat oesophageal epithelium which touched incidentally on the topic of the plane of cell cleavage. The results of a more general pilot survey of the location and orientation of mitotic figures in a variety of epithelia with which the present author was associated have already been reported (Doig & Smart, 1966). The subject of mitotic orientation is also of some interest outside the epithelial context as the generation of the form of a tissue or organ, or whole animal for that matter, may stem not only from the migration of post-mitotic cells to a position away from their site of origin, but also from the laying down of cells in positions predetermined by the plane of division.

MATERIALS AND METHODS

The thoracic regions of mouse embryos of 11, 13, 15, 18 and 20 d post-concepcional 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 thoracic segments of the oesophagus of new-born, 7- and 14-d-old, and 6-month-old post-natal mice were similarly treated.
The oesophageal 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. For the location counts all recognisable mitotic figures were included, while for the orientation counts only those whose orientation could be unambiguously determined were used. The figures represent the pooled counts from 2 animals of each age group. The animals were all killed at the same time of day, between 10 a.m. and noon.

Criteria for location of mitotic figures

During the simple columnar phase a figure was designated ‘apical’ if it lay predominantly within the apical half of the epithelium and ‘basal’ if it lay predominantly in the basal half. In the stratified phases the location of a figure was described by the layer in which it occurred. Figures lying partly in one layer and partly in another were considered to lie in the deeper layer. All visible stages of the mitotic cycle were used in these counts. As they do not differ essentially from the orientation counts which were made on a more restricted group of figures (vide infra), they are not listed separately, but can be inferred from the figures in the fourth column of Table 1.

Criteria for orientation of mitotic figures

During mitosis the partition between the daughter cells forms at the middle of the spindle at right angles to its long axis. The orientation of the plane of division may be judged, therefore, from the orientation of the chromatin in metaphase, anaphase and telophase.

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 anaphase and telophase in which the separating chromatin masses could be distinguished without superimposition as in the diagram in Fig. 1a.

Metaphase plates seen edge on were divided into three groups termed ‘vertical’, if they made an angle with the basal surface of the epithelium which was obviously more than 45°, ‘horizontal’ if this 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’ and ‘horizontal’ thus refer to the orientation of the plane of division of the cells and not to the orientation of a long axis of the spindle.

Cells dividing vertically in a plane parallel to that of the section as in Fig. 1b, and in an oblique plane projecting out of the section as in Fig. 1c were not included in the orientation counts, as except for metaphase plates seen ‘full face’, their orientation was difficult to recognise. The incidence of oblique and vertical division given in the counts is therefore too low. An attempt was made to correct this by doubling the counts of vertically and obliquely orientated figures before calculating the final percentages of the different orientation. This assumed that vertical and oblique divisions were occurring as frequently in the plane at right angles to the section
Fig. 1. Diagrams showing orientation of mitotic figures in relation to plane of section. (a) Mitotic figure with spindle lying within the plane of the section; the partition between the daughter cells will form at right angles to the plane of the section, giving vertical division. (b) Mitotic figure with spindle at right angles to the plane of the section. In this case the partition between the daughter cells will form in a plane parallel to that of the section, giving vertical division as in (a). (c) Mitotic figure also lying in a plane at right angles to the plane of the section but obliquely orientated within this plane and consequently giving rise to oblique partitioning. In both (b) and (c) the mitotic figure tends to be incompletely included in the section, or to be seen as super-imposed chromatin masses. As no attempt was made to include such figures in the counts the estimate obtained of vertical and oblique partitioning was too low and required correction as explained in the text.

Fig. 2. Histograms showing orientation of mitotic figures in the various layers of the mouse oesophageal epithelium at different stages of stratification.
as in the actual plane of the section. Reference to Table 1 in which both crude and corrected counts are given shows that this correction does not in any case give rise to a pattern greatly different from that given by the crude figures.

FINDINGS

In its simple columnar phase (up to about 11 d) mitotic figures in the oesophageal epithelium are all located at the apical surface (Table 1, fourth column), and are orientated to give a plane of division at right angles to its surface (Table 1; Fig. 2).

On becoming two cells thick (about 13 d) about 85 % of mitotic figures are located in the basal of the two layers (Table 1). About 85 % of these are orientated to give a plane of division parallel to the epithelial surface. Of the 15 % of figures found in the apical layer, on the other hand, the majority retain the original orientation giving a plane of division at right angles to the tissue surface (Table 1). By 15 d the epithelium is 3 cells thick. The percentages of mitotic figures in the apical and basal layers and the pattern of orientation are similar to that found at 13 d. A small population of mitotic figures at this stage can be attributed to the middle or intermediate layer, and their orientation is less consistent than that of the other layers.

Table 1. Orientation of mitotic figures in the various layers of the mouse oesophageal epithelium at different stages of development

(The figures in parentheses in the fourth column represent the total count broken down into the constituent vertical, oblique and horizontal orientations in that order. The corrected percentages are calculated from a total obtained by doubling the number of vertical and oblique mitoses for reasons explained in the text.)
Orientation of mitotic figures

With the appearance of a fourth layer of squamous cells at the epithelial surface about 18 d of post-conceptional age, mitotic figures are virtually restricted to the basal surface and about 80% of these are horizontally orientated. At subsequent ages the basal layer retains the mitotic monopoly, while there is a progressive reduction in the percentage of horizontally orientated figures from about 50% in the perinatal period to 20% or so thereafter.

Discussion

In the simple columnar phase of its development the mitotic pattern in the oesophageal epithelium was similar to that found in other columnar epithelia, the nucleus appeared to migrate to the apical pole of the cell, and in over 90% of cases the mitotic figures were orientated to give a plane of cleavage at right angles to the surface with daughter cells lying in the plane of the epithelium. Sauer (1936) demonstrated that when a columnar cell enters mitosis the rounding up of the cytoplasm which accompanies this event results in the cytoplasm retracting from the basement membrane towards the terminal bars where the cell is most firmly adherent to its neighbours. The usually basally located nucleus is carried with the cytoplasm and thus occupies an apical position. Sauer also showed that in a columnar epithelium the mitotic process includes the fashioning of a new segment of terminal bar between the daughter cells from the mid-portion of the spindle fibres. In a simple epithelium, therefore, the process of cell division includes a mechanism for orientating the mitotic figure and reconstituting the intercellular attachments. Simple crowding does not seem to upset this process. In the embryonic neural epithelium, for example, nuclear crowding is extreme, pseudostratified nuclei lying 8–10 deep, and yet nuclear migration to the cell apex and vertical division is retained (Sauer, 1935; Doig & Smart, 1966).

The widespread rotation of mitotic figures to give horizontal cleavage and presumably a different type of intercellular attachment for the deeper daughter cells, observed when the oesophageal epithelium stratifies, therefore, reflects an active modification of the mitotic process and is not the result of mechanical pressures of adjacent cells. The existence of a mechanism for orientating mitotic figures has long been suspected. Wilson (1925), investigating the complicated yet predictable cleavage patterns of vertebrate and invertebrate ova, was of the opinion that the mitotic spindle adjusted its position prior to partitioning to give the appropriate cleavage plane, and that cell movement after division played little part in building the geometrical patterns which characterise these early stages of development. Although some thought has been given to the matter, the factors controlling spindle orientation in the early stages of development are unknown (Waddington, 1956). In the present study it is interesting to note that a gradient seems to exist between the deep and superficial layers in the early stage of stratification. For example, in the specimens at 15 d of post-conceptional age, horizontal cleavage has an incidence of 78% in the basal layer, 32% in the intermediate layer and 9% in the superficial layer (Table 1, last column). This may reflect the decay of some influence arriving at the basal surface of the epithelium.

Once stratification was established the pattern of orientation changed. The pro-
portion of horizontal cleavage declined until shortly after birth; thereafter they constituted about 20% of the corrected count. This implies that in the mature oesophageal epithelium the superficial layers receive cells not only from the superficial daughter cells of horizontal cleavages but also from the other cells of the basal layer, whose number is being augmented by the daughter cells of vertical divisions, but whose area is remaining constant. It is worth noting, without necessarily implying a connexion, that the drop in the percentage of horizontally cleaving cells occurs about the same time as the appearance of keratinisation (Table 1).

Marques-Pereira & Leblond (1965) reached a broadly similar result in their study of the migration of ³H-thymidine labelled cells in the oesophageal epithelium of adult rats. They were of the opinion, however, that horizontal cleavage contributed only 4% of the cells reaching the superficial layers from the basal layer, the remaining 96% originating randomly from post-mitotic basal cells migrating upwards. The results from the adult mouse epithelium would suggest that in this species any random component of superficial cell origin would stem from the possible random orientation of the mitotic figures. Whatever the reason for the difference in the estimates of the different types of cleavage between rat and mouse may be, it is apparent that in the mouse horizontal cleavage does not seem to play as important a part in the maintenance of stratification as it does in its initiation.

SUMMARY

The location and orientation of mitotic figures were recorded in the oesophageal epithelium of embryonic and adult mice as it developed from a single layer of cells to a stratified system. During the initial simple columnar phase all mitotic nuclei lay at the apical pole of the cell and 94% of those were orientated to give horizontal cleavage. At the onset of stratification when two layers of cells were present 85% of mitotic figures were found in the basal layer and 85% of these were orientated to give horizontal cleavage with one daughter cell in the basal layer and one in the superficial layer. The percentage of horizontally orientated figures declined as development proceeded; in the perinatal period about 50% of figures were so orientated; by 7 days the proportion had dropped to 20%, where it seemed to remain.

It is concluded that the introduction of stratification is associated with a rotation of mitotic figures to give horizontal cleavage, whereas in the mature epithelium only about one fifth of superficial cells originate from horizontal cleavage and the majority are recruited from the daughter cells of previous vertical cleavages.

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REFERENCES


