CHANGES IN THE SIZE AND STRUCTURE OF THE NUCLEOLUS OF COLUMNAR CELLS DURING THEIR MIGRATION FROM CRYPT BASE TO VILLUS TOP IN RAT JEJUNUM

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SUMMARY

An image analyser was used to measure the area of the nucleolus and its component parts in columnar cells at six levels of the jejunal epithelium, corresponding to stages in cell migration from crypt base to villus top.

In columnar cells of crypt base, which function as stem cells for the epithelium, the nucleolus is large (3.1 \( \mu m^2 \)), irregular and reticulated. As cells migrate up the crypt, divide and differentiate, the nucleolus decreases in size (1.7 \( \mu m^2 \)) and becomes spherical, but remains reticulated. In the fully differentiated cells of the midvillus, however, the nucleolus becomes small (0.9 \( \mu m^2 \)) and compact. At the villus top, as the cells display early signs of degeneration, the nucleolus is further compacted (0.5 \( \mu m^2 \)).

Most nucleolar components also decrease in size. Pars fibrosa (about 19% of the nucleolar area in crypt base) and pars granulosa (about 70%) decrease in proportion to the rest of the nucleolus, except in mid-villus and villus top where loss of pars granulosa predominates. In contrast, the total area of fibrillar centres remains constant (about 0.1 \( \mu m^2 \)), even though individual centres are small and numerous in crypt base, larger and fewer at higher levels, and they coalesce into a single structure in villus top. The other nucleolar components are also segregated into distinct, but adjacent, areas at this level.

The changes in size and structure of the nucleolus taking place during the migration of columnar cells can be correlated with the maturation of the cells and the loss of their ability to synthesize ribosomal RNA.

INTRODUCTION

The epithelium of small intestine is continually being renewed. Thus, in the rat, the columnar cells arising from mitosis in the crypts migrate up to the extremity of the villi in about 2 days and are shed to the lumen (Leblond & Messier, 1958). In the course of this migration, columnar cells exhibit marked structural changes (Cheng & Leblond, 1974) and produce enzymes that complete the digestion of sugars and proteins (Nordstrom, Dahlqvist & Josefsson, 1968; Riordan & Forstner, 1971). The transformation of the reticulated nucleolus of crypt base cells into a compact structure in villus cells is a remarkable change (Adamstone & Taylor, 1972; Altmann, 1980; Leblond, 1981). However, this evolution of the nucleolus has not been described in a precise, quantitative manner, nor has it been related to the other changes that columnar cells undergo in the course of their migration. In this work, the sizes of the nucleolus and its component parts were measured at six levels of crypt and villus. The evolution
in the nucleolus was then discussed in relation to the other structural and functional changes taking place in the migrating columnar cells.

MATERIALS AND METHODS

Six male Westar rats weighing 150-200 g and fasted overnight were sacrificed under chloroform. A 1–2 cm long piece of jejunum was excised about 2 cm below the Treitz ligament, opened and flattened on a piece of cardboard with the mucosal surface facing upwards. After cleaning off mucus and debris with a gentle jet of lukewarm Hank's solution, the sample was immersed for 30 min in a solution containing 3% glutaraldehyde, 0.5% formaldehyde and 5% sucrose in cacodylate buffer at pH 7.2. Pieces of jejunal wall 1 mm wide were cut out and placed in a solution of the same fixative for one more hour. The pieces were postfixed in 1%, osmium tetroxide solution, dehydrated in a series of ethanol, cleared in propylene oxide and embedded in low-viscosity Epon (Spurr) in such an orientation that villi and crypts could be sectioned longitudinally. The sections were routinely stained with uranyl acetate and lead citrate.

In addition, three 45 g, male, non-fasted Sherman rats were perfused through the left ventricle of the heart with 3% glutaraldehyde in 0.8 M-phosphate buffer at neutral pH; and pieces of jejunum were processed as above.

Using only crypts and villi cut along their long axes, the jejunal epithelium was investigated at six levels identified by the position of their cells. The position of a cell in the crypt was defined by the number of nuclei separating it from the crypt bottom (Cairnie, Lamerton & Steel, 1965; Cheng & Leblond, 1974). Three crypt levels were arbitrarily defined as follows, in the 150-200 g rats: (a) 'crypt base', comprising the cells occupying the six lowest positions; (b) 'crypt top', the cells in the uppermost six positions; and (c) 'mid crypt', the cells in the intervening 10–25 positions.

Similarly, three villus levels were identified: (a) 'villus base', comprising the cells in the first ten positions above the crypt mouth; (b) 'villus top', the cells in the last ten positions at the extremity of the villus; and (c) 'mid villus', the cells in the intervening 40–60 positions. A record was also kept of whether a cell was in the lowermost, intermediate or uppermost third of the mid villus.

The number of nucleoli in columnar cells was examined in the light microscope by Louise Quentin (unpublished) using the method of Shea & Leblond (1966). She found from 1 to 5 nucleoli per nucleus, but the mean numbers at the six levels were between 3.0 and 3.5 without significant difference between any two levels. Hence the number of nucleoli did not change as cells migrated from crypt base to villus top; and, therefore, any variation in the size of nucleoli should not be related to changes in their number.

The present investigation consisted in locating a field at a given level under low magnification (× 5000). The nuclei of the columnar cells present were examined, whereas those of mucous, entero-endocrine and Paneth cells were ignored. The largest nucleolus within a field was selected, since it was likely to be cut through its central region. It was then photographed at × 19,500 and printed at × 64,000. A total of 125 photographs of nucleoli were analysed quantitatively, with a minimum of 10 per level.

Nucleolar parts were identified as follows. The pars fibrosa was defined as the densest component arranged in the form of cords or spread out fields, in which packed 4–5 nm dots and filaments could be distinguished. A few of the 15–20 nm granules seen mostly in the pars granulosa were occasionally present. Fibillar centres were light grey, fairly homogeneous, approximately circular areas, slightly denser than the nuclear sap and surrounded partly or completely by cords of pars fibrosa. In contrast, 'interstitial spaces' were light areas that, in density and overall appearance, resembled the nuclear sap. The pars granulosa occupied the rest of the nucleolus and consisted of areas less dense than the pars fibrosa and mainly composed of 15–20 nm granules, which were mixed with 4–5 nm filaments in widely varying proportions.

A Zeiss MOP-3 image analyser was used to measure the area occupied in the micrographs by the nucleolus and its components. The nucleolar micrographs from the six levels of the epithelium were randomly arranged; the nucleolus was outlined with a colour pen (Fig. 1) and the nucleolar components were outlined in different colours. By tracing the outlines with the MOP pencil, the areas were measured in the following order: whole nucleolus, vacuolar spaces, fibillar centres and pars fibrosa. The pars granulosa occupied the rest of the nucleolus and its area was obtained by subtraction.
RESULTS

Structure of nucleoli at six levels of the jejunal epithelium

A description of the nucleolus at each level will be preceded by a brief summary of the features of columnar cells (Cheng & Leblond, 1974; Falconer & Altmann, 1979; Leblond, 1981; Falconer, 1982).

Crypt base. The columnar cells present at this level function as stem cells for the epithelium; they are small proliferative cells characterized by embryonic features: a smooth plasma membrane, a cytoplasm packed with free ribosomes but poor in other organelles, and a pale nucleus with diffuse chromatin. The nucleolus is larger than at any other epithelial level. Its shape is irregular and differs from cell to cell. It is composed of a network of winding bands separated by interstitial spaces whose content is similar to the nuclear sap. Such a network has been described in other nucleoli by names such as reticulum and nucleolonema. Its darker portions constitute the pars fibrosa, which consists mainly of cords composed of closely packed 4–5 nm filaments. The less dark portions constitute the pars granulosa, which is composed of 15–20 nm granules or, more frequently, of such granules in association with filaments.
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(granulo-fibrillar areas) (Fig. 2). Small, nearly circular areas slightly denser than the interstitial spaces and partly or completely surrounded by dark cords of pars fibrosa are the fibrillar centres (Fig. 2c).

Mid crypt. Mid crypt columnar cells, even though rich in free ribosomes and actively proliferating as in crypt base, show an increase in the number and size of cytoplasmic organelles and microvilli. Mid crypt nucleoli are reticulated and of irregular shape, but are somewhat smaller and have a less prominent network than crypt base cells.

Crypt top. In the crypt top, columnar cells no longer divide; they are slightly enlarged and have an increasing number of organelles, while in the nucleus the chromatin may condense to some extent and invaginate into the nucleolus (Fig. 3). Nucleoli tend to be round instead of irregular; they are further condensed and diminished in size, while interstitial spaces are decreased, so that the network arrangement is less distinct. Fibrillar centres are fewer, but larger and more completely surrounded by pars fibrosa than at lower crypt levels.

Villus base. At the crypt mouth, cells enlarge, as their height increases by more than 50%. The lateral plasma membrane shows infoldings and the apical microvilli approach their full length and number. Cytoplasmic organelles continue increasing in size and number. Chromatin masses are prominent, often in the vicinity of the nucleolus, but usually do not invaginate into it. The trend toward decrease in the size of nucleoli continues, while interstitial spaces are diminished in size and number and, therefore, the network pattern is barely distinguishable. Fibrillar centres are fewer, but larger than in crypt nucleoli.

Mid villus. Towards the border between villus base and mid villus, columnar cells complete their differentiation; they acquire a full complement of organelles, secrete intestinal enzymes (Nordstrom et al. 1968; Leblond, 1981), and absorb digested food. In the meantime, the nucleolus loses its reticulated character and becomes compact (Figs. 5–7). In the lowest third of the region, a few nucleoli may still show traces of reticulation, but most of them have condensed to a point where nearly all interstitial spaces are lost, but dense fibrillar cords still occur (Fig. 5). In the intermediate and uppermost third of the mid villus, nucleoli are characteristically compact (Figs. 6, 7). The number of fibrillar centres decreases to only one or two, rarely three or four, but they are prominent; within them, a small region of condensed material is often visible (Fig. 7). The pars granulosa is usually relegated to the periphery (Fig. 6).

Villus top. In the few cells of the villus top region, changes indicative of degeneration are observed. The cells are partly separated from one another. In the cytoplasm,

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Fig. 2. Reticulated nucleolus of crypt base columnar cell. The nucleolus is attached to the chromatin (ch) adjacent to the nuclear envelope, which may be distinguished at the base of the figure (ne). The nucleolus consists of a network of irregular cords separated by interstitial spaces (i). The dense part of the cords (composed of fine fibrils) constitutes the pars fibrosa (f). Cords composed of granulo-fibrillar material (g) make up the pars granulosa. The interstitial spaces (i) consist of material that appears similar to the nuclear sap. The fibrillar centres (c) are slightly denser than the interstitial spaces and are completely or partly surrounded by pars fibrosa.
Nucleolus in migrating intestinal cells

Table 1. Area of nucleolus and components in columnar cells at various levels of crypt and villus (μm²)

<table>
<thead>
<tr>
<th></th>
<th>Nucleolus</th>
<th>Pars fibrosa</th>
<th>Fibrillar centres</th>
<th>Interstitial spaces</th>
<th>Pars granulosa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crypt base</td>
<td>3.14 ± 0.585</td>
<td>0.570 ± 0.138</td>
<td>0.106 ± 0.024</td>
<td>0.232 ± 0.041</td>
<td>2.148 ± 0.432</td>
</tr>
<tr>
<td>Mid crypt</td>
<td>2.43 ± 0.191</td>
<td>0.492 ± 0.064</td>
<td>0.095 ± 0.007</td>
<td>0.160 ± 0.032</td>
<td>1.688 ± 0.143</td>
</tr>
<tr>
<td>Crypt top</td>
<td>1.73 ± 0.143</td>
<td>0.324 ± 0.094</td>
<td>0.071 ± 0.021</td>
<td>0.098 ± 0.013</td>
<td>1.238 ± 0.119</td>
</tr>
<tr>
<td>Villus base</td>
<td>1.77 ± 0.073</td>
<td>0.257 ± 0.038</td>
<td>0.087 ± 0.018</td>
<td>0.049 ± 0.016</td>
<td>0.983 ± 0.057</td>
</tr>
<tr>
<td>Mid villus</td>
<td>0.85 ± 0.116</td>
<td>0.208 ± 0.028</td>
<td>0.101 ± 0.014</td>
<td>0.013 ± 0.002</td>
<td>0.529 ± 0.072</td>
</tr>
<tr>
<td>Villus top</td>
<td>0.49 ± 0.124</td>
<td>0.132 ± 0.019</td>
<td>0.102 ± 0.024</td>
<td>0.005 ± 0.004</td>
<td>0.256 ± 0.049</td>
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Analysis of variance. Comparison of results between adjacent levels within columns

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<tbody>
<tr>
<td>Crypt base</td>
<td>P &lt; 0.005</td>
<td>n.s.</td>
<td>n.s.</td>
<td>P &lt; 0.01</td>
<td>P &lt; 0.005</td>
</tr>
<tr>
<td>Mid crypt</td>
<td>P &lt; 0.005</td>
<td>P &lt; 0.01</td>
<td>n.s.</td>
<td>P &lt; 0.005</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>Crypt top</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>P &lt; 0.05</td>
<td>n.s.</td>
</tr>
<tr>
<td>Villus base</td>
<td>P = 0.01</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>P = 0.005</td>
</tr>
<tr>
<td>Mid villus</td>
<td>P = 0.05</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>P = 0.005</td>
</tr>
<tr>
<td>Villus top</td>
<td></td>
<td></td>
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<td></td>
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</tbody>
</table>

Analysis of variance. Comparison of results between all non-adjacent levels within columns

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<tr>
<th></th>
<th>all s.</th>
<th>all s.</th>
<th>none</th>
<th>all s.</th>
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<td></td>
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<td>except</td>
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<tr>
<td>v. base/v. top</td>
<td>v. base/v. top</td>
<td>s., significant; n.s., not significant; v., villus.</td>
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the Golgi apparatus may be disintegrated; cisternae of rough endoplasmic reticulum may swell; and lysosomes accumulate. The nucleolus undergoes further compaction to reach minimal size (Figs. 8, 9). Usually, a single fibrillar centre, rarely two, are embedded within *pars fibrosa* material, beyond which is a small group of granules. Thus, *pars fibrosa* and *granulosa* are segregated into distinct, but closely apposed units.

Figs. 3–4. Condensing nucleoli in crypt top (Fig. 3) and villus base (Fig. 4) columnar cells.

Fig. 3. The crypt top cell nucleolus is smaller and the reticulated arrangement less distinct than in crypt base and mid crypt cells. Cords and interstitial spaces are less distinct, but fibrillar centres are more prominent than at lower levels.

Fig. 4. In villus base, the nucleolus further decreases in size and fibrillar centres occupy a larger proportion of its area than in the crypt. The reticulated arrangement is still discernable due to the presence of a few interstitial spaces (v).
Table 2. Circularity index of the nucleolar outlines in columnar cells at various levels of the jejunal epithelium*

<table>
<thead>
<tr>
<th></th>
<th>No. of samples</th>
<th>Mean circularity index†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crypt base</td>
<td>11</td>
<td>2.8</td>
</tr>
<tr>
<td>Mid crypt</td>
<td>17</td>
<td>3.3</td>
</tr>
<tr>
<td>Crypt top</td>
<td>11</td>
<td>1.8</td>
</tr>
<tr>
<td>Villus base</td>
<td>14</td>
<td>1.8</td>
</tr>
<tr>
<td>Mid villus</td>
<td>50</td>
<td>1.5</td>
</tr>
<tr>
<td>Villus top</td>
<td>19</td>
<td>1.3</td>
</tr>
</tbody>
</table>

* The circularity index, \( \frac{\text{perimeter}^2}{4\pi \times \text{area}} \), was obtained by measuring perimeter and area simultaneously with the image analyser Zeiss MOP-3. (It is the inverse of the form index provided by this instrument.) A perfectly circular structure should give a reading of 1, whereas the greater the deviation from circularity, the higher the reading should be.

† By analysis of variance, the circularity indices for crypt base and mid crypt differed, though not significantly \((0.1 > P > 0.05)\). They both differed from all others in a highly significant manner \((P > 0.001)\). The difference between villus top and either crypt top or villus base reached the accepted level of significance \((P = 0.05)\).

(Figs. 9, 10). Fibrillar centres and extranucleolar chromatin are often connected, although their staining density remains quite different (Fig. 11).

Quantitative examination of nucleolus and components

Nucleolar size and shape. The nucleolar area (Table 1) averaged 3.1 \(\mu m^2\) in crypt base cells and decreased progressively through crypt and villus to less than 0.5 \(\mu m^2\) in villus top cells – an 84% drop (Fig. 12). Comparison of the figures by analysis of variance (Table 1) revealed that the decrease was significant either between any two non-adjacent levels or between any two adjacent levels except crypt top and villus base. Within the mid villus region, the nucleolar area decreased from 1.09 ± 0.08 \(\mu m^2\) in the lowermost third, to 0.80 ± 0.07 \(\mu m^2\) in the intermediate third and 0.79 ± 0.18 \(\mu m^2\) in the uppermost third, but the differences were not significant \((0.10 > P > 0.05\) between the first two; \(P > 0.10\) between the last two).

(Figs. 5-7. Compact nucleoli from mid villus columnar cells.

Fig. 5. In the lowest third of the mid villus region, the contrast between cords and interstitial spaces is no longer seen, although dense strands of pars fibrosa \((f)\) are still apparent. Fibrillar centres \((c)\) are prominent.

Fig. 6. In the uppermost third of the mid villus region (as in the middle third) there is very little change, resulting in a characteristic compact nucleolus. The pars fibrosa tends to be spread out. The pars granulosa is mainly composed of granules with only rare fibrils. The two fibrillar centres stand out.

Fig. 7. In this other view of a nucleolus from the uppermost third of the mid villus region, two fibrillar centres are again present, but one of them shows a condensed area (arrow). The rest of the nucleolus consists of pars fibrosa and a small pars granulosa.
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The mean area of the nucleolus is represented by column height at six successive levels along crypt and villus. The mean total area of each nucleolar component is indicated within the columns.

The nucleolar area gradually decreases from crypt base to villus top. Similarly, there is a gradual decrease in the size of interstitial spaces, pars fibrosa and granulosa. No significant change is observed in fibrillar centres.

Figs. 8-11. Segregated nucleoli in villus top columnar cells.

Fig. 8. Pars fibrosa (f) and granulosa (g) are segregated into distinct areas. The nucleolus shows a prominent fibrillar centre (c). Most of the centre is, as usual, stippled with tiny black dots that may be cross-sections of fibrils. At the left of c, a more uniformly grey field may correspond to the Pb region described by Recher et al. (1976) in nucleoli segregated under the influence of actinomycin D. The material at upper left (arrow) seems to be a nucleolar fragment.

Fig. 9. Some extranucleolar chromatin (ch) is located close to the fibrillar centre (c), which includes a condensed portion in its middle. The pars fibrosa encircles half of the fibrillar centre. A strip of pars granulosa is at the right.

Fig. 10. The sequence: chromatin, fibrillar centre, pars fibrosa and pars granulosa is clearly visible.

Fig. 11. This nucleolus is cut along the length of a broad fibrillar centre (c), which at both ends joins densely stained extranucleolar chromatin (ch). The fibrillar centre is flanked on both sides by pars fibrosa (f).
Fig. 13. Plotting of the mean total area of fibrillar centres and interstitial spaces in three levels of the crypt and three levels of the villus. The levels are located on the abscissa at their approximate distance from the crypt–villus junction (small vertical line). There is a significant decrease in interstitial spaces from crypt base to villus top. In contrast, the total area of fibrillar centres shows no significant changes.

Since nucleoli appeared irregular in the cells of the two lower crypt levels and more or less round higher up, their circularity index was measured (Table 2). The mean index was high in crypt base and mid crypt; thus confirming that circularity was usually lacking at these levels. At successively higher levels, however, there was a progressive decrease in the index, which approached unity in villus cells; and, therefore, the nucleolar outline became more and more circular.

Pars fibrosa. Throughout crypt and villus base, the pars fibrosa was mainly in the form of cords (Figs. 3, 4). In the mid villus, however, it became spread out into irregular patches (Figs. 5–7). A single patch was often seen in villus top nucleoli (Figs. 9–10). The total area of pars fibrosa gradually decreased from crypt base to villus top (Fig. 12), but the percentage of the nucleolar area it occupied remained
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around 19% from crypt base to mid villus. However, the percentage increased to 30% at higher levels.

Fibrillar centres. The mean total area occupied by fibrillar centres in a section of nucleolus oscillated around 0.1 μm², without significant difference between the results at any two levels (Table 1). Nevertheless, the size and number of fibrillar centres varied markedly: small and numerous in crypt base, they became larger and fewer at higher levels. This evolution culminated at the villus top where a single large fibrillar centre was usually present.

Interstitial spaces. Interstitial spaces were conspicuous in crypt base cells (Fig. 2), where they comprised 7% of the nucleolar area. Their decrease at higher levels was rapid (Table 1, Fig. 13), leading to almost complete disappearance in mid villus and villus top.

Pars granulosa. Through crypt and villus base, the pars granulosa included few purely granular regions; the granules were usually associated with filaments to form the so-called granulo-fibrillar regions (e.g. see Fig. 3). In mid villus top, however, granules seemed to occur with few or no associated filaments (e.g. see Figs. 8, 12). The total area of pars granulosa decreased from crypt base to villus top (Table 1), although it occupied a constant proportion (about 70%) of the nucleolar area. At higher levels, the pars granulosa occupied a smaller proportion of the nucleolar area, that is, 62% in mid villus and nearly 50% in villus top.

DISCUSSION

Relation of nucleolar changes to the features of migrating columnar cells in rat jejunum

The columnar cells of crypt base are embryonic-looking, proliferative, and function as stem cells for the epithelium (Leblond, 1981). Their nucleolus was found to be large and reticulated, and after injection of [3H]uridine, showed high incorporation of label (unpublished observations). This was a clear indication of a high rate of ribosomal RNA (rRNA) production (Bernhard & Granboulan, 1968; Mirre & Stahl, 1981). The production of rRNA by crypt base columnar cells was attributed to the building of ribosomes for synthesis of the proteins required for the formation of new cells. A similar situation probably holds for the reticulated nucleoli observed in proliferative embryonic cells (Adamstone & Taylor, 1977; Leblond, 1981), trophoblast cells (Martin & Spicer, 1973) and cancer cells (Busch & Smetana, 1970). However, non-dividing cells, such as the major neurons of cortex and cerebellum, Sertoli cells, endodermal cells, etc. also have a large reticulated nucleolus. Such cells were known to synthesize protein actively; thus, neurons produce proteins for the axoplasmic flow (Droz, 1975), Sertoli cells for their secretions (Wilson & Griswold, 1979), and endodermal cells for the yolk sac (Azevedo & Coimbra, 1980). It thus appears that a reticulated nucleolus is a common feature of cells active in protein synthesis.

Differentiation of columnar cells. This proceeded in two main phases. First in mid crypt, where the proliferation rate is high, the [3H]uridine uptake of the fairly large, reticulated nucleolus was high, indicating again active rRNA production (unpublished
observations). Secondly, in crypt top and villus base, mitosis ceased; the size of the nucleolus decreased and the uptake of $[^{3}H]$uridine dropped.

**Maturation of columnar cells.** This was completed at about the junction of villus base and mid villus, where active production of intestinal enzymes is revealed by a high uptake of $[^{3}H]$fucose (Leblond, 1981). The nucleolus of mid villus cells was compact and did not incorporate $[^{3}H]$uridine (unpublished observations); it had, therefore, stopped producing new rRNA. Presumably, the cells relied on previously built ribosomes for the synthesis of the protein moiety of the enzymes.

**A terminal stage.** This was reached by columnar cells in the villus top, where they showed signs of degeneration and a segregated nucleolus which, as in mid villus, did not incorporate $[^{3}H]$uridine (unpublished observations) and, therefore, did not produce rRNA. Segregated nucleoli were also observed after administration of actinomycin D and other drugs, which combined with DNA to prevent RNA transcription (Simard, 1970; Scheer, Trendelenburg & Franke, 1975; Daskal, 1979). The lack of rRNA production meant that, in villus top as in mid villus columnar cells had to rely on previously built ribosomes; but perhaps fewer and fewer ribosomes remained available as migration proceeded. Indeed, protein synthesis gradually decreased from the base to the extremity of the villus (Altmann, 1976). Hence, the signs of degeneration observed in villus top might result from the deficiency in newly formed proteins.

**Changes in nucleolar components**

A major observation was the pronounced decrease in the overall size of the nucleolus during the migration of columnar cells. The nucleolar volume (calculated on the assumption of spherical size from the formula $0.752S_{3}/S$, where $S$ is the mean surface of the measured area) decreased from crypt base to villus top by a factor of 16. Yet, fibrillar centres, the sites of nucleolar organizer DNA (Chouinard, 1975; Recher, Sykes & Chan, 1976; Mirre & Stahl, 1976, 1978, 1981; Geossens, 1979) occupied a total area that remained statistically the same at all levels of the epithelium (Fig. 13), even though the number of fibrillar centres decreased. It was not known whether fibrillar centres were continuous with one another as in the 'elongated nucleolar organizer' of *Spirogyra* cells (Godward, 1956; Ashraf & Godward, 1980) or were distinct units connected by extended DNA as in meiotic mouse oocytes (Mirre & Stahl, 1981). In either alternative, they must be united into a single structure when nucleolar compaction is completed.

In contrast, pars fibrosa and granulosa decreased markedly during the cell migration. The *pars fibrosa* was known to arise from the transcription of rRNA (reviewed by Mirre & Stahl, 1981). After injection of $[^{3}H]$uridine, the label appeared first over the 45 S rRNA-rich fibrils of *pars fibrosa* and later over the 32 S rRNA-rich granules of *pars granulosa*, where it gradually decreased (Granboulan & Granboulan, 1965; Karasaki, 1965; von Gaudecker, 1967; Recher et al. 1976). From these and other findings (Mirre & Stahl, 1981) it is concluded that *pars fibrosa* components are converted into *pars granulosa* components, which in turn are released from the nucleolus. Accordingly, the decrease in *pars fibrosa* observed during columnar cell migration was attributed to a reduction in the rate of transcription, while the conversion of its com-
ponents into pars granulosa continued. The decrease in pars granulosa was attributed to the release of its components exceeding the entry of material from pars fibrosa.

The decrease in the area of pars fibrosa and granulosa up to villus base was not associated with significant changes in the proportion of the nucleolar area which they occupied, that is about 19% for pars fibrosa and 70% for pars granulosa. Presumably the successive steps in rRNA metabolism occurred at the same rate. In mid villus and villus top, however, the proportion of pars fibrosa increased up to about 30% and the proportion of pars granulosa decreased to about 50% of the nucleolar area. Hence, even though RNA transcription had stopped some granules might still be released at these levels, implying that some 32 S rRNA passed out of the nucleolus. This was not likely to be sufficient, however, for the building of new ribosomes in villus cells.

In conclusion, the columnar cells of jejunal epithelium show profound nucleolar changes during the few days of their life. In crypt cells the reticulated nucleolus actively produces rRNA, but soon after cells reach the villus the nucleolus becomes compact and ceases producing rRNA, even though the activity of the cells in absorbing food and synthesizing intestinal enzymes is at its peak.

There are indications that the nucleoli in the cells of other renewing systems undergo similar evolution during the formation of blood cells (Busch & Smetana, 1970), keratinocytes (Karasek, Smetana, Hrdlicka, Dubinin, Hornak & Oehlert, 1972), sebaceous cells (Karasek, Hrdlicka & Smetana, 1973), spermatozoa (Leblond, 1981) and epithelial cells of oesophagus, stomach and colon (Falconer, 1982). In these cases young, immature cells usually have a reticulated nucleolus and mature cells, a compact one.

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Nucleolus in migrating intestinal cells


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