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Morphogenesis of the intestinal villi of the mouse embryo: chance and spatial necessity*

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INTRODUCTION

The notion that local differences in the rate of tissue growth contribute to morphological changes during embryonic life has been put forward by several authors. D'Arcy Thompson (1917) says in his book *On Growth and Form*: " . . . we are . . . justified in thinking of form as the direct resultant and consequence of growth: of growth, whose varying rate in one direction or another has produced, by its gradual and unequal increments, the successive stages of development and the final configuration of the whole material structure". Landsmeer (1968, 1978) emphasizes the importance of the reciprocal topological influences of the tissues during embryonic life, i.e. the growth of one element produces a new spatial arrangement which influences the growth of the neighbouring elements. The nature of this particular influence is, however, not clear. Blechschmidt & Gasser (1979) also strongly support the idea that local changes trigger changes in adjacent regions during morphogenesis. Dynamic factors, such as differences in growth rate and differential changes in physical factors acting between growing masses, are considered to be input stimuli for differentiation.

More specifically for the morphogenesis of the gastro-intestinal tract, Miete (1960) postulated that the shape and rotation of the human embryonic stomach are the result of the unequal growth of the gastric walls. Pleeging (1975) found that mitosis occurred more frequently in the duodenum than in the rectum of the mouse embryo and attributed the formation of the intestinal loops to local growth differences. Sbarbati (1979) and Sbarbati & Strackee (1980, 1982) showed that the volumes of the intestine and stomach, and of their components, i.e. the epithelium and mesenchyme, grow at different rates in mouse embryos between the twelfth day and birth. In particular, the intestinal epithelium grows faster than the gastric epithelium and, for both, the rate of volume increase is higher than that of the surrounding mesenchyme. These authors suggested that the dynamic interaction between the two layers growing at different rates plays an important role in the morphogenesis of the intestinal wall. The outer layer might exert a restraining action on the rapid growth of the inner layer, thus contributing to the folding of the mucosa.

Further study (Sbarbati, 1981) of the maturation of the intestinal wall in the mouse embryo led me to conclude that, unlike the chick intestine (Coulombre & Coulombre, 1958; Burgess, 1975; Hilton, 1902; Grey, 1972), the mouse intestine does not start by forming previllous ridges which then segment, but immediately forms epithelial elevations which project into the lumen and later acquire a core of

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mesenchyme. These structures, which will be called villi here, are characteristic for both the small and the large intestine, although they are shorter in the latter. This pattern of development was also observed by Hilton (1902) and Kammeraad (1942) for the rat embryo. The scanty literature on this subject treats the organogenesis of the small and large intestine separately and emphasizes the formation of the villi in the former and the formation of the depressions between the villi (called crypts) in the latter. This distinction is based on the different functions that the cells lining the villi and crypts will have in later stages of development, but it is not useful for the study of the morphogenesis of the organ because it gives the erroneous impression that the process of shaping of the intestinal wall differs as well, whereas there is marked similarity between the organogenesis of the rat duodenum (Mathan, Moxey & Trier, 1976) and that of the colon (Eastwood & Trier, 1974).

The aim of the present study was to test the following hypotheses: (1) similar morphogenetic processes, leading to formation of villi, occur in both the small and the large intestine of the mouse embryo in the period between 12 and 16 days after fertilization; (2) these morphogenetic processes are brought about by the dynamic interaction between the epithelium and mesenchyme; in particular, differences in the growth rates and changes in physical forces acting between the growing tissues generate new forms within a framework of 'spatial coherence' (Landsmeer, 1968).

MATERIALS AND METHODS

Mouse embryos of the CPB-S strain were used in this study. The main part of the material consisted of series, already present in our department, of mouse embryos aged between 12 and 16 days after fertilization (a.f.) and sectioned at a constant thickness of 10 μm (Goedbloed, 1972).

Cross sections of the duodenum and of the large intestine were selected as originating from corresponding straight parts of the intestinal tube at different ages. For orientation purposes, the intestine of three embryos (aged 12·9, 13·8 and 15·9 days a.f.) were tri-dimensionally reconstructed (Sbarbati, 1981) and used to determine the level of cross sectioning. For the same purpose, use was also made of the intestinal reconstructions published by Pleeging (1975).

The duodenal cross sections were selected from the area caudal to the entrance of the ductus choledochus into the intestinal wall (Sbarbati, 1981). Selection of cross sections of the large bowel was more difficult, because in embryos younger than about 14 days (Pleeging, 1975) the rectum is not completely separated from the urogenital structures but terminates with them in an enlarged common portion, the cloaca. In this stage, the large intestine has a broader precloacal part that is not present in later stages after the formation of the caudal part of the organ has been completed. Furthermore, in comparable portions, the longitudinal axis of the organ is not always perpendicular to the plane of sectioning. Both the cross sectional area of the epithelium and mesenchyme and the outer circumference of the tube were estimated in the duodenum as well as in the large intestine by the point-counting method (Sbarbati, 1979).

To avoid over-estimation of the parameters under study, only cross sections taken from straight parts of the intestinal tube were measured (Sbarbati, 1979). The following procedure was used for these estimations. I took m, $m = 1, 10$ as the consecutive cross sections of the intestine, $n_m(t)$ as the number of lattice points falling on the cross sections, $\nu_m(t)$ as the number of intersections of the outer

intestinal circumference with a regular set of parallel lines, and A as the distance between the lattice points or the lines.

For the ages under study, n_m and ν_m were determined by counting. For the cross sectional area and for the outer circumference of the intestinal tube I used, respectively, the formulas:

$$C(t) = \frac{1}{10} \sum_{m=1}^{10} n_m(t) A^2, \text{ and}$$

$$\psi(t) = \frac{1}{10} \sum_{m=1}^{10} \nu_m(t) \frac{\pi}{2} A.$$

Selected portions of the small and large intestine were taken from new embryos aged 13·5–14·5 days a.f. and used for light microscopy (1 μm sections) or scanning electron microscopy (SEM).

Mice were allowed to mate overnight. It was assumed that fertilization occurred at midnight. The presence of a vaginal plug was considered confirmation of pregnancy. The age of the embryos was determined according to Goedbloed's (1972) timetable. The embryos were removed from the uterus, perfused via the liver with a solution of 2% glutaraldehyde, osmicated, thoroughly washed in cacodylate buffer, and dehydrated in ethanol.

Light microscopy

The embryos were embedded in Epon, sectioned at constant thickness (1 μm), and stained with toluidine blue.

Scanning electron microscopy

Critical point drying was performed in CO_2. The abdominal cavity was then opened and the intestinal loops broken along pre-fixed cuts. The segments were mounted on an aluminium stub and coated with gold in an argon atmosphere in a sputter-coater.

RESULTS

Morphogenesis of the intestinal wall

Duodenum

About 12 days after fertilization (Fig. 1) a typical cross section of the duodenum showed the inner layer, the epithelium, with its round or slightly elliptical internal and external contours. The epithelium was 1–2 cells thick and surrounded by a loose mesenchymal layer which was more densely packed in the area of the muscular layers. The long axis of the mesenchymal cells became circularly oriented around the epithelium. The transition between the intestinal mesenchyme and mesenterial tissue was not sharp.

One day later (Fig. 2) the outer profile of the epithelium was still rather elliptical, and this layer had a thickness of 2–4 cells, varying locally in that the long sides of the ellipse were thicker than the short ones. The lumen had a slit-like appearance, or sometimes a triangular shape. In the mesenchyme the lamina propria and the site of the muscular layers were recognizable.

With increasing age the epithelium formed elevations (up to 6–8 cells high) projecting into the lumen (Fig. 3). Tri-dimensional reconstructions of epithelial

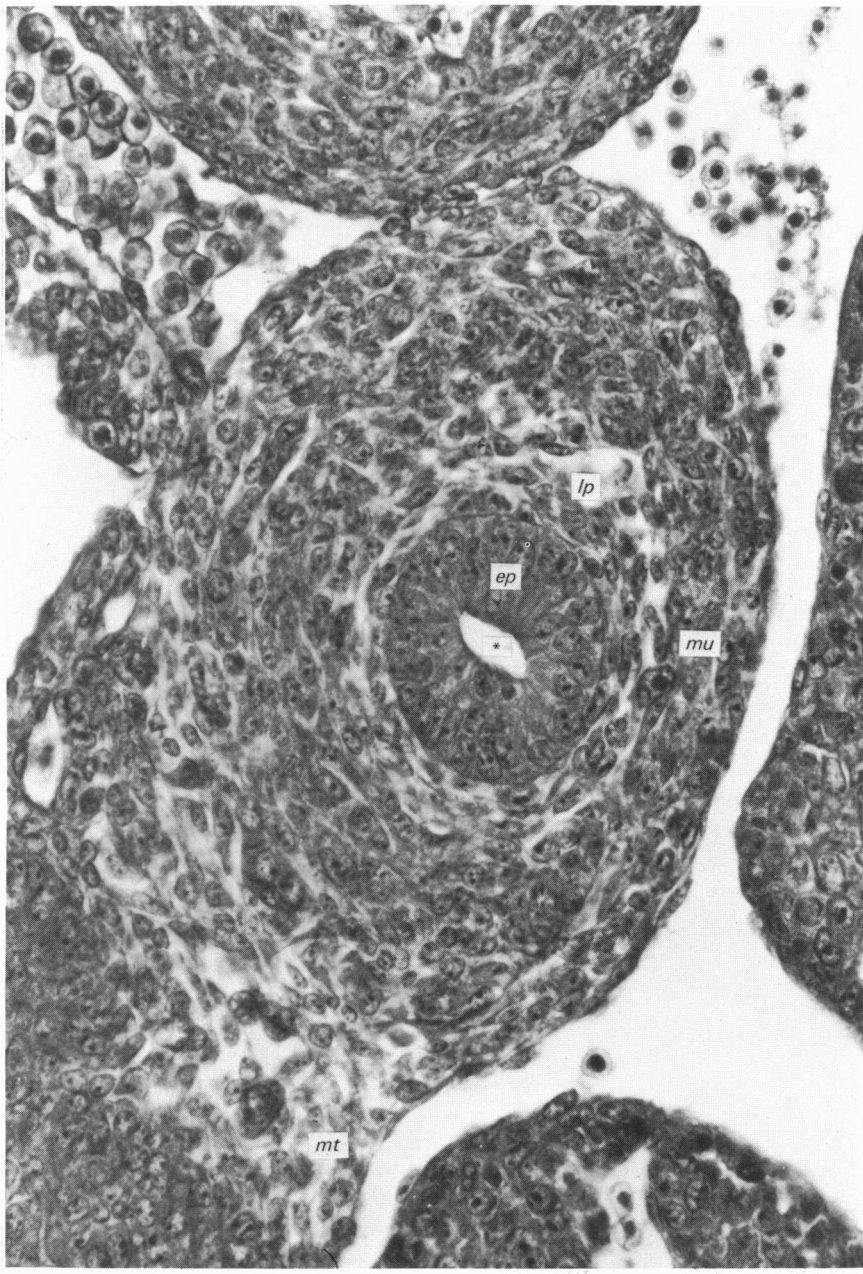

Fig. 1. Cross section of the duodenum at 12 days a.f. *ep*, epithelium; *lp*, lamina propria; *mu*, muscular layer; *mt*, mesentery; *, lumen. (Ref. no. 112–7.1.2). ×200.

tubes of embryos aged around 13–14 days a.f. and SEM pictures of the longitudinally broken duodenal wall (Sbarbati, 1981) provided no indications of the presence of regular, long epithelial ridges, as described for the chick, but showed quite irregular elevations into the lumen. Around 14 days a.f., degenerating cells were observed at the top of the epithelial elevations (Fig. 3*b*). These cells became rounded and were extruded into the lumen.

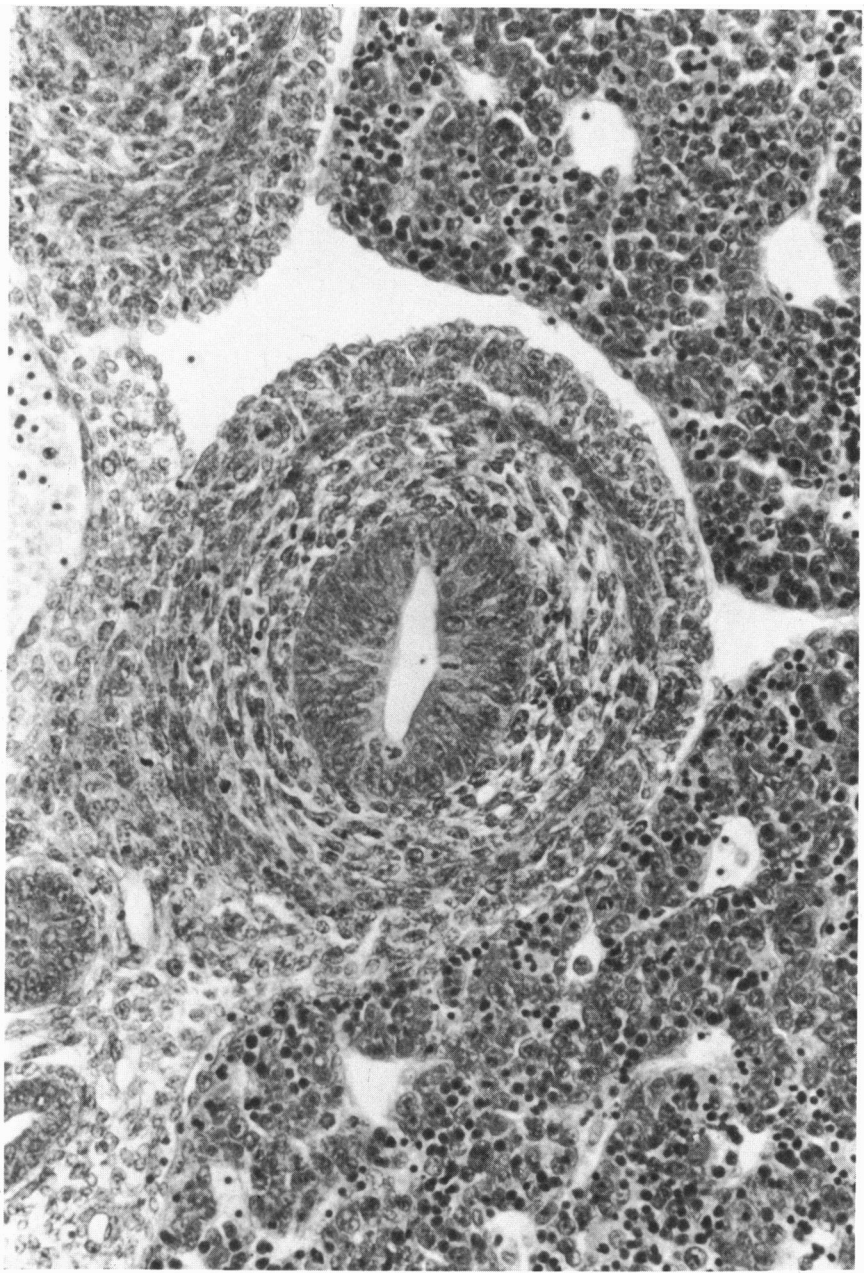

Fig. 2. Cross section of the duodenum at 12·9 days a.f. (Ref. no. 118–18.1.2). ×128.

A survey of a thin (1 μm) cross section (Sbarbati, 1981) indicated that the cells around the lumen were not regularly arranged. The borderline between epithelium and mesenchyme (basal lamina) at the base of the epithelial elevations gradually became straight and then curved slightly inward.

At about 15 days a.f. (Fig. 4) the epithelial elevations had a mesenchymal core constituted by cells of the lamina propria. In cross sections of the intestine during

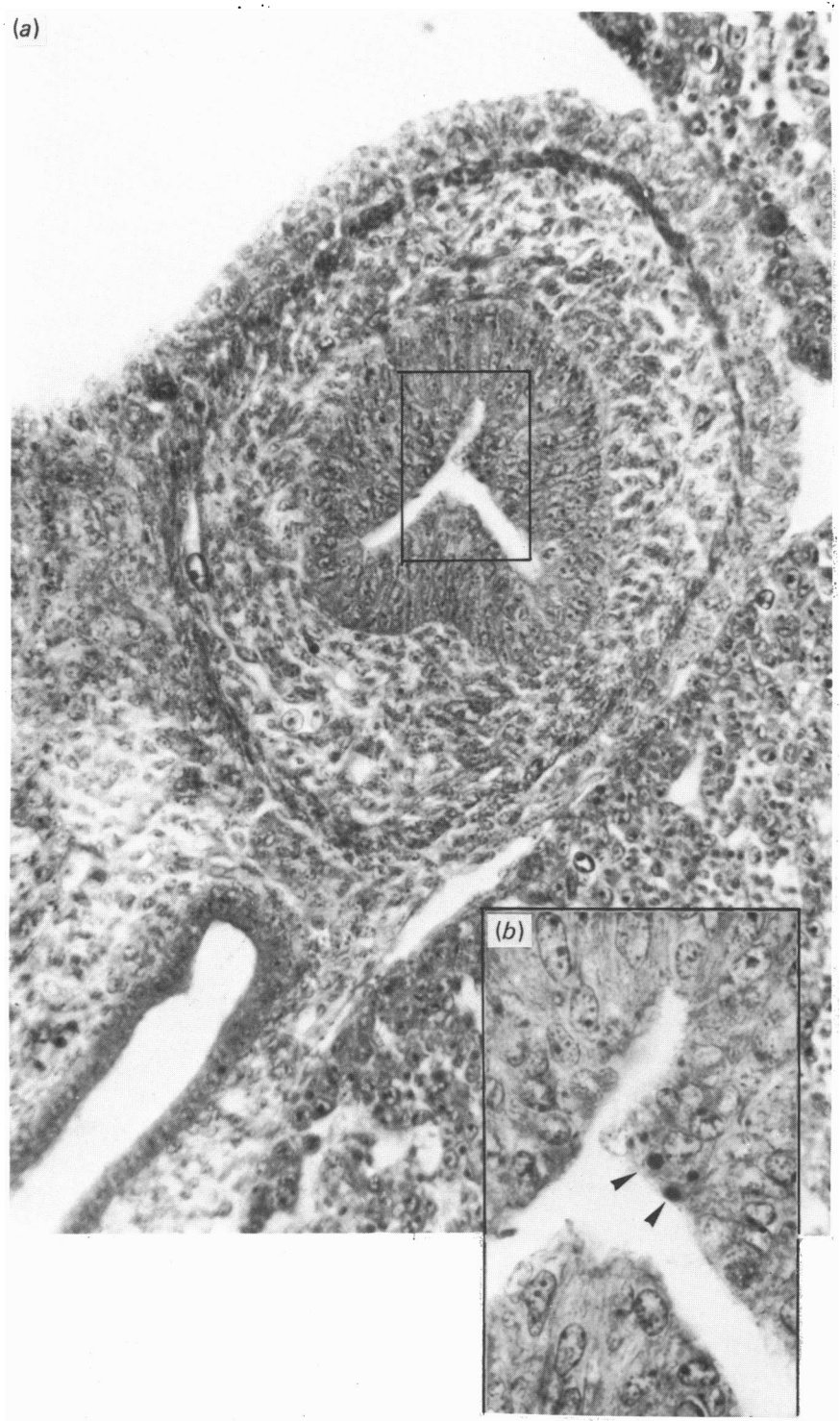

Fig. 3. (*a*) Cross section of the duodenum at 13·8 days a.f. (*b*) Detail of the tops of the epithelial elevations projecting into the lumen. Arrowheads, degenerating cells (Ref. no. 122–14.2.10). (*a*) × 128. (*b*) × 800.

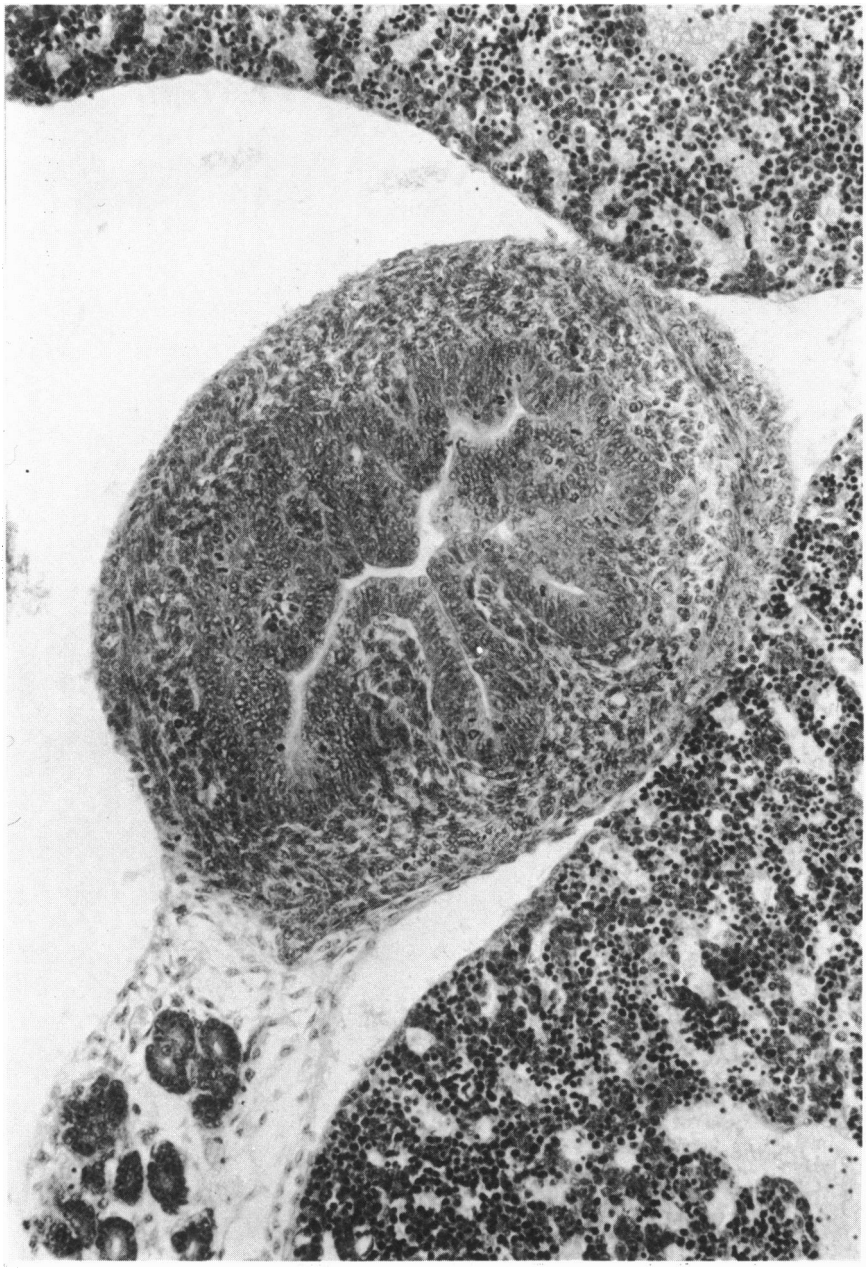

Fig. 4. Cross section of the duodenum at 15 days a.f. (Ref. no. 128–29.2.6). ×80.

the elliptical stage the long axis of these mesenchymal cells was circularly oriented around the basal lamina, whereas in this stage the long axis of the mesenchymal cells was oriented parallel to the axis of the newly formed villus. The epithelium gradually became a single layer. The pattern shown by embryos aged about 16 days (Fig. 5) was that of an increasing number of sectioned duodenal villi in the cross sections. The villi had grown considerably in length and remnants of the cavity could be seen between them.

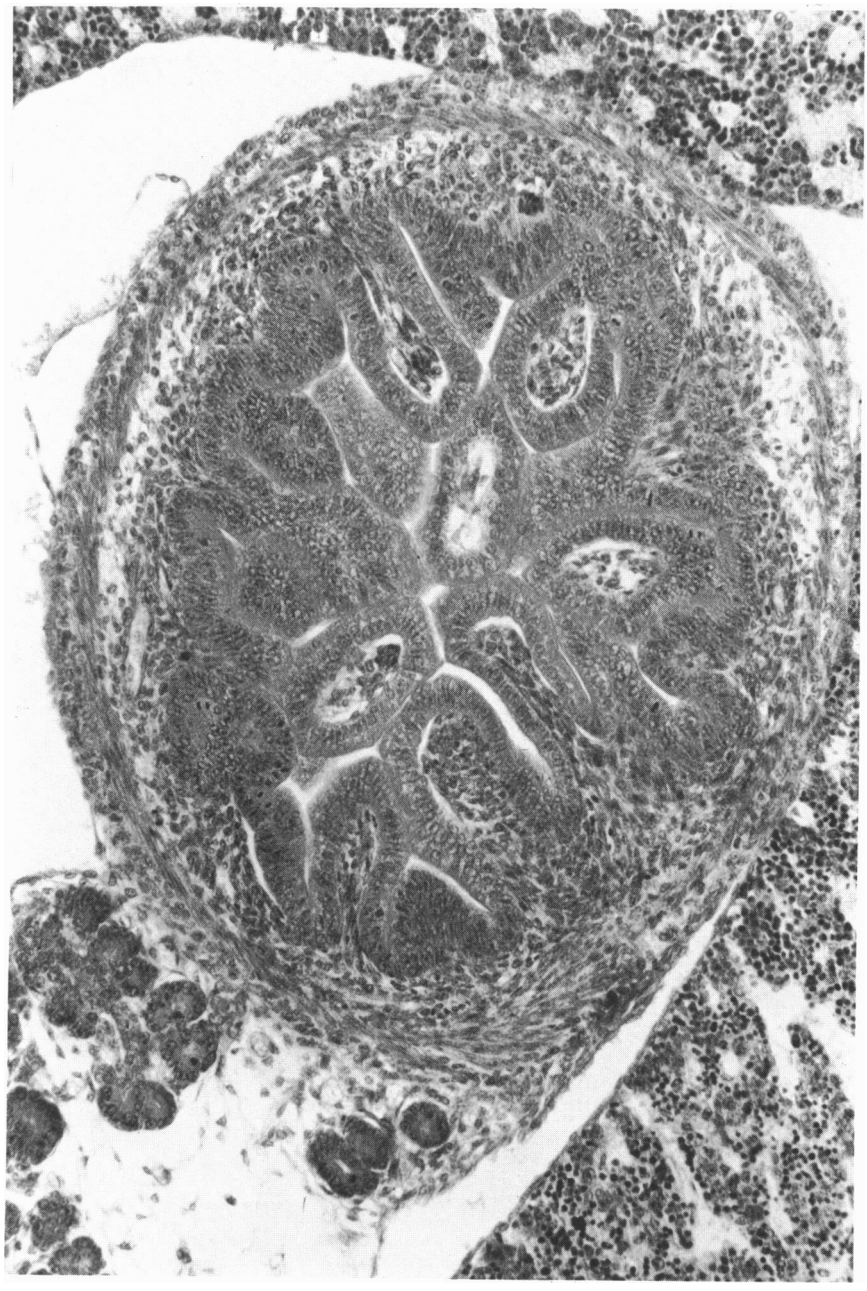

Fig. 5. Cross section of the duodenum at 15·9 days a.f. (Ref. no. 136–48.2.3). ×80.

Large intestine

A cross section of the organ at 12 days a.f. showed the epithelium with a height of 1 to 2 cells (Fig. 6). The external contour was rather round, the lumen either elliptical or triangular. As can be seen in Figure 6, local differences in the epithelial height gave rise to three small elevations projecting into the lumen. In the mesenchyme (Figs. 7–8), the cells gradually became oriented with their long axis parallel to the

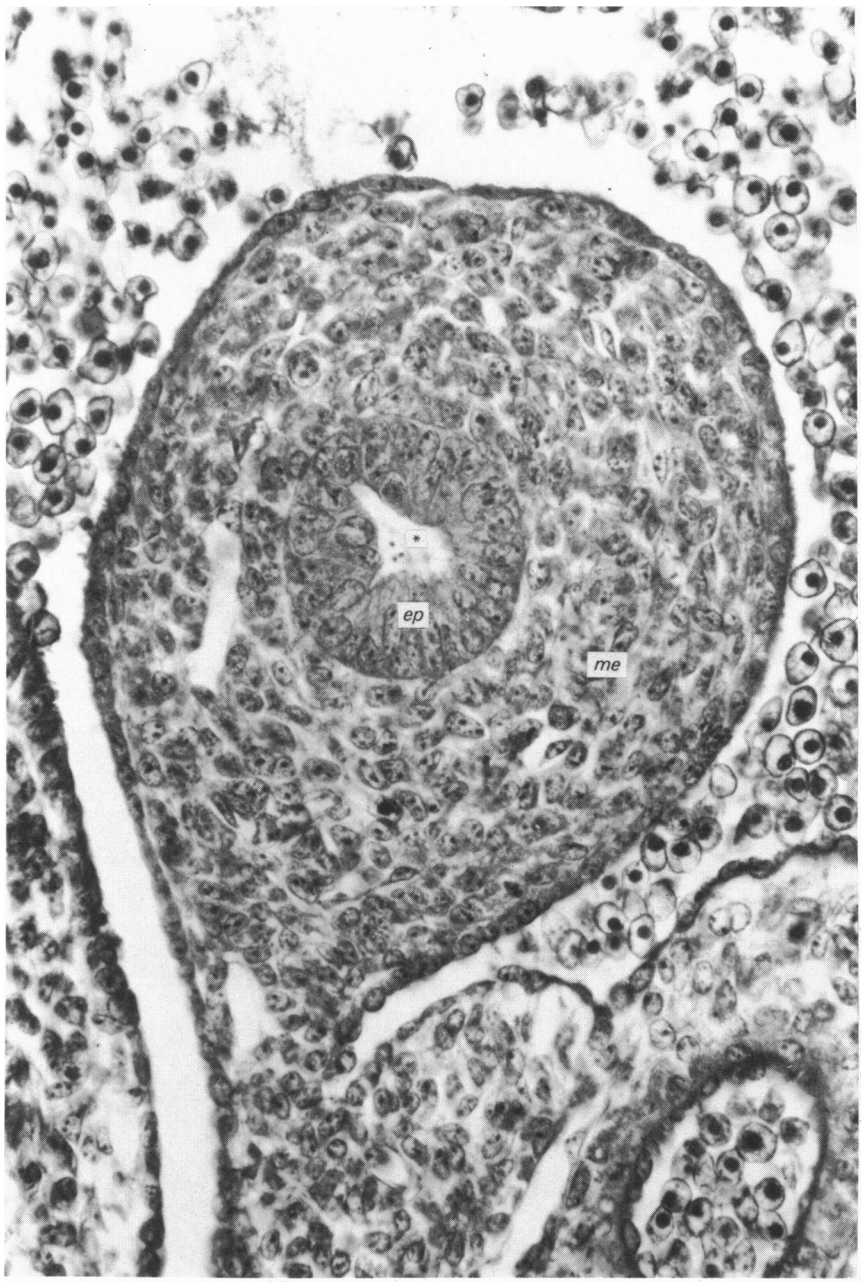

Fig. 6. Cross section of the large intestine at 12 days a.f. *ep*, epithelium; *me*, loose mesenchymal layer; *, lumen. (Ref. no. 112–7.1.2). ×200.

plane of cross section and they were more closely packed in the area of the muscle layers.

At about 14 days, the epithelium was 1–4 cells thick and the elevations were more pronounced (Fig. 8), sometimes filling the tiny lumen almost completely (Sbarbati, 1981). At 15 days (Fig. 9) the formation of the mesenchymal cores had started at the base of the epithelial elevations, and degenerating cells were present at the top.

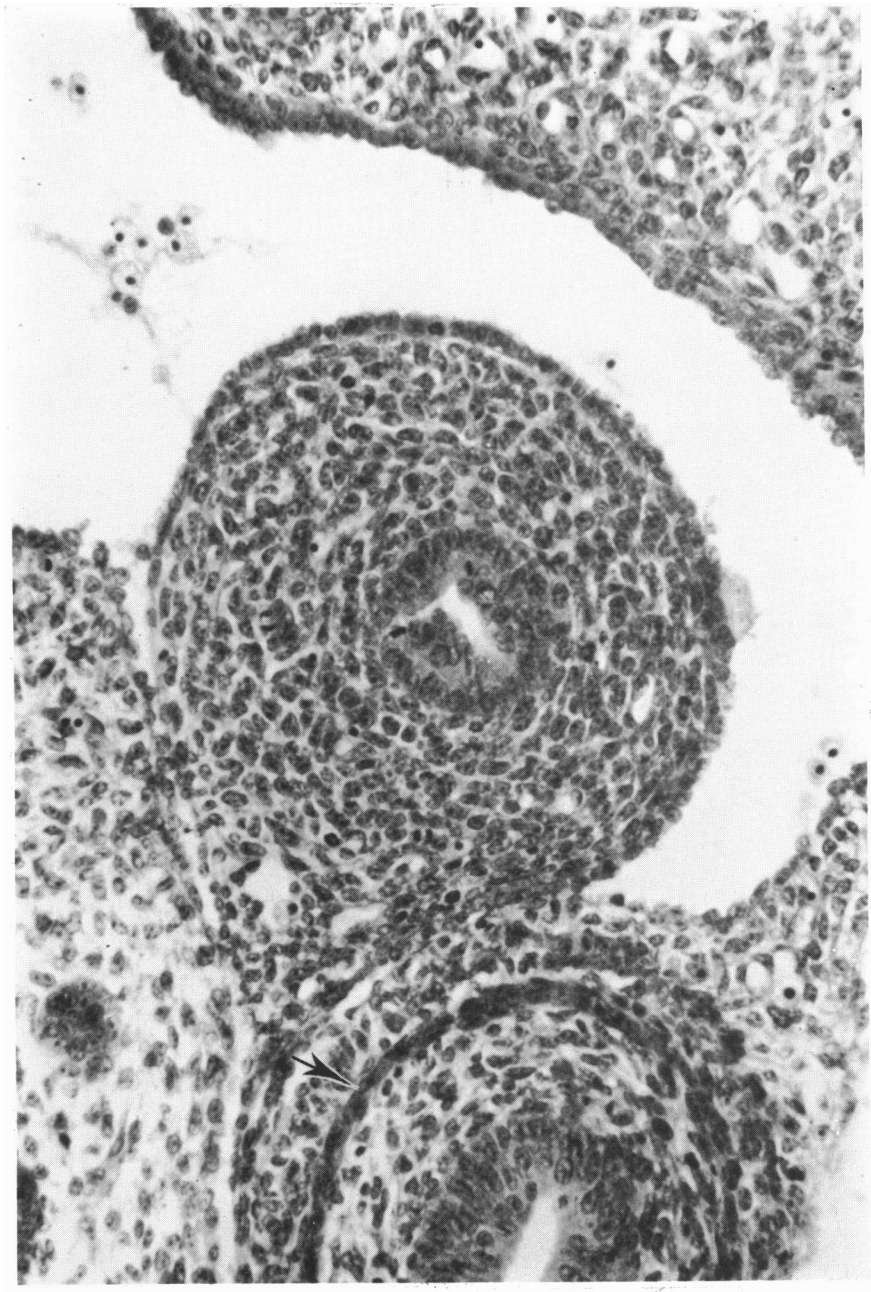

Fig. 7. Cross section of the large intestine at 12·8 days a.f. Note the more advanced development of the muscle layer (arrow) in the lower cross section of the small intestine. (Ref. no. 115–13.1.10). × 128.

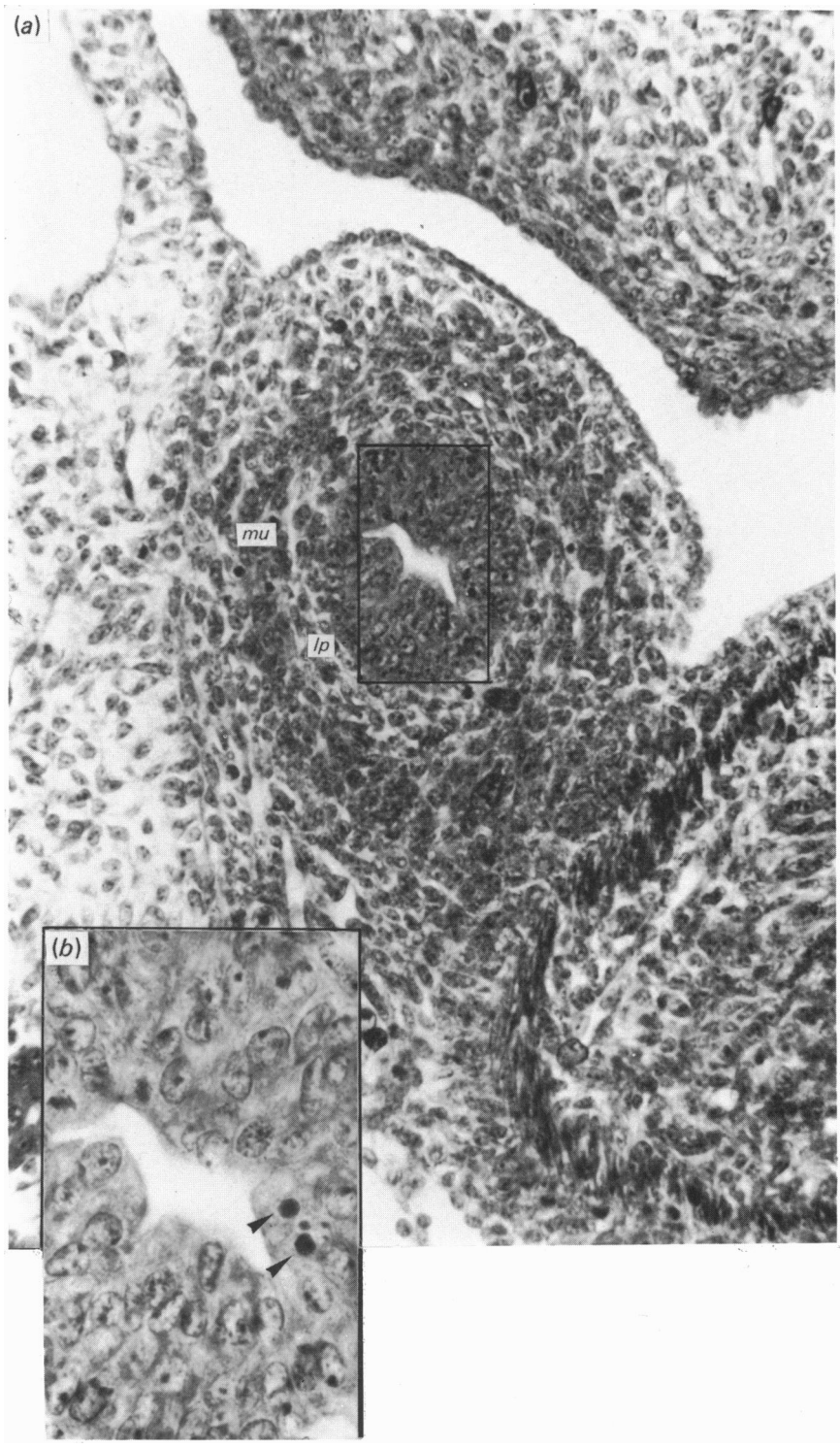

Fig. 8. (*a*) Cross section of the large intestine at 13·8 days a.f. (*b*) Detail of the epithelium with degenerating cells (arrowheads). *lp*, lamina propria; *mu*, muscular layer. (Ref. no. 122–16.2.7). (*a*) × 128. (*b*) × 800.

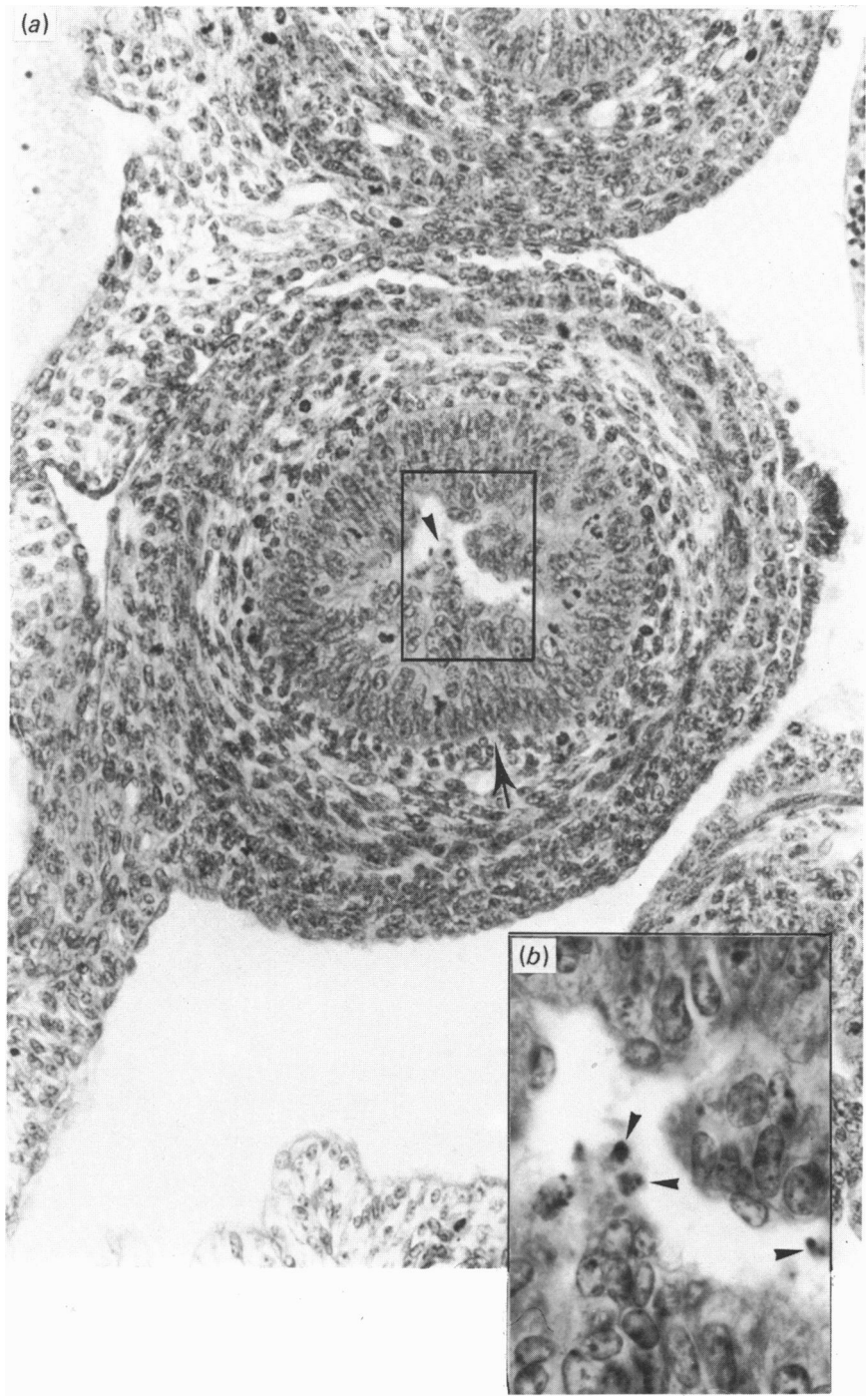

Fig. 9. (*a*) Cross section of the large intestine at 15 days a.f. Degenerating cells are visible at the top of one epithelial elevation (arrowhead), while at the base the process of formation of the mesenchymal core has begun (arrow). (*b*) Detail of epithelial elevations. Arrowheads, degenerating cells. (Ref. no. 128–30.1.2). (*a*) × 128. (*b*) × 800.

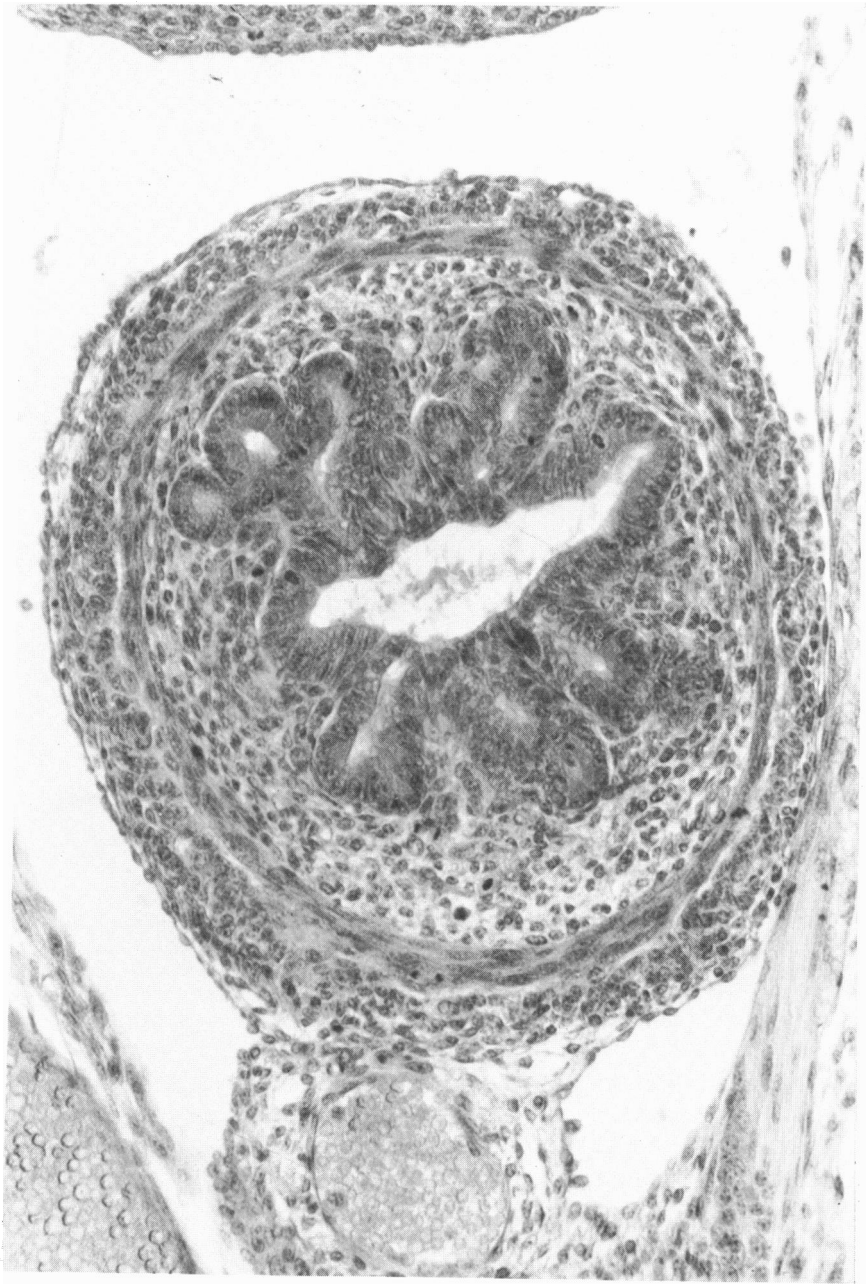

Fig. 10. Cross section of the large intestine at 15·9 days a.f. (Ref. no. 136–57.2.5). × 128.

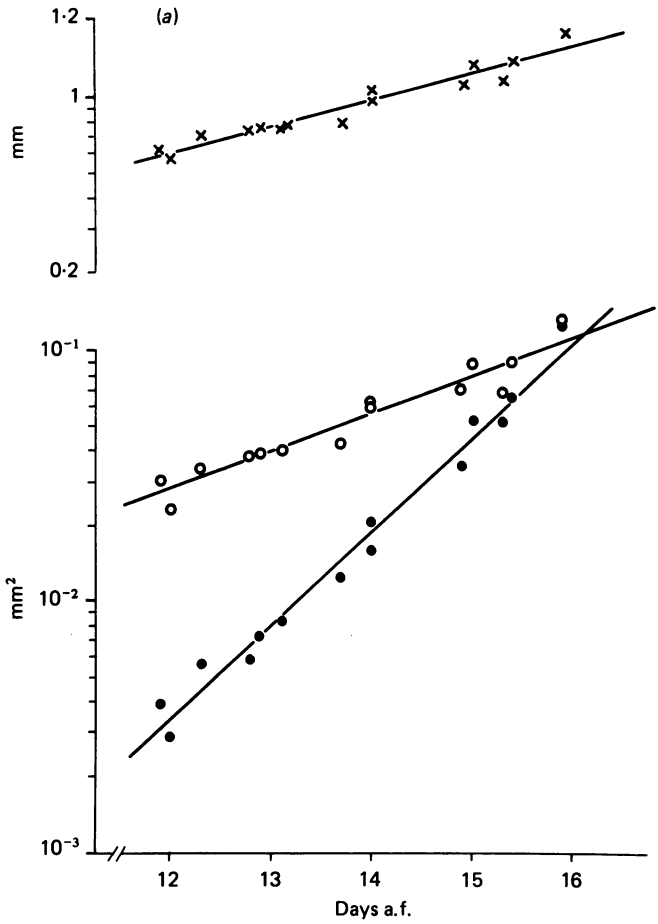

Fig. 11. (*a–b*). Increase in cross sectional area of the epithelium (•), mesenchyme (○), and external circumference (×) of the duodenum (*a*) and large intestine (*b*) plotted on a logarithmic scale against time. At a given time the specific growth rate is the derivative of the logarithmic function.

In the following stage (16 days, Fig. 10), cross sections of the large intestine showed villi which were shorter than those of the duodenum and did not completely occupy the lumen. Whereas in the large intestine the newly formed villi had a broad base and a slender top, in the duodenum they had a narrow base and a large rounded top.

Increase of the cross sectional area and the outer circumference

Figure 11*a* shows, on a logarithmic scale, the data on the increase of the cross sectional area of the epithelium and mesenchyme of the duodenum and the external circumference between the twelfth and sixteenth days after fertilization. Figure 11*b* gives the corresponding data for the large intestine. A polynomial regression analysis of the logarithmically transformed data was performed and the F-test was applied to determine the highest degree of the polynomial that was significant at the 95 % confidence level. The data are described by:

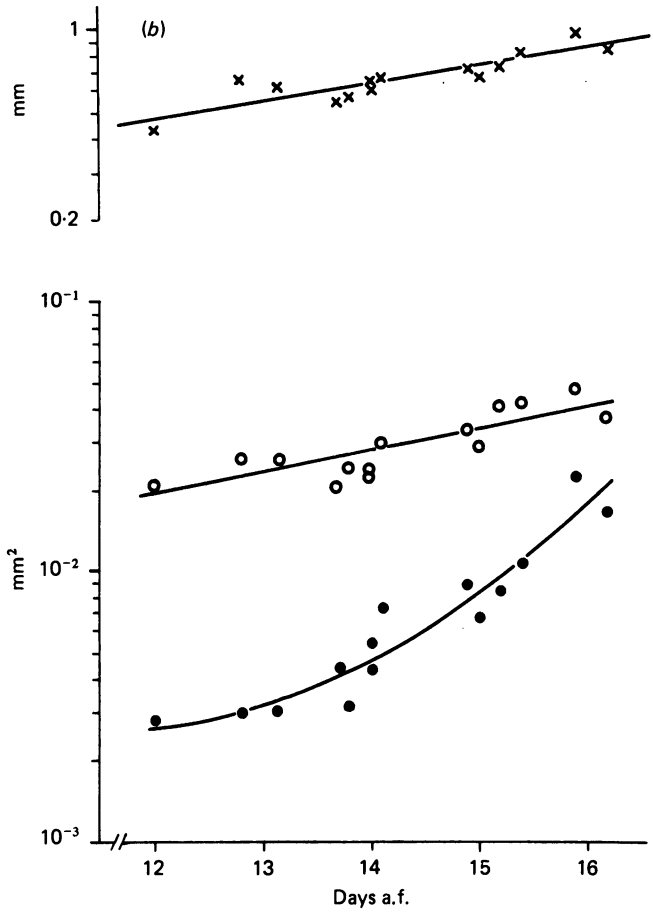

Fig. 11. (*b*). For legend see opposite.

Epithelium of duodenum: $\ln C_E(t) = -16{\cdot}05 + 0{\cdot}8639t$
Mesenchyme of duodenum: $\ln C_M(t) = -7{\cdot}693 + 0{\cdot}3437t$
Circumference of duodenum: $\ln \psi(t) = -3{\cdot}401 + 0{\cdot}2421t$

Epithelium of large intestine: $\ln C_E(t) = 5{\cdot}731 - 2{\cdot}054t + 0{\cdot}09017t^2$
Mesenchyme of large intestine: $\ln C_M(t) = -6{\cdot}041 + 0{\cdot}1765t$
Circumference of large intestine: $\ln \psi(t) = -2{\cdot}569 + 0{\cdot}1511t$.

The specific growth rates are given by the derivative of the logarithmic function (Sbarbati, 1979).

Figure 12 shows the curves of the allometric growth of the mesenchyme plotted against that of the epithelium for both the small and large intestine, i.e. the logarithm of the estimates of the cross sectional area of the mesenchyme plotted against the logarithm of the analogous estimates of the epithelium. The data are described by the following regression equations:

Small intestine: $\ln C_E(t) = -1{\cdot}289 + 0{\cdot}4019 \ln C_M(t)$
Large intestine: $\ln C_E(t) = -1{\cdot}765 + 0{\cdot}3448 \ln C_M(t)$

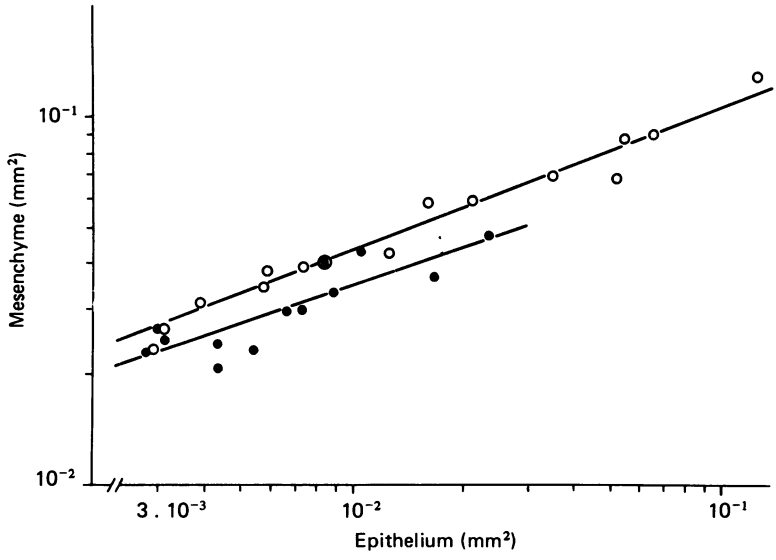

Fig. 12. Curves of allometric growth: logarithm of the cross sectional areas of the mesenchyme plotted against the logarithm of the cross sectional areas of the epithelium of the duodenum (○) and large intestine (●).

DISCUSSION

The findings concerning this series of events during the maturation of the duodenal and colonic walls in the period between the twelfth and sixteenth days a.f. validate the hypothesis that in both organs similar morphogenetic processes mould the villi.

Differential growth rate

The results pertaining to the increase in cross sectional area of the epithelium and mesenchyme are in agreement, for both the small and the large intestine, with the results concerning the growth in volume of the epithelium and mesenchyme in the whole intestine (Sbarbati & Strackee, 1980). In particular, they provide the following information: (*a*) the rate of increase of the cross sectional area of the epithelium is higher than that for the mesenchyme in both duodenum and colon; (*b*) the absolute increase of the cross sectional area of the epithelium and mesenchyme is greater for the duodenum than for the large intestine; (*c*) the allometric growth of the cross sectional area of the epithelium and mesenchyme shows similar characteristics for the duodenum and the colon, i.e. the allometric curves show similar slopes.

Figure 13 is a schematic representation of the development of the small and large intestines: note the difference in the absolute increase of the cross sectional area of the epithelium and mesenchyme and their changes in shape. These results suggest a shift in time of the development of the two intestinal parts, i.e. the area and shape of the colon seem to be time-shifted to the left with respect to those of the duodenum. The finding that the ratio between the growth rates of the epithelium and mesenchyme (Fig. 12) is similar in the two parts of the intestine during the period under study validates the hypothesis that the dynamic balance of the growth of the two layers contributes to the shaping of the wall and to the formation of the villi. The growth of the two tissues within the geometric framework constituted by their

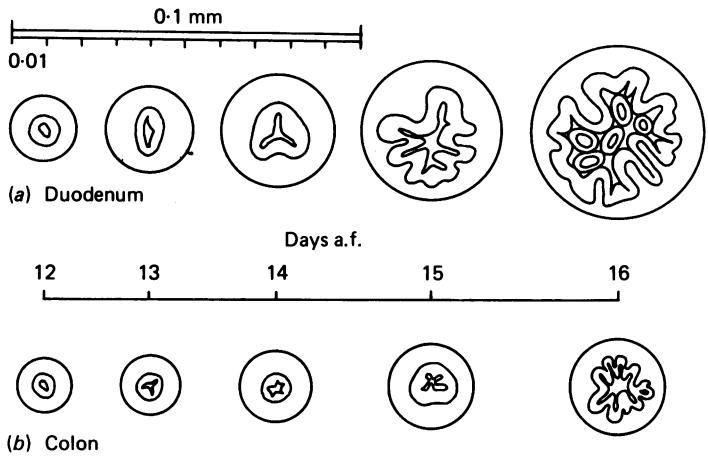

Fig. 13 (*a–b*). Schematic representation of the cross section of the duodenum (*a*) and large intestine (*b*). The dimensions are proportional to the values of the estimated parameters (see Fig. 11*a–b*).

reciprocal position in space involves physical (and chemical) reactions which lead to new local adaptations: "Un processus d' élaboration est soumis à une limite spatiale, laquelle une fois atteinte incite une nouvelle transformation" (Landsmeer, 1968). In a system constituted by growing masses, physical laws should hold; because chemical reactions have an important physical component, morphogenesis can be considered a dynamic process which includes chemical processes as well (Blechschmidt & Gasser, 1979).

What does the system aim at?

The assumption is now made that the important concept formulated by Blechschmidt & Gasser (1979), i.e. that: "at any particular moment during development, an organism functions according to the features its organs have attained at that time" holds also for single organs, and leads to the conclusion that at least one of the functions of the organ's tissues during morphogenesis is the generation of new forms resulting from their mutual interaction.

The aim of the chain of the reciprocal adaptations of the epithelium and mesenchyme during morphogenesis is at any given time: (1) to enlarge the surface between epithelium and lumen (Figs. 14, 16) and (2) to keep the epithelial mass/epithelial surface ratio constant (Fig. 15). A boundary condition for the process is the preservation of the lumen (Mandelbrot, 1977). These ideas find support in the earlier literature: "Another phenomenon, and one which is visible throughout the whole field of morphology, is the tendency (referable doubtless in each case to some definite physical cause) for mere bodily surface to keep pace with volume, through some alteration of its form. The development of villi on the lining of the intestine (which increase its surface much as we enlarge the effective surface of a bath-towel) . . . all these and many more are cases in which a more or less constant ratio tends to be maintained between mass and surface, which ratio would have been more and more departed from with increasing size, had it not been for such alteration of surface-form. . . . In fact, a deal of evolution is involved in keeping due balance between surface and mass as growth goes on" (D'Arcy Thompson, 1917).

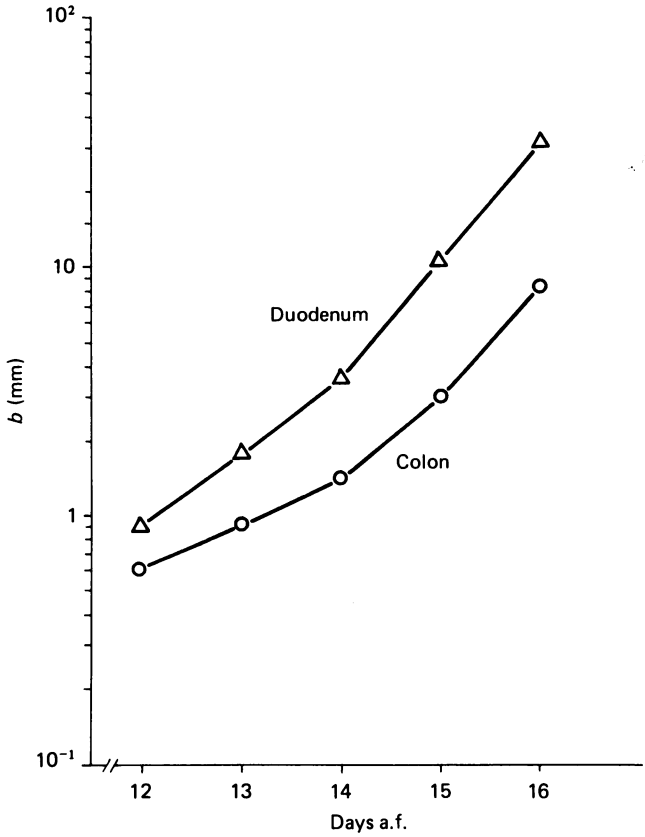

Fig. 14. Estimates of the length of the borderline (*b*) between the epithelium and the lumen in the cross sections in Fig. 13. Duodenum (△) and large intestine (○) show a rapid increase of this dimension in time. The log of the length is plotted against time.

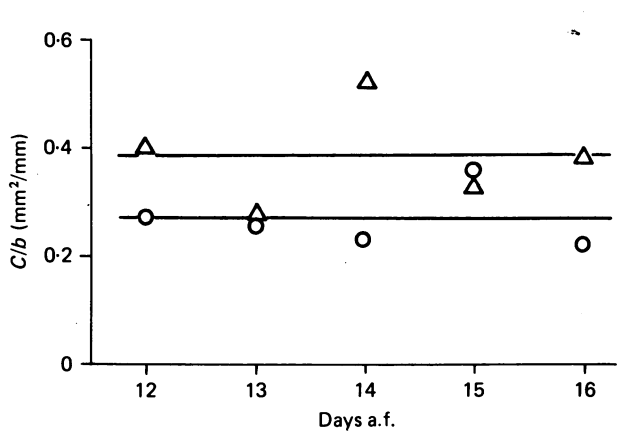

Fig. 15. The constancy in time of the epithelial mass/epithelial surface ratio is evident from this plot of the course of the ratio between the epithelial cross sectional area (*C*) and the borderline (*b*) between the epithelium and lumen. △, duodenum; ○, large intestine.

One of the main functions of the small and large intestines in postnatal life is the absorption of digested food and water. To perform this function efficiently the intestine needs a vast surface of epithelial cells of the absorptive type. The formation of the villi and the growth in length of the intestinal tube during prenatal life provide the morphological basis for the development of this function. The results presented in Figures 14 and 16 suggest that at any given time the maturation of the intestinal wall tends to enlarge the inner epithelial surface, i.e. it is a step towards the adult form. It is therefore possible to suggest that at any time during morphogenesis the intestine has an absorptive function peculiar to that stage of development.

Some statistical aspects of growth

A possible interpretation of the process of formation of the epithelial elevations projecting into the lumen is as follows: one may start by considering the stage in which the epithelium is several cells thick and the lumen is narrowed to a slit; the cells around the lumen are not arranged in regular layers (Shaw Dunn, 1967). In cross sections, the mitotic figures are randomly scattered (Eastwood & Trier, 1974), i.e. the proliferating epithelial cells have random spatial positions. This means that the probability of finding a proliferating cell in a particular region is independent of the occurrence of the same event outside that region.

The event called cell division can be considered, at least in first approximation, a random process in time, i.e. the probability of a cell division occurring at a certain time is independent of the occurrence of the same event in the past. In other words a random process is such that the occurrence of an event is completely independent of the occurrence of previous events. Examples of random spatial processes are dust particles on a plate or raisins in a cake; examples of random time processes are the cars coming out of a tunnel on a highway or the spontaneous firing of a visual neuron in the dark.

In a random (Poisson) process (e.g. Lindley, 1969) the probability density of the time between any two successive events is given by

$$f(t) = \lambda e^{-\lambda t},$$

where λ is non-negative and is the rate of occurrence of the events. The density has a maximum at $t = 0$ and then diminishes steadily with time. This is interesting, because it means that small intervals between successive events are more probable than longer ones. Sbarbati (1981) shows the simulation of a spatially random pattern of dividing cells at time t and the simulation of the proliferation of cells according to the model of random proliferation in time. In both cases the tendency of the events to 'cluster' is evident. The phenomenon of division synchrony in groups of cells is described in the literature. According to Snow (1977) they "represent clones with a common ancestor, indicating that over a period of several divisions progeny of cells do not move away from one another to any great extent. These synchronous clones in one embryo are rarely observed in the same site in other embryos". The occurrence of clones might be seen in relation to the property of random events to occur in clusters. Returning to the intestinal epithelium, one may speculate that 'clustering' in space and/or time occurs as the result of random processes and produces epithelial elevations into the lumen.

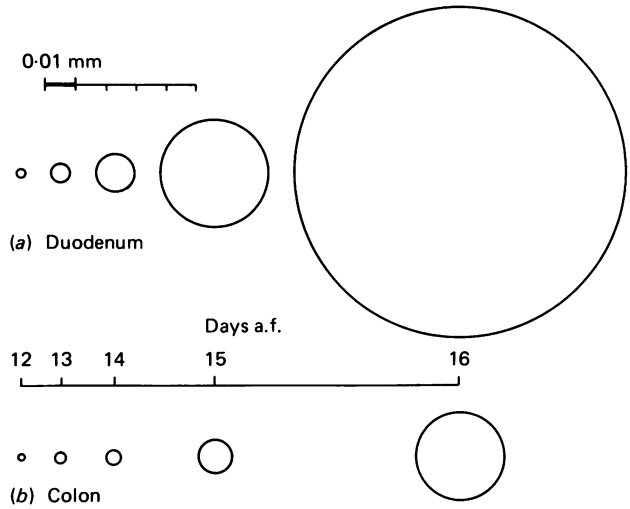

Fig. 16 (*a–b*). Hypothetical dimension of the intestinal lumina of the duodenum (*a*) and large intestine (*b*) if the epithelium were to grow unrestrained without the formation of villi. The circumferences represent the values of the estimates plotted in Fig. 14.

A proposal for dynamic growth

The following interpretation is proposed of the picture shown by the morphogenetic processes involved in the maturation of the duodenal and colonic wall. In early stages of the organogenesis, a cross section of the intestine shows the squamous epithelial cells arranged in a monolayer. The cells then become cuboidal, next cylindrical, and finally the epithelium becomes stratified. It is generally observed that when the growth of the epithelium is hindered because of the position of this layer, the epithelium becomes relatively thick (Blechschmidt & Gasser, 1979). In early stages, the outer layer is composed of loosely packed, randomly oriented mesenchymal cells and by the serosa. Because the growth rate of the epithelium is more rapid than that of the mesenchyme (Fig. 11*a–b*) the epithelium exerts growth-pressure on the mesenchymal cells, which thus tend to become oriented with their long axis around the epithelial tube. The circular arrangement of the stromal cells shows the pressure of the inner layer and demonstrates that the motor for the work of forming the embryonic intestine resides in the epithelium (Blechschmidt & Gasser, 1979). The vascularized area near the epithelium forms the lamina propria, and in the surrounding area the musculature differentiates. The formation of the muscular layer results in increased circular resistance to the rapid expansion of the epithelium: the mesenchyme is stretched by the growth-pressure of the epithelium and becomes more and more a restraining apparatus. The lumen is filled with fluid which resists expansion of the epithelium into it. All the successive reciprocal spatial adjustments of the epithelium and mesenchyme can be explained in terms of transitions within states of equilibrium between the physical forces acting at the interfaces between epithelium and mesenchyme, and epithelium and lumen. The elliptical shape of the epithelium in cross sections is the simplest non-circular shape conceivable for a tube in rapid expansion and constrained between two forces of opposite sign. The elliptical epithelium maintains the shape of the lumen with less expenditure of energy

than would be the case if it continued to grow round (Fig. 16) and at the same time produces enlargement of the interface between epithelium and lumen (Fig. 14).

Around 13–14 days the epithelium shows elevations projecting into the lumen. Evidence has been presented (Sbarbati, 1981) that these structures originate as elevations, and not as ridges which then become segmented. Hilton (1902) describes the formation of the villi in the small intestine of the white rat as "thick epithelial processes into which later the connective tissue cores extend", and concerning the villi in the large intestine he says: "they may be first recognized as little elevations of epithelial cells, a core of connective tissue pushes up into these cell masses and the villi grow upward and become like those in other parts of the intestine".

The cells at the top of the elevations degenerate and their arrangement becomes loose. This phenomenon could be due to the increasing distance between these cells and the vascularized lamina propria, which makes it impossible for them to receive an adequate blood supply. This loss of cells at the top of the elevations may affect the base by altering the dynamic balance between the growth-pressure exerted by the epithelium and the restraining action of the mesenchyme. As a result, the cells of the lamina propria are simultaneously 'pushed and pulled' into the epithelium by the two opposite forces, and thus come to form the mesenchymal core of the newly formed villus. This push–pull effect is expressed in the orientation of the mesenchymal cells, whose long axis lies parallel to the axis of the villus. The mesenchymal stroma offers a gradually enlarging surface for the epithelial cells, which become rearranged into a single layer.

CONCLUSION

Landsmeer (1968) stated that: "des phénomènes de differentiation . . . doivent avant tout, être consideré comme des nécessités d'ordre spatial". It is believed that chance and necessity are closely related in nature and that random processes can produce ordered structures.

Equilibrium is said to be a state of the biological system where the difference is minimal between the actual values of the variables and their optimal values, according to the purpose of the system. When biological matter is not in equilibrium, it organizes itself in an attempt to reach equilibrium, and the interaction between self-organizing structures leads to the formation of new structures. The random proliferation of a group of epithelial cells leads to a highly ordered structure like the villus; in other words, the chance gives rise to a structure, i.e. a spatial arrangement with a specific function.

Mandelbrot (1977) gives amazing examples of simulation of highly complex patterns occurring in nature, for instance the profiles of coast lines, based on the laws of chance. Some of the features generated in this way might serve as examples of the formations of the epithelial elevations according to the concept presented here.

Morphogenesis can thus be seen as a continuous attempt of living matter to reach the adult form via successive states of equilibrium.

SUMMARY

The duodenum and colon are adjacent organs with a different morphogenesis, but they show a common developmental pattern, the formation of the villi.

The aim of the present study was to test the hypothesis that the morphogenesis of the villi in both organs is brought about by the dynamic interaction between the epithelium and the mesenchyme, in particular by differential changes in their growth rates within the geometric framework constituted by their interrelated spatial positions.

Mouse embryos of the CPB-S strain aged between 12 and 16 days after fertilization were used. They were sectioned at constant thickness (10 μm and 1 μm). Cross sections of the duodenum and large intestine were selected as originating from corresponding straight parts of the intestinal tube at different ages. The following parameters were estimated by means of a morphometric method: cross sectional area of the epithelium and of the mesenchyme, outer circumference of the intestinal tube and length of the borderline between the epithelium and the lumen.

The results show that the epithelium and the mesenchyme of the duodenum grow differently from the epithelium and the mesenchyme of the colon, e.g. they present different cross sectional areas and specific growth rates. Nevertheless in both cases the mesenchyme grows more slowly than the epithelium and the ratios between the specific growth rates of the layers (e.g. the allometric curves) are similar throughout growth. These findings support the hypothesis that the dynamic interaction of the layers produces qualitatively similar shapes. The mutual interaction of the epithelium and mesenchyme produces at any time (1) enlargement of the surface between epithelium and lumen and (2) constancy of the ratio epithelial mass/epithelial surface. A boundary condition for the process is the preservation of the lumen.

The hypothesis is put forward that chance plays a role during morphogenesis and that random generation of cells in time and/or space might be involved in the origin of biological structures such as villi.

REFERENCES

BLECHSCHMIDT, E. & GASSER, R. F. (1979). *Biokinetics and Biodynamics of Human Differentiation. Principles and Applications* (ed. A. R. Burdi). Springfield, Illinois: Charles C. Thomas.

BURGESS, D. R. (1975). Morphogenesis of the intestinal villi. II. Mechanisms of formation of the previllous ridges. *Journal of Embryology and Experimental Morphology* **34**, 723–740.

COULOMBRE, A. J., & COULOMBRE J. L. (1958). Intestinal development. I. Morphogenesis of the villi and musculature. *Journal of Embryology and Experimental Morphology* **6**, 403–411.

D'ARCY THOMPSON, W. (1917). *On Growth and Form.* 1961. Cambridge: Cambridge University Press.

EASTWOOD, G. L. & TRIER, J. S. (1974). Epithelial cell proliferation during the organogenesis of the rat colon. *Anatomical Record* **179**, 303–309.

GOEDBLOED, J. F. (1972). The embryonic and postnatal growth of rat and mouse. I. The embryonic and early postnatal growth of the whole embryo. A model with exponential growth and sudden changes in growth rate. *Acta anatomica* **82**, 305–336.

GREY, R. D. (1972). Morphogenesis of the intestinal villi. S.E.M. of the duodenal epithelium of the developing chick embryo. *Journal of Morphology* **137**, 193–214.

HILTON, W. A. (1902). The morphology and development of intestinal folds and villi in vertebrates. *American Journal of Anatomy* **1**, 459–504.

KAMMERAAD, A. (1942). The development of the gastro-intestinal tract of the rat. I. Histogenesis of the epithelium of the stomach, small intestine and pancreas. *Journal of Morphology* **70**, 323–352.

LANDSMEER, J. M. F. (1968). Les cohérences spatiales et l'équilibre spatial dans la région carpienne. *Acta anatomica, Suppl.* 54=1 **70**.

LANDSMEER, J. M. F. (1978). Spatial aspects of extremity development. *XIX Morphological Congress Symposia Charles University Prague*, pp. 149–159.

LINDLEY, D. V. (1969). *Introduction to Probability and Statistics; Part I. Probability.* Cambridge: Cambridge University Press.

MANDELBROT, B. B. (1977). *Fractals: Form, Chance and Dimension.* San Francisco: W. H. Freeman & Co.

MATHAN, M., MOXEY, P. C. & TRIER, J. S. (1976). Morphogenesis of the fetal rat duodenal villi. *American Journal of Anatomy* **146**, 73–92.

MIETE, M. (1960). Some aspects of the embryonic development of the human stomach. Doctoral Dissertation, University of Leiden.

PLEEGING, J. H. (1975). De ontogenese van de darm bij *Mus Musculus* L. Doctoral dissertation, University of Groningen.

SBARBATI, R. (1979). Quantitative aspects of the embryonic growth of the intestine and stomach. *Journal of Anatomy* **129**, 795–803.

SBARBATI, R. & STRACKEE, J. (1980). Aspects of the growth of epithelium and mesenchyme in the intestine of mouse embryos. In *Cell Proliferation in the Gastro-intestinal Tract* (ed. D. R. Appleton, J.P. Sunter & A. J. Watson), pp. 22–26. Bath: Pitman Medical.

SBARBATI, R. (1981). Of mice and mazes – The prenatal development of the gastro-intestinal tract in the CPB-S mouse. Doctoral Dissertation, University of Leiden.

SBARBATI, R. & STRACKEE, J. (1982). A simple polynomial model of the growth of the gastro-intestinal tract in the mouse embryo. *Journal of Anatomy* **134**, 809–815.

SHAW DUNN, J. (1967). The fine structure of the absorptive epithelial cells of the developing small intestine of the rat. *Journal of Anatomy* **101**, 57–68.

SNOW, M. H. L. (1977). Gastrulation in the mouse: growth and regionalization of the epiblast. *Journal of Embryology and Experimental Morphology* **42**, 293–303.