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Mini Review

A Comprehensive Model of the Crypts of the Small Intestine of the Mouse Provides Insight into the Mechanisms of Cell Migration and the Proliferation Hierarchy

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A comprehensive model has been formulated for the proliferative behaviour of the crypts of the small intestine based on individual cell to cell relationships rather than on the average effects of all cells. The model accommodates a wide range of cell kinetic data and provides an insight into the mechanisms involved in cell movement within the columnar sheet of cells and into the relationship between the stem cells and their progeny. The model permits the number of stem cells and transit generations to be estimated. The number of stem cells is predicted to be not less than 4 and not more than 16 per crypt with cell cycle times of between 12 and 32 h respectively. Certain conclusions can be drawn concerning the mechanisms involved in the initial cell displacements after cell division. The model also allows an estimation of parameters which cannot be measured directly such as the degree of cell generation disorder and the amount of dispersion of cells within a cell lineage.

Introduction

Many cell kinetic experiments have been conducted over the years on the crypts of the small intestine (see Fig. 1). These range from simple mitotic (MI) and labelling indices (LI) to mitotic accumulation (stathmokinetic) experiments, percent labelled mitosis (PLM) and continuous labelling (CL) experiments and studies on how the labelling patterns change with time. These have resulted in a fairly thorough understanding of the migration rates and parameters such as the average cell cycle times, at least in the mouse for which most data are available. Such experiments have been thoroughly reviewed elsewhere (Wright & Alison, 1984; Potten & Hendry, 1983; Potten *et al.*, 1983). One problem is that these data have not been considered in relation to the hour by hour functioning of the crypt, i.e. these data have not generally been considered in the context of the crypt as a multicellular proliferative unit (Fig. 1). Hence, such simple questions as: (1) where does the new daughter cell position itself in relation to its mother after division? (2) how is the steady emigration of cells from the crypt controlled? (3) how does the crypt and its cells respond to injury? and (4) how is the shape of the crypt maintained? have not been investigated. The cell kinetics of the system have now been thoroughly investigated

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1986)—and the way in which the lengths of the clusters (or number of runs) changes with time, is a potentially powerful piece of information which is particularly dependent on the mechanisms involved in cell movement. Few runs indicate that there are many long sequences of like-labelled cells and many runs indicate many alternating labelled and unlabelled cells. Hence, this parameter provides information on the degree of vertical clustering of cells, i.e. the vertical arrangement of cells, rather than being a direct kinetic parameter. Neither this parameter nor the idea of investigating the behaviour of the tissue on a cell to cell and hour by hour basis has been comprehensively studied before.

Although a vast amount of basic data is available it often differs slightly from case to case as different animals or techniques have been used within different laboratories. For this reason we did not attempt to make use of data not generated by ourselves that were not relevant to our animals. Thus the comprehensive accommodation of different data sets is restricted. Nevertheless we have now accumulated a considerable range of information on the crypts of the ileum in male 11-16 week old B6D2F1 (Pat) mice. Information accumulated includes the following: (1) the distribution of various cell types as measured in thin sections or conventional paraffin sections; Paneth cells, columnar cells, goblet cells; (2) the distribution of DNA synthesizing cells (labelled cells) and the changes in these distributions with time after labelling which can be used to measure a migration velocity; (3) the changes in the distribution of labelled cells when tritiated thymidine is administered repeatedly (continuous labelling); (4) the distribution of mitotic figures and the changes in the number and distribution of mitotic figures when stathmokinetic agents are used; (5) the clustering of labelled cells and mitotic cells both vertically up the crypt and circumferentially around the crypt; (6) the changes in the percentage of labelled mitotic (PLM) figures with time after labelling; (7) the distribution of the number of histologically recognizable dead or dying cells under normal and perturbed states; (8) estimates of the number of clonogenic cells from radiation studies. Other data available but so far not analysed include the changes in various parameters throughout the day (circadian variations). This list includes a fairly extensive set of studies using virtually all possible cell kinetic approaches and hence we feel that we can be reasonably comprehensive in our analysis. Although most of the information generated by these experiments has been considered our modelling approach has so far not included the detailed PLM or cell death studies.

The conclusion drawn from many experiments over the years is that there is a steady flow of cells from the crypts and on to the villi and that the velocity of the cells increases progressively as they move up the crypt. Consequently the cells at the origin of this movement must be ultimately responsible for all cell replacement. These cells, upon which the crypt, all of its cells and indeed the entire epithelium depend, can be regarded as the stem cells of the system. They are few in number and are located near the bottom of the crypt (Wright & Alison, 1984; Potten & Hendry, 1983; Potten *et al.*, 1983; Loeffler *et al.*, 1986; Cheng & Leblond, 1974; Leblond & Cheng, 1976; Bjerknes & Cheng, 1981). Their descendants are displaced progressively up the crypt and divide as they move. Hence the crypt can be regarded

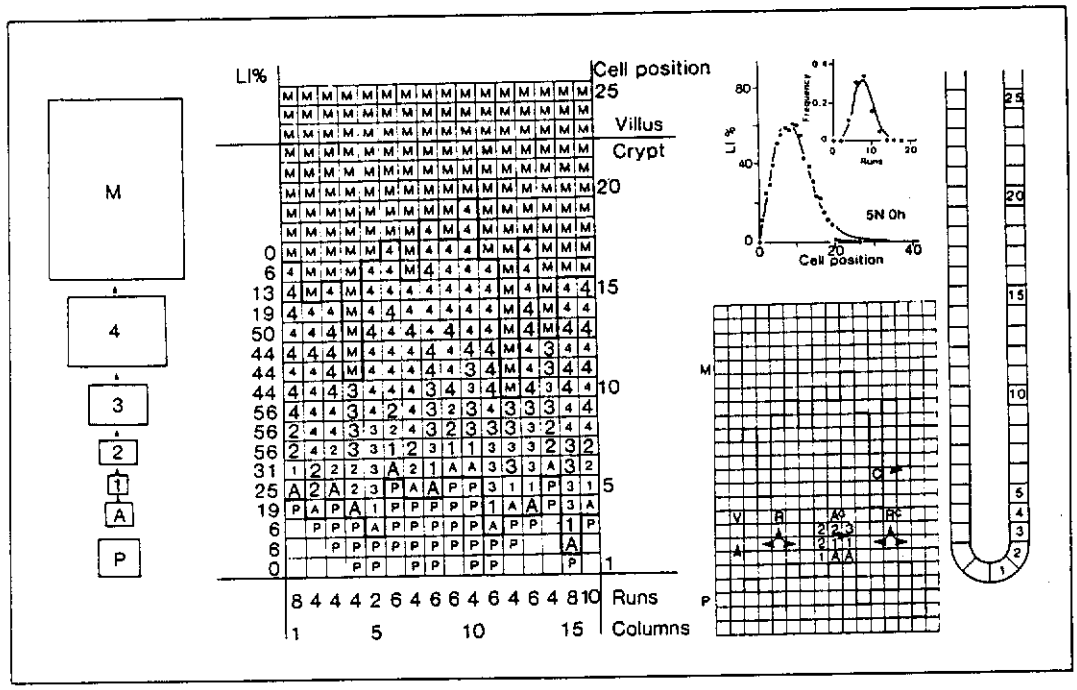

FIG. 2. Diagrammatic representation of the crypts in the small intestine of the mouse. The entire crypt can be represented by a matrix of cells (16×25 in this case). The matrix represents a crypt opened up and then flattened. In longitudinal section the crypt would represent a closed cylinder and the right-hand diagram shows the cell numbering system (see Fig. 3 for a more detailed representation of a longitudinal section). The proliferative zone is delimited by the strong solid lines, the upper one representing the T/M boundary (see text) and the lower one the Paneth/stem cell (P/S) boundary. Besides the non-proliferative Paneth (P) cells and post mitotic (M) cell sthe crypt contains an hierarchy of proliferative cells originating from the stem (A) cells which are positioned above the highest P cell. These transit proliferative cells can be subdivided into 4 separate generations (T_1–T_4), numbered 1-4 in the diagram. Within the middle band of the crypt about half the cells would be replicating their DNA and hence are labelled by tritiated thymidine. A typical labelling index distribution (LI%) is shown and the labelled cells are indicated by the large numbers. Small matrix: on division the daughter cells could position themselves one above the other (vertical V), or randomly (R) select a position, or move beneath the oldest neighbouring cell (A^C), or a cut-off (C) could act in conjunction with an initial random cell displacement (R^C) Graphs: A typical model fit (solid line) to LI and runs data is shown for the five neighbour model. This figure has been taken with modification from Loeffler *et al.* (1986).

The precise values for all three parameters are largely unknown but the model is able to restrict the range of possible parameters as will be discussed below. The model so far does not take into account the turnover of the Paneth cells or the differentiation of cells in the goblet lineage. Both effects are relatively minor. The former, in effect, slightly reduces the output from the stem cell compartment—roughly equivalent to a small reduction in stem cell number (N_A) or a small lengthening of stem cell cycle time (T_{cA}); the latter in effect reduces the number of transit generations slightly by extinguishing some branches of the lineage prematurely, i.e. resulting in slightly less than integer values for L.

When mitotic activity is inhibited by radiation (Potten & Hendry, 1983), or a cocktail of cytotoxic drugs in combination with radiation (Kaur & Potten, 1986*b*) or by stathmokinetic agents (our own unpublished data) prelabelled cells or mitotic figures continue to move up the crypt at least for a short time after treatment.

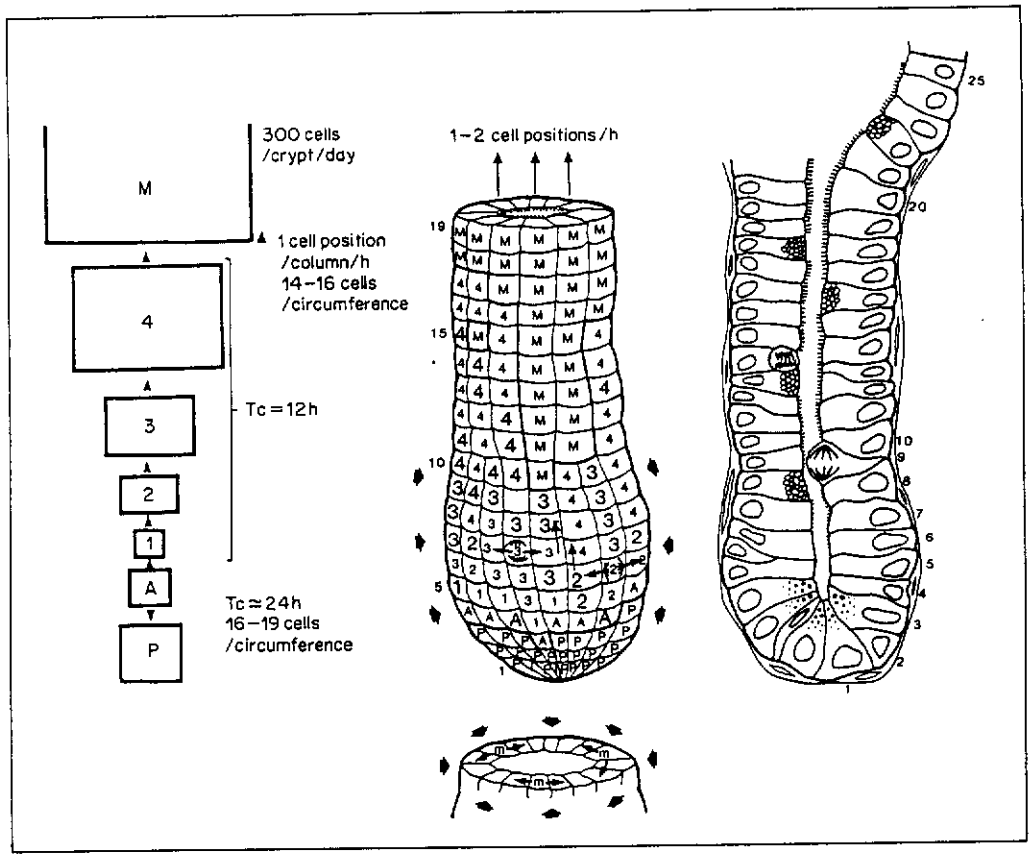

FIG. 3. If the matrix is reassembled to form the crypt, or if the information within the matrix is transferred to an actual crypt the central diagram would result. The right-hand diagram shows a more typical longitudinal section through the crypt showing two centripetal mitotic figures (close to the lumen), the crypt numbering procedure, the Paneth and goblet cells and the pericryptal fibroblast sheath which represent the most intimate non-epithelial element. Motitic activity results in many new daughter cells positioned within the circumferential plane. This is counterbalanced by a pressure from outside the crypt (heavy arrows) which squeeze the crypt resulting in the fusing of neighbouring columns and the movement of cells out of the top of the crypt.

some sorting (repositioning) of the cells according to their age occurs higher up the crypt.

The micro-environment at about the 13th position may act as a sort of *cut-off* for cell proliferation preventing any further re-entry into S phase of cells above this point. This would restore some age order in the crypt at the level of the T/M boundary and in fact provides a completely acceptable set of rules by which the model can be run to generate good fits to all the data. This would result in some cells undergoing fewer transit divisions than others, i.e. the number of transit divisions would not be precisely predetermined.

A final mechanism to explain the positioning of newborn cells assumes that the cells somehow detect the age of other cells in their local milieu and position themsleves beneath the oldest nearest neighbour (see Fig. 2): a selection perhaps based on the diffusion gradient of some cell age-dependent diffusible factor, or on age-dependent adherence of cells to the basal lamina. Models based on a scanning

one lateral or circumferential which is related to mitosis, and one squeezing the crypt, like a tube of toothpaste, which counter balances the mitotic force (see Fig. 3). The LI and MI distributions thus would reflect the distribution of the mitotic force and where the indices decline at the bottom and top of the crypt the squeezing force would become dominant and the number of columns would be expected to fall—as is indeed observed, i.e. this hypothesis would provide an explanation for the shape of the crypt. One experiment showed 16 cells per circumference at the top of the proliferative zone in normal crypts compared with nearly 19 a few cell positions from the bottom. In another study we observed a 25% reduction in the diameter of the top of the crypt. We assume here that cells do not change their shape and their area of attachment to the basal lamina during their life in the crypt. At present the matrix model does not account for this reduction in columns near the top of the crypt. However, this tapering towards the top of the crypt, the extinguishing of lineages due to goblet cell differentiation and the loss of cells after cytotoxic exposure all should be more readily accommodated when the model is adapted to be based on a more flexible network where the cell to cell contacts, rather than matrix position, are recorded.

When LI, runs and MI data are considered the age-dependent model (looking at 3 neighbours) would operate satisfactorily within the known biological and architectural constraints for the crypt with between 4 and 16 stem cells per crypt having stem cycle times between 12 and 32 h and between 4 and 6 transit generations (see Fig. 4). If 5 neighbours are considered the "window" of acceptable values is smaller (12-16 stem cells, with a T_C of 12-32 h and 4 or 5 transit generations) (Loeffler *et al.*, 1986). If the cut-off model is considered, the "window" is slightly larger. It is

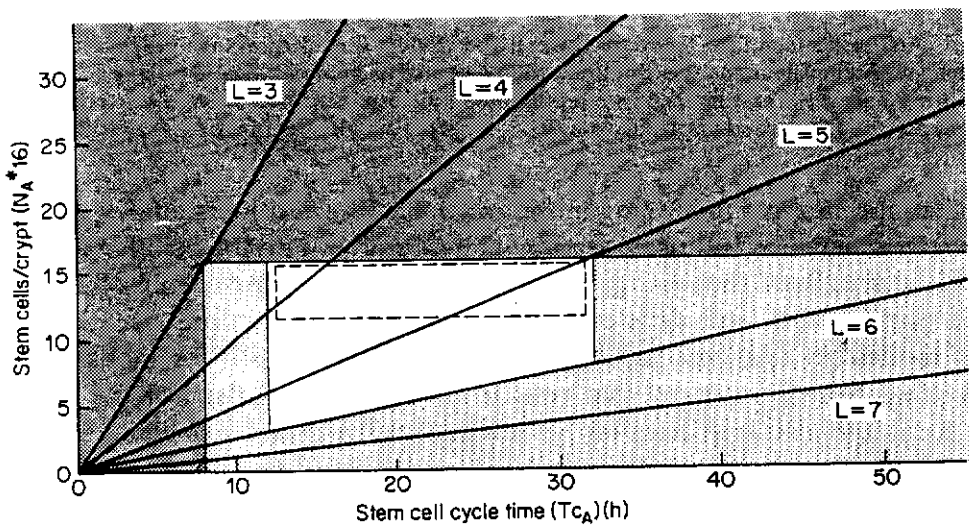

FIG. 4. Diagram showing the relationship between the number of stem cells per crypt (N_A) (*unlikely to be above 16), the stem cell cycle time (T_{cA}) and the number of transit generations (L). The heavily shaded area represents unlikely configurations of values, the lightly shaded area possible but unlikely values, while the unshaded "window" represents the most likely range of values: the smaller dashed window the 5N model, the larger window the 3N model. Reproduced with permission from Loeffler *et al.* (1986).

as a cone shaped structure and we would postulate that in this model system both position and generation age are detected by the cells and are important in determining the proliferative and differentiative behaviour of these crypt cells. Radiation and drug treatment experiments in the crypt are analogous to the elegant grafting experiments of developmental biologists, in both cases the cell to cell inter-relationships are experimentally manipulated.

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