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# A model of the control of cellular regeneration in the intestinal crypt after perturbation based solely on local stem cell regulation

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**Abstract.** The control mechanisms involved in regeneration of murine intestinal crypts after perturbations are presently not well understood. The existence of some feedback signals from the cells on the villus to the cells in the crypt has been suggested. However, some recent experimental data point to the fact that regeneration in the crypt starts very early after perturbation, at a time when the villus cell population has hardly changed. In particular, this early cell proliferative activity is seen specifically at the bottom of the crypt, i.e. in the presumed stem cell zone and furthest from the villus.

The objective of this study was to investigate whether a new concept of regulation operating solely at the stem cell level could explain the present mass of accumulated data on the post-irradiation recovery, which is an extensively studied perturbation from the experimental point of view. In order to check its validity, the new concept was formalized as a mathematical simulation model thus enabling comparison with experimental data. The model describes the cellular development from stem cells to the mature villus cells. As a basic feature it is assumed that the self-maintenance and the cell cycle activity of the stem cells are controlled by the number of these cells in an autoregulatory fashion. The essential features of the experimental data (i.e. the recovery with time and the consistency between different types of measurements) can be very well reproduced by simulations using a range of model parameters. Thus, we conclude that stem cell autoregulation is a valid concept which could replace the villus crypt feedback concept in explaining the early changes after irradiation when the damage primarily affects the crypt. The question of the detailed nature of the control process requires further investigation.

The epithelium lining the small intestinal tract consists of a layer of columnar cells folded into villi and crypts. Cell function is restricted to the villi and cell proliferation is restricted to the

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crypts. The crypts contain about 250 cells in total of which 150 are rapidly proliferating with cell cycle times of the order of 12 h. Over the last 2 decades it has become increasingly apparent that the cell replacement in the crypts is achieved according to cell lineages, or cell hierarchies, with relatively few lineage ancestor cells, or stem cells, located near the base of the crypts. (Potten & Hendry 1983, Wright & Alison 1984, Potten 1990, Potten & Loeffler 1990). The precise number of stem cells per crypt and the mechanisms by which their proliferation is controlled remain somewhat obscure. There are two main problems associated with addressing the question of the number of stem cells and their control. The first is that there are no morphological features that enable one to identify these cells and hence count them. As a consequence of this we are forced to use functional assays for studying stem cell behaviour. This inevitably leads us to the second problem which is that we invariably attempt to study stem cell properties by observing the entire crypt or the progeny of the stem cells at long times after perturbation.

The stem cell population maintains its size at each cell division and gives rise to a population of dividing transit cells which form the lineage and effectively amplify the proliferative behaviour of the stem cell population. The number of generations within the dividing transit population is believed to be between four and six. The number of generations in the transit population is either a fixed intrinsic property of the cells or is determined by the external environment (a cutoff system). We assume here, and in a past, an intrinsic control on the number of transit generations, i.e. a control within the cell lineage or cells. One of the uniquely attractive features of the gastrointestinal system is the ability to relate the hierarchical position of a cell to its topographical position within the crypt. In this way the stem cells are located at or near the base of the crypt, the crypts function as independent closed entities for long periods of time, perhaps for the entire life of the animal.

**Perturbations of the system**

A variety of chemicals and radiation has been used in the past to disturb the system in order to study the consequences. Here, we consider the effects of irradiation. It is clear from the extensive literature on irradiation effects on the intestine that the system is very robust and capable of regenerating itself following even severe levels of cytotoxic insult, i.e. cellular depletion. The effects of a single dose of irradiation on the small intestinal crypts of one strain of mice have recently been extensively reviewed (Potten 1990) and the sequence of events that occurs can be summarized as follows.

The earliest changes that can be detected in histological sections are the appearance of dying cells that morphologically display pycnosis. In some studies using the electron microscope these cells have been clearly demonstrated to be morphologically identical to cells in apoptosis (Potten 1977, 1990). These cells appear within 2 or 3 h after irradiation and are restricted largely to the base of the crypt, i.e. the stem cell zone (Potten 1977, Ijiri & Potten 1983, Hendry *et al.* 1982). They appear following small doses of irradiation which indicates that the cells that are susceptible to this form of death are very radiosensitive. The number of cells that appear capable of dying in this way is restricted to about six per crypt (Potten 1977). Concomitant with these changes are changes in cell kinetics (Tsubouchi & Potten 1985, Potten 1991a,b). The progression of cells from $G_2$ to mitoses also is very sensitive. This results if a fall in the mitotic activity at an early time and in an accumulation of cells in $G_2$ (the mitotic block or delay). Again this phenomenon appears to the most prominent (longest delay) in the cells at the base of the crypt (Chwalinski & Potten 1986). Once this block in $G_2$ is overcome there is evidence that the cells progress through the cycle more rapidly than in the steady state (Potten 1990). The overall cell cycle time for the crypt is shortened somewhat but the cycle time for the

cells at the base of the crypt is shortened fairly dramatically (Potten 1990). Such changes in cell cycle duration can be observed within hours of irradiation and are clearly evident over the first few days post irradiation.

From clonal regeneration studies it is evident that moderate doses of irradiation (8–12 Gy) cause a reproductive sterilization in a large proportion of the regenerative, or clonogenic, cells in the crypt. Providing at least one clonogenic cell survives the crypt will eventually be re-established. As a consequence of: **1** the acute cell death, **2** the $G_2$ arrest and the subsequent fall in mitotic activity and **3** the reproductive sterilization of many of the clonogenic cells (all of which are associated with the stem cell population), the production of cells for the transit population and subsequently the villus is decreased. As a consequence of this the crypt shrinks in size and eventually the villus likewise shrinks. Observations based on the association between the reduced villus cellularity and the increased proliferation in the crypt led to the formulation of the concept of negative feedback control of proliferation, i.e. signals from the villus influencing the crypt (Cairnie 1976, Wright & Al-Nafussi 1982, Rijke *et al.* 1974, 1976).

Sato *et al.* (1972) were the first to suggest a model for regeneration after irradiation which included a feedback from the villus on the crypt cells. Several investigators have concluded subsequently that a feedback from the villus to the crypt should act on cell cycle times and/or the self-maintenance of stem cells (Britton, Wright & Murray 1982, Kicherer 1983, Tucker & Hendry 1990). A more complicated model with the above features was suggested by Yakovlev & Zorin (1988). Meinzer *et al.* (1990) also assumed a feedback from the villus and in addition took crypt fission into account. There is one model which operates with regulation within the proliferating crypt population (Taylor, Withers & Hu 1988). However, in this model no distinction is made between stem cells (A) and dividing transit cells (T).

Since these initial studies much further information has been obtained and it is now clear that some of the earliest phases of regeneration occur in the crypt at times when there is no detectable change in the villus cell population and minimal changes in the crypt cellularity (Potten 1990, 1991a,b). These observations clearly pose problems for the villus feedback model. We consequently set out to investigate whether the experimental observations could be accounted for by regulation solely within the stem cell compartment. The observations referred to above are as follows:

**1** The cell numbers in the crypt are reduced by more than 50% at day 2 and then overshoot at day 3 (Figure 1a).

**2** The overall labelling index (LI) begins to increase from its minimum values at about 15 h after irradiation. There are more specific changes in the stem cell region that occur even earlier than this, at a time coincident with local crypt base cell death.

**3** Similar patterns of change as seen with LI can be observed with mitotic activity.

**4** Movement of the leading edge of a LI frequency plot with time after pulse labelling the cells in the crypt can be used to measure the rate of migration of cells from the crypt (Potten *et al.* 1982, Kaur & Potten 1986a). Cell movement from the crypt continues at all times after irradiation, albeit at reduced rates. Immediately following irradiation the migration velocity is about 60% of the control value. Between 3 h and 24 h following irradiation it continues at between 23% and 30% the control rate. At the early time cell migration continues while mitotic activity is suppressed (Potten *et al.* 1982, Kaur & Potten 1986b, Chwalinski & Potten 1986). By 2 days after 8 Gy cell migration is nearly back to normal. Using these migration velocities and the measured changes in cellularity, the crypt emigration rate and cell production rate from division can be calculated (Potten, Taylor & Hendry 1988). The cell production is reduced to almost 0 at day 1 (Figure 1b).

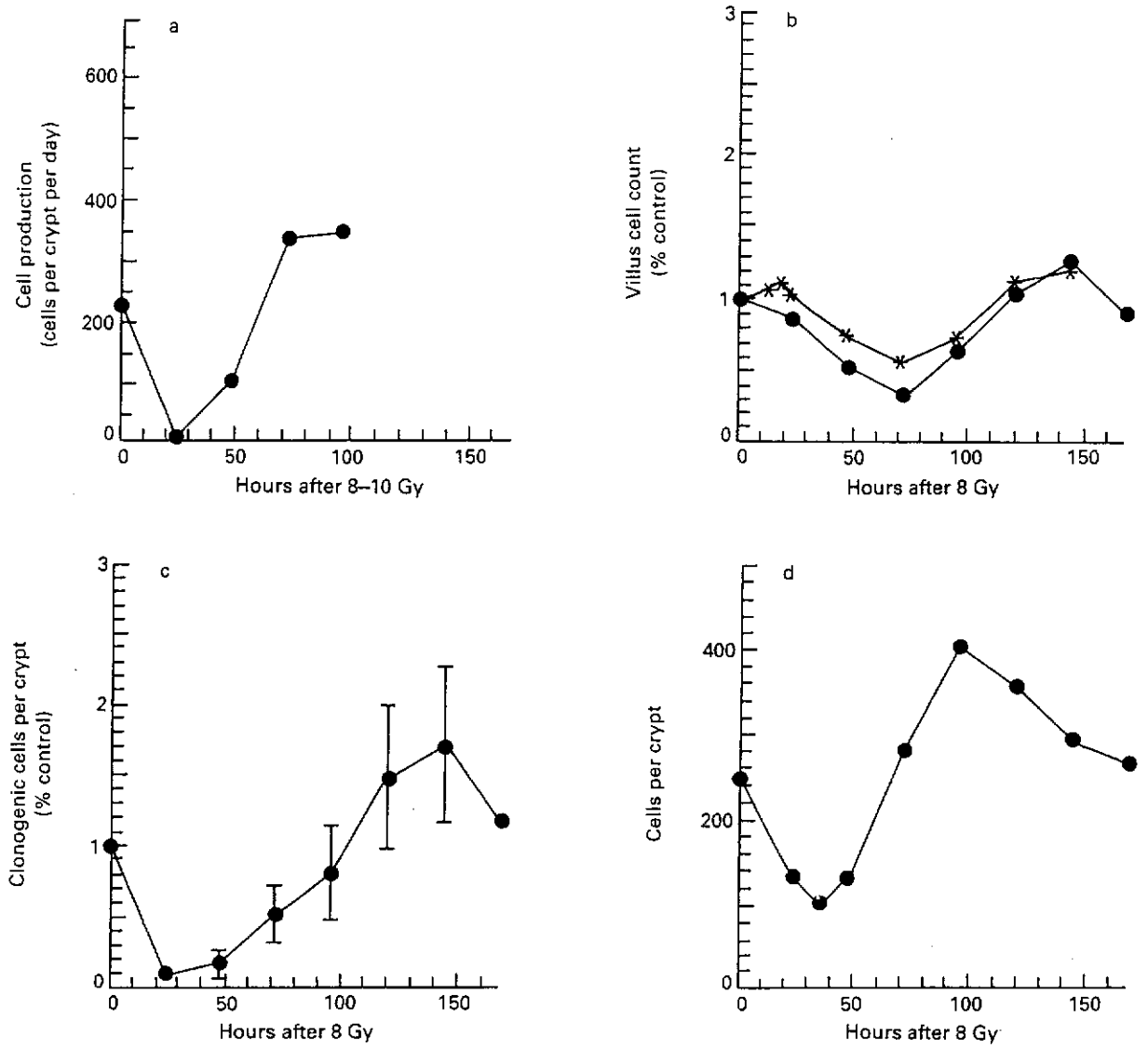


**Figure 1.** Time course of changes in experimental data. **a** Cell production per day after 8 Gy. The cell production is calculated from the changes of the crypt cellularity (Figure 1**d**) and the cell output (migration velocity × crypt circumference at the crypt top). During the first day after 8 Gy the cell production breaks down entirely (Table 2 in Potten 1990). **b** Changes in the villus cellularity (number of cells in the crypt-villus column) as a percentage of control values. Closed circles, data from Sato *et al.* (1972) for 10 Gy to CFF1 mice; (*) = data for 8 Gy to BDF1 mice (Potten unpublished data). **c** The number of clonogenic cells per crypt as a percentage of the control value at various times after 8 Gy measured using the split-dose technique (Potten *et al.* 1988). At day 4 the control value is re-established. **d** Number of cells per crypt over the first 7 days after 8 Gy $\gamma$-irradiation of BDF1-mice (Table 2 in Potten 1990). The reduction over the first 2 days is followed by an overshoot on day 4 and 5. This observation is used as an input in the model simulations.

5 The villus cellularity begins to fall after times in excess of 1 day (Sato *et al.* 1972, Figure 1c). Villus height shows little change over the first 3–4 days following irradiation (Potten 1990).

6 In studies where the number of clonogenic cells are estimated using split dose techniques an expansion in clonogenic numbers begins about 22 h after irradiation (Potten *et al.* 1988).

7 Percentage labelled mitosis (PLM) studies indicate that there are changes in the cell cycle duration soon after irradiation (Tsubouchi & Potten 1985; Potten 1991a,b) and a significant shortening of the cell cycle time in the bottom of the crypt on day 2, at a time when the crypt may have shrunk in size but the villus cellularity is still high (Potten 1990).

**Table 1.** Summary of some basic experimental observations after total-body irradiation of BDF1 mice

| **Dose of total-body irradiation: 8 Gy** | |
|---|---|
| Number of cells per crypt: | reduced to 50% at day 2; overshoot on day 4 (Potten *et al.* 1988). |
| Histologically recognizable cell death (apoptosis): | up to six cells in cell position 4–7 (Potten 1977, Hendry *et al.* 1982, Ijiri & Potten 1983, 1987). |
| Overall labelling index: | reduced on day 1, overshoot on day 3 (Potten *et al.* 1990, Potten 1990). |
| Cell migration:* | continuing migration from crypt to villus during mitotic inhibition; reduced migration until day 2; subsequently accelerated migration (Potten *et al.* 1988, Kaur & Potten 1986b). |
| Change in crypt geometry: | circumference reduced on days 1–2; overshoot on day 4 (Potten *et al.* 1988). |
| Mitotic inhibition: | ~8 h for middle crypt cells (~1 h/Gy); up to 20 h for bottom crypt cells (~2 h/Gy)(Chwalinski & Potten 1986, Potten 1990b). |
| Cell production: | dramatically reduced after 1 day; overshoot on days 3–4 (Potten *et al.* 1988). |
| Cell cycle times (percentage labelled mitosis): | accelerated for middle crypt cells (down to 8 h, while normally 12–13 h); bottom crypt cells (10 h, compared with normal: ~24 h) (Potten 1986, 1990). |
| Clonogenic cell number: | drastic reduction on day 1 down to 1–5 per crypt; normalization on day 4 (Potten *et al.* 1988). |

*Cell migration is evaluated from the velocity of the leading edge of the labelling index versus cell position graphs obtained 40 min, 3 h, 6 h, 9 h after labelling at different times after irradiation.

These data have mostly been reviewed by Potten (1990). The studies have all been performed on the same F1 strain of mice.

Some of these changes can be observed in Figure 1 (see Figure 4 also). A summary of these experimental observations is given in Table 1. In order to address the question of the general controls of the system we believe that it is necessary to disturb the system. We have approached this by using irradiation. We do not wish to investigate in detail the mechanisms of damage but merely to use the damage induced as way of disturbing the system and reducing the cellularity to a certain level from which it can regenerate. We have made use of published data but we have also generated some new data using the same BDF1 mice specifically for the purpose of this study. We have also developed a new simulation model to investigate a range of possible regulatory mechanisms. We have previously modelled the steady state intestinal mucosa using a two-dimensional matrix model over short time scales and a stochastic branching model also of the steady state over longer time scales. Neither are capable of addressing the question of regulatory mechanisms and as a consequence we have evolved here a compartment model of the crypt hierarchy (Figure 2) which applies to a population of average crypts under normal and perturbed conditions. We have considered only the time frame that includes the regeneration of the crypt from its minimum size. In practice this means between 1 day and 6 days after irradiation.

The results of our modelling suggest that there is a possible alternative to the negative feedback model. We suggest that the crypt stem cell population is self regulating and that such local regulatory mechanisms can explain the experimental post-irradiation observations over the time frame outlined above.

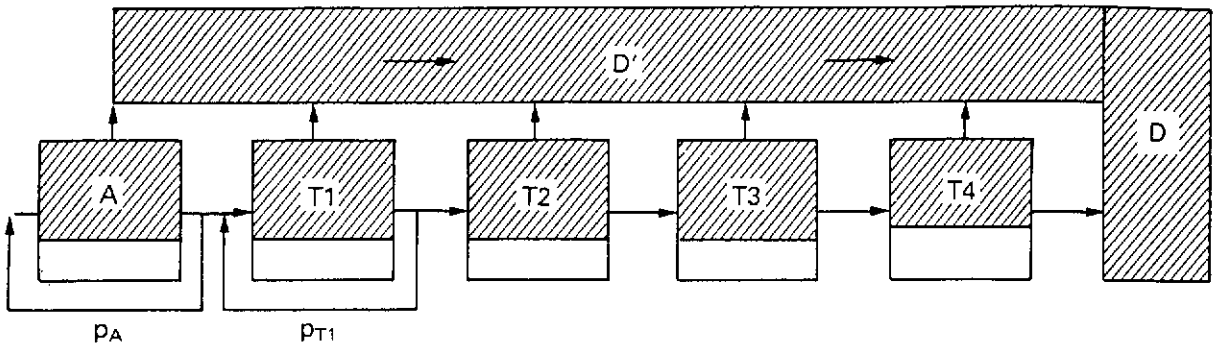


**Figure 2.** The compartment model. There is a sequence (pedigree) of cell stages (A, T1, . . . , T4, D) connected by arrows indicating cell fluxes. To describe one possible consequence of irradiation where cells are removed from proliferation and become non-proliferative an additional compartment D' has been introduced (technically the two D compartments are handled similarly). $P_A$ and $P_{T1}$ denote the self-maintenance probability of A and T1 respectively. The shaded areas denote the proportion of cells that prematurely differentiate as a consequence of radiation exposure.

## MODEL DESCRIPTION

We now wish to describe the essential assumptions constituting the model. This involves a description of the cellular development and of the regulatory processes assumed to contribute to the regeneration after a perturbation. It is not our aim to give a precise description and modelling of the damage (e.g. cell death, repair mechanisms etc.), we merely require a reasonable estimate of the number of surviving cells at each stage from which regeneration starts once the damage and repair processes have occurred and their net effect becomes apparent.

### Cellular development

We assume that the cells in the crypt exhibit an age structure which permits a distinction between normal steady state stem cells (A), a sequence of dividing transit cells (T1, . . . , T4) and non-dividing differentiated cells (D). As the behaviour of many crypts and consequently large numbers of cells is considered, a compartment model of this pedigree type is justified. We assume that only A and T1 cells can self-maintain (Potten & Loeffler 1990) while all other cells (T2, T3, T4) progress towards more mature stages once having undergone a cell division. Non-dividing differentiated cells (D) are produced at the end of this process (Figure 2).

### Regulation process

Cell production and regeneration of the crypt are assumed to be regulated properties. We assume that primarily the stem cells and the early transit cells (A, T1) are subject to controls. A basic feature of the subsequent model is that two distinct parameters are being controlled: the cell cycle time and the self-maintenance probability.

A second basic assumption is that both of these parameters depend on the number of stem cells alone rather than on any other cell stages (e.g. transit or differentiated). To formalize this basic concept several approaches can be used; for convenience we used the following formula:

**1** *Cell cycle time of stem cells.* It is assumed that the cell cycle time of A cells ($T_A$) shortens if the number of A cells declines. The minimum value of the stem cell cycle time $T_A^{min}$ can be reached under maximum stimulation (Figure 3a).

$$T_A(A) = \begin{cases} A^n & \text{for} \quad A^n \geqslant T_A^{min} \\ T_A^{min} & \text{for} \quad A^n < T^{min} \end{cases} \tag{1}$$

n = sensitivity coefficient for the cycle time;
$n > 0, 1 > T_A^{min} > 0.$

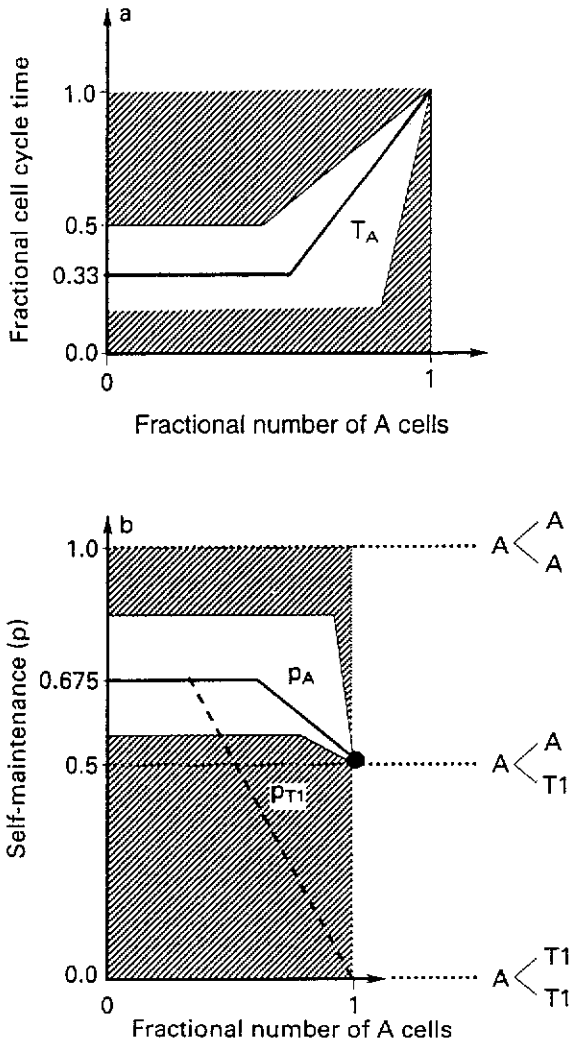


**Figure 3.** Regulatory functions. **a** The cell cycle time of the A cells as a function of the relative number of A cells (see equation 1). The number of stem cells (A) and the cycle times have been normalized to their steady state value. The minimum value of the cycle time ($T_A^{min}$) is assumed here to be 1/3 i.e. the proliferative activity can increase three-fold (or for example, the cycle time can be reduced from 24 h to 8 h). Realistic values for $1/T_A^{min}$ range between 2 and 5. For reasons discussed in the text the shaded areas can be excluded. For all subsequent simulations the full line is used as standard. **b** The self-maintenance probability as a function of the relative number of A cells normalized to the control value (see equations 2 and 3). The standard relationships used throughout the subsequent simulations are given for the A cells (full line) and for the T1 cells (dashed line). The self-maintenance function $P_A$ may not cut through the shaded areas for reasons discussed in the text. Schematically the average outcome per A-cell divisions in terms of new A and T1 cells is illustrated.

The number of stem cells (A) and their cycle times are normalized to their steady state values (i.e. $T_A(A = 1) = 1$).

In contrast, we assume that T1 cells like all other transit cells have a fixed cell cycle time which is not subject to similar regulation.

**2** *Self-maintenance of cells.* It is assumed that the self-maintenance property of stem cells and T1 cells can be quantified by a self-maintenance probability $P_A$ and $P_{T1}$: e.g. $P_A = 1$ implies that both daughter cells of an A cell become A cells (symmetric division) and that no differentiation to T1 cells takes place; $P_A = 0.5$ implies asymmetric divisions and therefore steady state; $P_A = 0$ implies that all A cells differentiate to T1 cells. The extreme theoretical values of $P_A$ and $P_{T1}$ are 1 and 0. We further assume that $P_A$ and $P_{T1}$ are functions of the number of A cells. The functions differ somewhat for A and T1 cells to account for the fact that T1 cells are not self-maintaining stem cells in the normal steady state.

A cells:

$$P_A(A) = \begin{cases} P_A^{min} & \text{for} \quad s_A^*(1-A) + 1/2 \leq P_A^{min} \\ s_A^*(1-A) + 1/2 & \text{else} \\ P_A^{max} & \text{for} \quad s_A^*(1-A) + 1/2 \geq P_A^{max} \end{cases} \tag{2}$$

$s_A^*$ = sensitivity coefficient;
$s_A > 0, 0.5 \leq P_A^{min} < P_A^{max} < 1.$

T1 cells:

$$P_{T1}(A) = \begin{cases} 0 & \text{for } s_{T1}*(1-A) \leq 0 \\ s_{T1}*(1-A) & \text{else} \\ P_{T1}^{max} & \text{for } s_{T1}*(1-A) \geq P_{T1}^{max} \end{cases} \quad (3)$$

$s_{T1}$ = sensitivity coefficient;
$s_{T1} > 0, 0.5 < P_{T1}^{max} < 1.$

This description implies that T1 cells have an ambiguous nature since they can act as real self-maintaining cells ($P_{T1} >= 0.5$) in case of demand or as pure transit cells (not self-maintaining $P_{T1} = 0$) in the steady state. This is consistent with the concept of a flexible stem cell property as recently proposed (Potten, Hendry & Moore 1987, Potten & Loeffler 1990). However, this description is only valid within the simple framework if $A > 0$. Figure 3b displays functions (2) and (3)

No other regulation processes were assumed. The cell kinetic properties of the transit and differentiating cells are assumed constant throughout regeneration.

**Choice of starting conditions**

The value of these assumptions on the regulatory processes were examined by testing the model prediction against real data for the post-irradiation recovery. The main dose of irradiation in the experiments was 8 Gy. This ensured a high level of damage and yet the survival of almost all individual crypts. The damage process was not of specific interest since it was solely a means of inactivating a large number of proliferating cells and of starting regeneration from the survivors. Thus only a rough modelling of the damage was performed. Specifically, three effects were considered or assumed.

**1** *Cell death.* Cell death is assumed to occur only in the stem cell compartment with a maximum of six dead cells as suggested by the detailed measurements of Potten (1977, 1990) and Ijiri & Potten (1983, 1987).

**2** *Irreversible proliferative inhibition or premature maturation.* Here a fraction of proliferative cells is removed from the proliferative pool but the damage is not sufficient to kill the cell (in the sense of removing them physically from the tissue). These cells then remain but do not resume proliferation. Consequently, these post-mitotic cells migrate in the crypts with all other cells but do not contribute further in terms of cell production and regeneration. No quantification of this process was available and therefore estimates had to be made. This was possible by using the model to fit the cell production determinations on day 1 and 2. This suggested that a remarkably large fraction of cells underwent this premature termination of proliferation. For convenience we treat these cells in the model as non-proliferating differentiating cells (D) and therefore call them D' cells. Figure 2 shows that they can originate from any proliferative stage.

**3** *Reversible proliferative inhibition (mitosis inhibition or delay).* Numerous proliferative cells survive irradiation and re-enter proliferation after some delay. Our own observations (Chwalinksi & Potten 1986) are in agreement with standard radiobiological knowledge in that the delay is dose- and cell-stage-dependent (Table 1). Stem cells are considered to require longer intervals to recover from this injury.

**Choice of model parameters**

Several cell kinetic parameters have been chosen on the basis of the available experimental data. They were fixed in advance and then kept constant throughout.

*Stem cells.* Twenty-four hours cell cycle time in steady state (S phase: 9 h); 8 h under maximum stimulation (S phase: 5 h).
*Transit cells.* Twelve hours cell cycle time in the model build-up phase before irradiation and in steady state; 8 h during regeneration after release from mitotic inhibition.
*Non-proliferating differentiating cells.* The total number of cells per crypt has been measured (Potten 1990). We use these values as external constraints on the simulations which gives us a simulation derived estimate of the non-proliferating cells for a given number of simulated proliferating cells. This implied a variable residence time for the differentiating cells in the crypt during regeneration and any extra cells are expelled to the villus.
*Villus.* A transit time of 2 days (±1 day) was assumed before cells were lost from the villus tip (Potten 1990).

Further model parameters were fixed in advance.

*Number of stems cells in steady state.* A number of 16 A cells was assumed in steady state. This is the maximum number consistent with the number of cells in the circumference of an ileal murine crypt. As a consequence of this eight T1 cells would be present on average in steady state crypts.
*Effect of irradiation.* Table 2 summarizes the parameters of the damaged crypt used as the starting point in the modelling.

The following model parameters could not be specified by independent previous measurements or by assumptions but had to be determined by comparing the data with simulations using the model. A systematic search for appropriate model scenaria covering a broad area of the corresponding parameter space was undertaken. The parameters of interest are specified below with the numerical values used in all subsequent figures.

*Self-maintenance.* Maximum self-maintenance probability: $P_A^{max} = P_{T1}^{max} = 0.675$ (the minimum values were fixed at $P_A^{min} = 0.5$ (steady state condition) and $P_{T1}^{min} = 0$ (transit cell condition)). Sensitivity coefficients: $s_A = 0.5$; $s_{T1} = 1$ (these values were chosen for simplicity as they would extrapolate $P_A$ and $P_{T1}$ to 1 had we not chosen the above maximum values).
*Cell cycle.* Sensitivity coefficient: $n = 2$. This corresponds to a very sensitive mechanism inducing an acceleration of the cell cycle of A cells from 24 h to 8 h (one third) upon a small reduction of the A cell number.

**Technical aspects of the model**

The time step used in the simulation is 1 h. Each compartment is therefore subdivided into subcompartments and every hour the cells move from one subcompartment to the following one. For simplicity the variation in the cell cycle time is allocated entirely to the $G_1$ phase. After passing through mitosis, cells are allocated randomly to the $G_1$-subcompartments of the subsequent cell stage. During regeneration the cycle times are shortened according to equation 1 for A cells. Technically this implies skipping subcompartments as cells proceed through the cell cycle.

From the mitotic subcompartments, A or T1 cells can either proceed into the $G_1$ subcompartment of the next compartment, T1 or T2, or they can re-enter their own respective com-

partment depending on the actual self-maintenance probability, $P_A$ and $P_{T1}$. This is a random process for each dividing cell. In the model spontaneous labelling is simulated by marking all cells in S phase at a specified time point.

The LI is defined as the number of labelled cells in all subcompartments normalized to the total number of cells. Total cell production in the model is determined from the number of mitoses per hour averaged over 24 h. The simulations shown below are based on cell numbers corresponding to 100 crypts; error bars denote the standard error of the means (SEM) of 100 independent simulations each containing the cells of 10 crypts.

## Experimental methods

*Animals*

Male B6D2F1 mice were used, between the ages of 10 and 12 weeks. They were housed under conventional animal house conditions with food and water *ad libitum* and a 12 h light-dark cycle (lights on at 0600 h).

*Irradiation*

The animals were irradiated too at a time during the morning using a caesium$^{-137}$ $\gamma$-irradiator which delivered a dose rate of about 4.5 Gy/min. All animals reported here were given 8 Gy, with air being delivered to the animals during irradiation (Potten 1990, Potten, Owen & Roberts 1990). In some experiments a dose of 2.5 or 12 Gy was delivered.

*Sampling*

Groups of at least four mice were killed at 35 different times after irradiation. In several cases the samples at particular points were repeated twice, or up to four times, providing pooled groups of eight to 16 animals. The sampling times were as follows: (0h) Control, 3, 4, 6, 8, 9, 10, 12, 14, 15, 16, 18, 20, 22, 24, 27, 30.5, 33, 36, 39, 42, 46, 48, 55, 60, 65, 72, 78, 84, 87, 96, 120, 144, 168, 192 h. The ileum was removed from each mouse and fixed intact in Carnoy's fixative for at least 20 min. It was then stored in 70% ethanol and bundles containing 10 segments from each mouse were prepared for histology as described elsewhere (Potten & Hendry 1985). Forty minutes before sacrifice of the animals they received 925 kBq of tritiated thymidine ([$^3$H]dT) (185 GBq/mM, Amersham Int.) intraperitoneally in 0.1 ml.

*Autoradiography*

The 5 $\mu$m paraffin sections were dewaxed and dipped in K5 (Ilford Ltd) emulsion diluted 1:1 with water. The slides were exposed for 7–14 days, developed and stained with haematoxylin and eosin.

*Scoring*

From the 10 ileal cross-sections from each mouse, 50 good longitudinal sections of half-crypts were selected using the following criteria: the presence of a lumen and Paneth cells at the base, and at least 17 nuclei up the side of the crypt. Each crypt section was considered as two individual crypt columns or two half-crypt sections. The cell at the mid-point at the crypt base was selected for the starting point for numbering and scoring. Each cell along the side of the crypt was analysed in turn, recording the data directly into a BBC microcomputer which recorded the position and characteristics of the cell, in the present case whether or not it was labelled (three or more grains) or mitotic, up to the crypt-villus junction.

From such scoring a frequency plot of LI or mitotic index (MI) at each cell position in the crypt could be obtained. From these frequency plots and the total labelled or mitotic cells and the total unlabelled cells an average LI or MI could be obtained for the whole crypt. In the sections obtained at each time after irradiation the number of cells in the column from the top of the crypt to the villus tip cell was counted in about 12–14 good villus profiles per mouse (i.e. 50 such columns of cells per group). This was used as a measure of villus cellularity and is the approach used by Sato *et al.* (1972) with whose data our results were compared.

At five selected times after irradiation (0, 24, 48, 72 and 96 h) a cell migration experiment was performed (Potten *et al.* 1982, Kaur & Potten 1986a). This involved injecting $^{3}$[H]dT into groups of 16 animals at these times and killing groups of four mice 40 min, 3 h, 6 h and 9 h later. These groups were processed and analysed in the usual fashion.

## RESULTS

The experimental data on the recovery of the intestinal crypts after irradiation are compared with model simulations. The objective is to show that the assumptions on the regulatory processes which involve only self-regulation of stem cells are sufficient to explain the data.

### Choice of starting values

Measurements showed that cell production on 1 day after 8 Gy irradiation dropped to almost zero. At this time the crypts still contained more than 50% of their normal cell numbers (Figure 1). Thus a large majority of these remaining cells were apparently not proliferating at this time, however, reversible inhibition of proliferation has ceased. This qualitative feature was investigated in more detail by model simulations for day 1 and 2. The range of numbers of T cells irreversibly removed from proliferation (premature maturation) is given in Table 2. For 8 Gy the range was estimated to be between 80 and 120 cells out of the approximately 150 proliferating cells present in a normal crypt. As an average for subsequent simulations 65% were removed from all proliferative stages (A, T1, . . . , T4) and considered to be non-proliferative (D', Figure 2). Similar estimates were obtained for 2.5 and 12 Gy. There was no other way of explaining the low cell production while many cells still persisted other than by assuming a large removal of cells to differentiation. This effect was far more important for the initial starting condition than the number of visibly dead stem cells or the reversible mitotic inhibition. As the irradiation damage was not modelled in detail the actual changes in the model LI during the first day is not presented. However, the starting conditions on day 1 actually measured in terms of LI, cell production, etc. match the model curves.

**Table 2.** Assumptions about the initial conditions for the model simulations of irradiated crypts

| | 2.5 Gy | 8 Gy | 12 Gy |
|---|---|---|---|
| Remaining number of A cells (steady state value: 16) | 10–12 | 1–5 | 1–3 |
| Number of T cells removed from proliferation (% of all (150) T cells present) | 24–36 (~20%) | 80–120 (~65%) | 100–160 (~90%) |
| Duration of mitotic inhibition maximum and (minimum) dependent on the generation | A: 5 h (3 h)<br>T1: 4 h (3 h)<br>T2: 3 h (2 h)<br>T3: 2 h (1 h)<br>T4: 1 h (1 h) | A: 21 h (14 h)<br>T1: 18 h (12 h)<br>T2: 15 h (10 h)<br>T3: 12 h (8 h)<br>T4: 9 h (6 h) | A: 28 h (21 h)<br>T1: 24 h (18 h)<br>T2: 20 h (15 h)<br>T3: 16 h (11 h)<br>T4: 12 h (8 h) |

**Recovery and regulation**

First we show a comparison of the model with the most comprehensive set of data available to us. This was obtained after 8 Gy whole body irradiation. Subsequently we show further comparisons with less complete data sets using smaller and higher doses. Model simulations start at day 1 and use the same parameters and regulatory functions in all simulations. When judging the comparisons it should be noted that it is the overall pattern of behaviour that is important rather than an accurate fit to specific data points.

**Recovery of the crypt after 8 Gy**

Figure 4 shows a quantitative comparison of the model calculations (b, d) and experimental data (a, c) after 8 Gy. Error bars (SEM) are given for the model simulations based on the stochastic effects in cell cycle progression and self-maintenance. Figure 4a and b compares data and model simulations for the overall flash LI at various times after irradiation, both exhibit a rapid increase between 1 and 2 days with an overshooting pattern. The model simulations show a satisfactory fit to the data.

A new set of cell migration data specifically generated for this comparison are shown in Figure 4c. This generated five label accumulation curves starting from 5 points in the flash LI curve shown in Figure 4a. The steepness of these accumulation curves is an approximate measure of the cell production which primarily depends on the number of proliferating cells and the cell cycle parameters. Figure 4d shows a corresponding model simulation which is in good agreement indicating that the regulatory processes in the model have contributed sufficient cell production.

This is further highlighted by Figure 5a, which displays the model cell production which can be compared with Figure 1a with which it is similar. Simulations of PLM data (data not shown) obtained after injection of [$^3$H]dT were also performed and showed an expected consistency since the cell cycle parameters used in the model where derived from the data.

Figure 5b shows the behaviour of the model villus compartment, which can be compared with Figure 1b. The decline of the villus cell numbers with a minimum on day 3 and a subsequent peak on days 6–7 is also matched by the model. When comparing the data with the model it should be noted that any spatial distortions of the villus have not been taken into account (Wright, Carter & Irwin 1989).

The data for model stem cell numbers are displayed in Figure 5c. They can be compared, with come caution, with the clonogenic cell numbers shown in Figure 1c. For a reasonable interpretation of this comparison some points should be noted. First, the measurements of clonogenic cell numbers are associated with considerable errors. Secondly, it is not clear with which cell population of the model the clonogenic cells should be compared. The construction of the model permits at least two optional interpretations: that only the steady state stem cells (A cells) are truly clonogenic, or that under specific situations, all cells which are able to self-maintain are clonogenic (A and T1). Curves for both views are plotted to demonstrate the range of options. Further aspects of this comparison will be discussed below.

**Behaviour of the crypt after 2.5 and 12 Gy irradiation**

For two additional sets of post-irradiation data model simulations were done with the same regulatory functions as above and with the initial conditions summarized in Table 2. Data and model simulations of crypt recovery after a relatively low dose of 2.5 Gy are shown in Figure 6a and b. Both show a rapid recovery within 1 day with a small subsequent overshoot. In the model this rapid recovery is due to the regulatory functions which allow a sensitive reaction even to small perturbations.

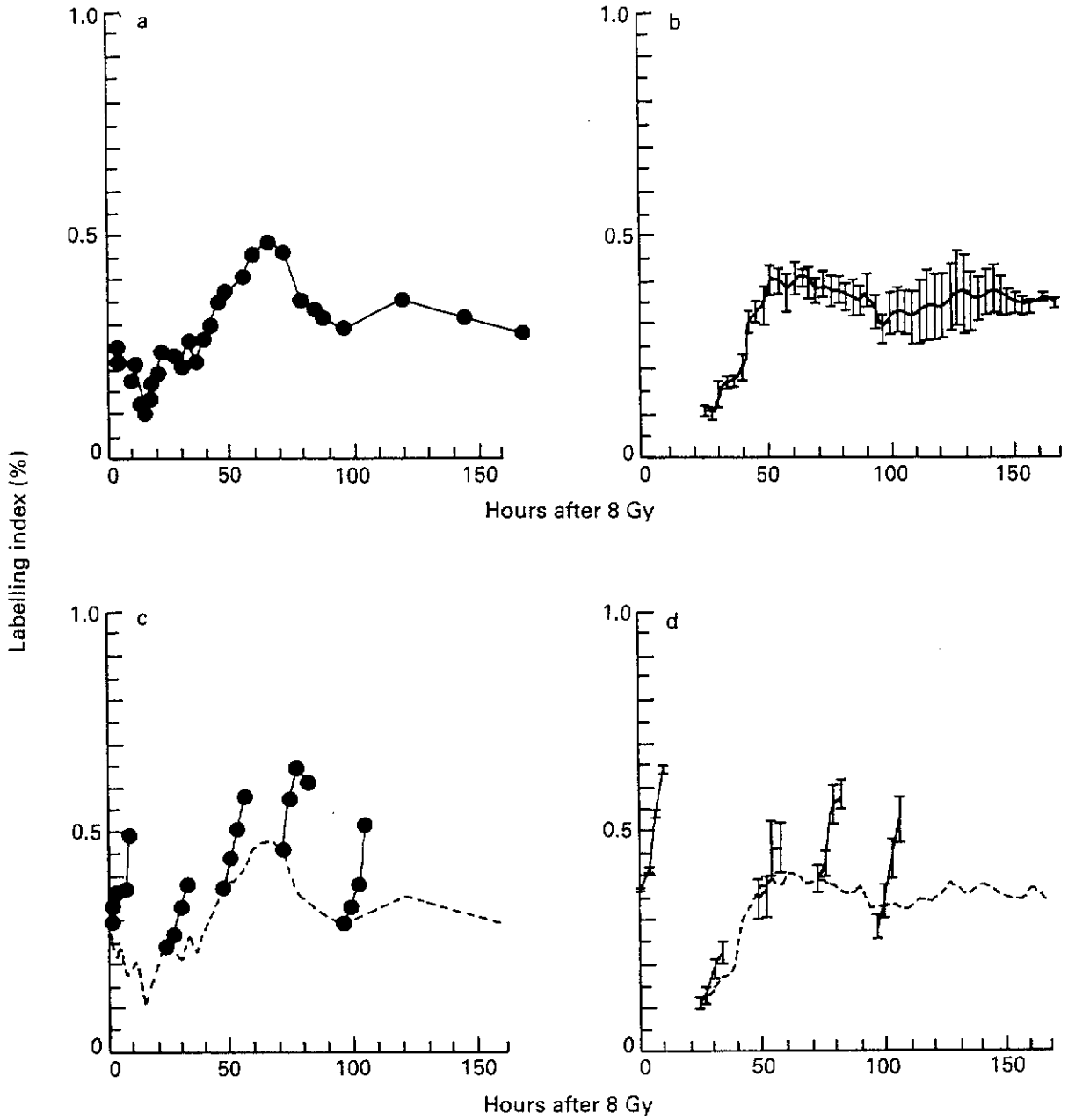


**Figure 4.** Comparison of experimental data and simulation results: labelling index (LI) data. Experimental data are shown in **a, c**. Model simulations are shown in **b, d** obtained from 10 independent simulations of 10 different crypts. **a** Experimental overall LI in the first 7 days after 8 Gy. The mice were killed 40 min after labelling. **b** Overall LI simulated by the model with the percentage of labelled cells being evaluated immediately after labelling. **c** Experimental overall LI scored 40 min, 3 h, 6 h and 9 h after labelling in a control group (0 h) as well as at 24, 48, 72, 96 h after irradiation to provide cell migration data after 8 Gy. The slope of increase in LI allows the crypt cell output to be estimated. The dashed line shows the overall LI from panel **a**. **d** Model simulations of the LI migration experiment shown in **c**. The dashed line shows the LI from panel **b**.

Figure 6c and d display a recovery from a high dose of 12 Gy. In this situation the surviving crypts almost certainly recovered from a single surviving clonogenic cell which represents the most extreme perturbation. The timing of the recovery and the overshoot in the model corresponds well to the experimental data providing a good qualitative test for the model and its regulatory functions.

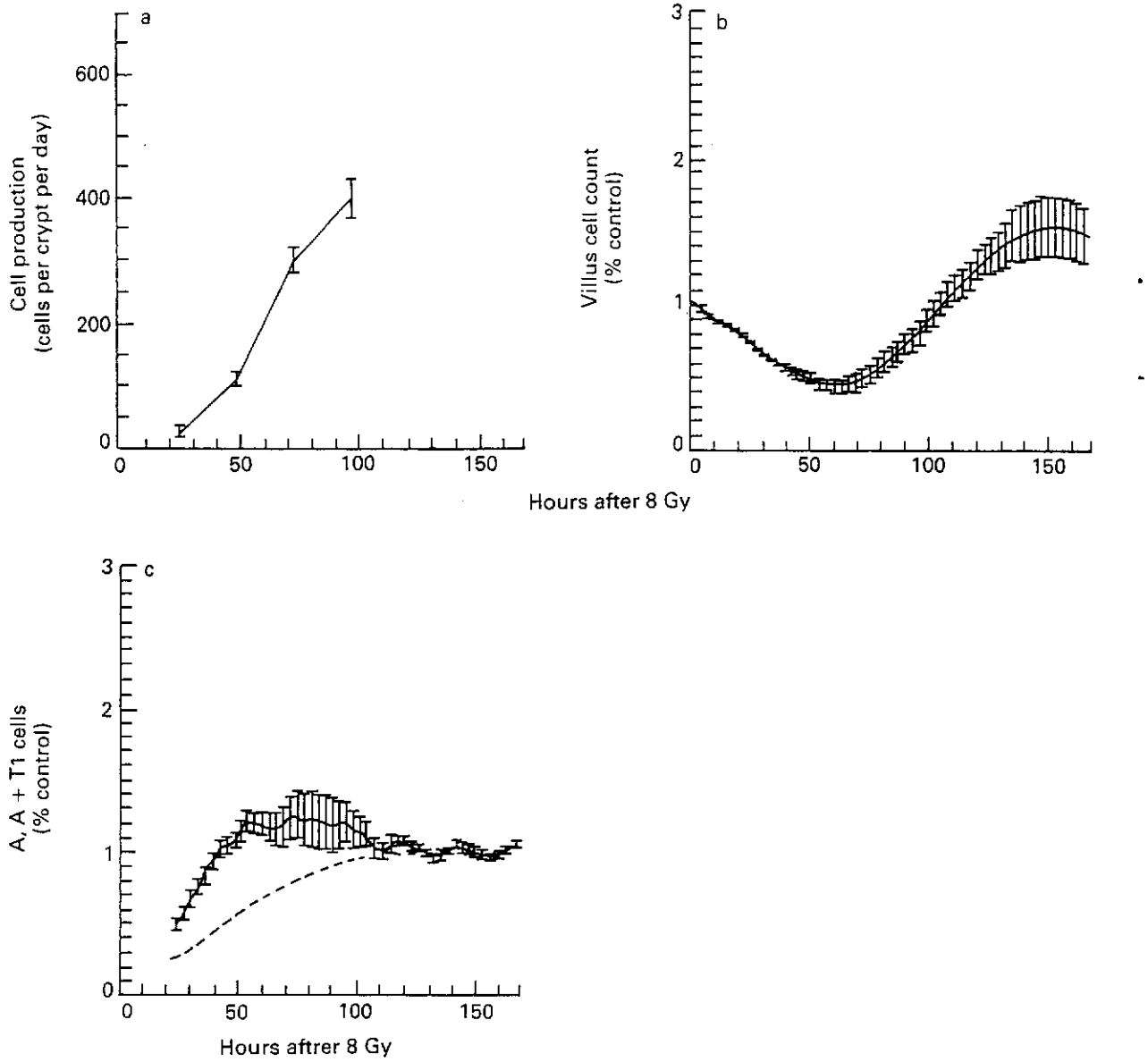


**Figure 5.** Simulation results after the initial damage from 8 Gy. **a** Cell production calculated with the model: number of mitoses averaged over 24 h. **b** Villus cellularity as a percentage of control values: the cells were assumed to have a transit time of 2 ± 1 day on the villus before they are lost. **c** A cells (– – –) and A + T1 cells (——) as percentage of the control values (steady state A-cell number: 16). This has to be compared with Figure 1a–c.

### Selection of parameters for the regulatory functions

There is little quantitative a priori knowledge upon which to base the proposed regulatory functions $P(A)$ and $T_A(A)$. However, from PLM measurements it has been shown that the cycle time for the stem cells is reduced until day 4 after irradiation when the stem cell number has nearly reached the normal value. From this we concluded a step function for $T_A(A)$ as shown in Figure 3a.

The rapid regeneration after low doses (2.5 Gy) of irradiation implies that the factors controlling the self-maintenance probability react quickly to small deviations from the steady state value. This supports the concept of sensitive reactions to relatively small changes in the number of A cells as displayed in Figure 3b and the interpretation made from actual experimental observations (Potten 1990, 1991a,b). The regeneration after 12 Gy appears similar to that after 8 Gy, but with a delayed recovery. This suggests that the self-maintenance probability rapidly

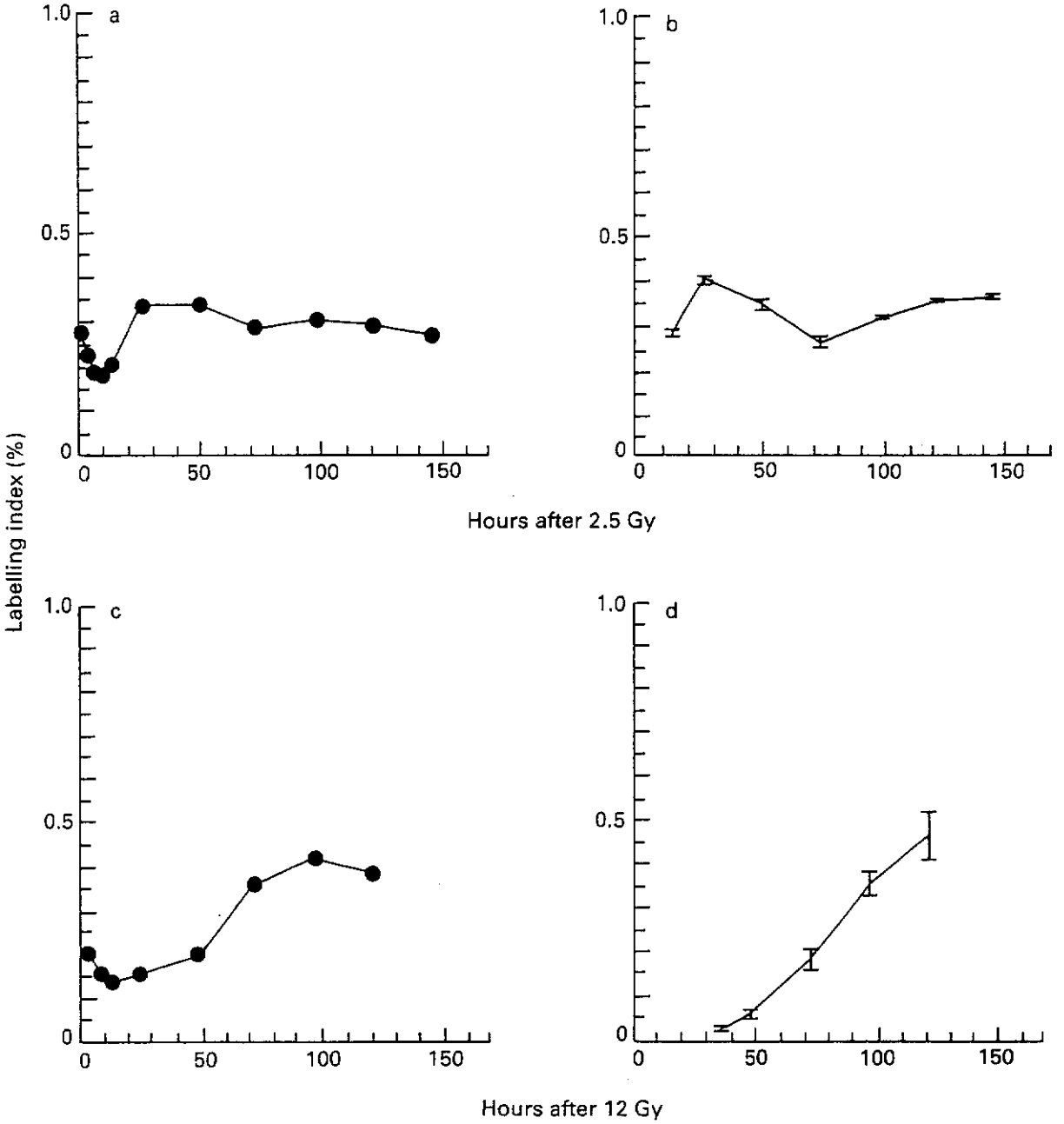


**Figure 6.** Comparison of **a**, **c** experimental data and **b**, **d** simulation results: labelling index (LI) for different doses. **a** Experimental overall LI after 2.5 Gy, **b** simulated LI after 2.5 Gy, **c** experimental overall LI after 12 Gy and **d** simulated LI after 12 Gy. Compare this with Figure 4a, b.

reaches a maximum when fewer and fewer A cells are present. We had to assume a plateau for the regulatory functions $P_A$ and $P_{T1}$ when only a few A cells remain. The maximum value of the self-maintenance probability can be deduced from the time course of the LI recovery. If the maximum value ($P^{max}$) is too small ($P^{max} < 0.57$) the LI regenerates clearly too late; if it is too high ($P^{max} > 0.8$) the regeneration is far too early in the model. Figure 3b gives an indication of the acceptable range of choices of the function $P_A$. Functions cutting through the shadowed area generate unsatisfactory fits with respect to the LI. The regulatory functions chosen as standard are the bold curves shown in Figure 3a and b. All model calculations were performed with these relationships.

## DISCUSSION AND CONCLUSIONS

The comparison of model and data shows good agreement in terms of the general shape. There are some difficulties in relating the model stem cells to the clonogen measurements. However, major aspects, including the timing of the recovery and the overcompensation or overshoot behaviour of the LI, can be explained by the model. Based on these comparisons we arrive at the following conclusions.

1 Irradiation leads to a dose dependent irreversible removal of cells from the proliferative compartments. Approximately 20, 65 and 90% of all proliferating cells stop proliferation after 2.5 Gy, 8 Gy and 12 Gy respectively and enter the differentiated compartment (premature maturation).
2 The essential phenomena involved in the recovery of irradiated crypts can be explained by regulatory processes solely depending on, and acting on, stem cells. This implies an autoregulation of the stem cells. In order to achieve a reasonable match of the model to the data one has to assume specific shapes for the regulatory functions. As a consequence, the model reacts sensitively to small changes in stem cell numbers. After mild damage with 2.5 Gy the damage is rapidly compensated for by an activation of proliferation and changes in self-maintenance of the stem cells. In fact, it is known from PLM measurements that a shortening of the cell cycle takes place in crypt-cell positions 1–5 and other cell kinetic changes have been detected in the stem cell region following very minor damage, e.g. killing of only a single cell (Potten 1990, 1991a,b). The model explains this onset of regenerative processes at a time when the villus cell population has hardly declined, a feature not explained by models only incorporating feedback signals from the villus to the crypt.
3 The model presented here is an extension of a previous steady state model (Loeffler *et al.* 1986, 1988). It preserves the assumption of an age-structured cell population with a pedigree-like lineage of transit cells. In addition, it generalizes the assumption about local interaction processes to include regulatory signals. Furthermore, the restrictive steady state assumption of a regular asymmetric stem cell division process ($P_A = 0.5$) has been given up in favour of a dynamically regulated self-maintenance.
4 The model presented here puts the T1 cells into an intermediate position between true steady state stem cells and other transit cells. In the steady state model T1 cells were considered as not self-maintaining. This still holds (i.e. $P_{T1}(A = 1) = 0$), although here we assume that the cells can exhibit self-maintenance under certain stimulation conditions (i.e. $P_{T1} > 0.5$ for $A < 0.5$). T1 cells can therefore be considered as potential stem cells in the sense of the definition proposed by us recently (Potten & Loeffler 1990). Therefore, the model gives a reasonable distinction between 'actual' stem-cells (A cells), 'potential' stem cells (T1 cells) and 'clonogenic' cells (which include both after severe stimulation).

The model simulations shown were undertaken with 16 'actual' stem cells. There are some indications, however, that perhaps the number of 'actual' stem cells may be smaller (Hendry *et al.* 1989). In which case the number of 'potential' stem cells would be larger to account for the first two or three transit generations. These 'potential' stem cells should possess a self-maintenance cell cycle which is regulated according to the crypt state much like the T1 cells in the present model. This would eventually lead to a concept of a continuum of decreasing self-maintenance capacity and increasing differentiation (Potten & Loeffler 1990).

An alternative explanation for the proposed stem cell concept of A and T1 cells might be

based on a stem cell niche hypothesis. Here, cells with stem cell capability only express this if anchored in such a niche (equivalent to A). If a perturbation removes cells from such niches other cells with a stem cell potential may seed them (T1 cells). Thus the niche concept would also explain why more potential stem cells can be found in post-perturbation bioassays than may be self-maintaining actually in steady state. Modelling of this process has been performed and gives similar results (not shown).

The above exercise was undertaken to illustrate that the current feedback mode (villus to crypt, mature cells to stem cells) are not the only interpretations of the data available and may in fact have some drawbacks. Such long-range feedback signals are indeed not required to explain the data. Preliminary examinations of data after cytotoxic drugs also seem to be consistent with the concept of self-regulation proposed here. On the other hand it is important to note that we do not conclude that long-range feedbacks can be totally dismissed. It is quite likely that a feedback of some sort exists, otherwise it would be difficult to explain why feeding or starvation affects proliferation and crypt size, even in blind loops of the gut (Wright & Alison 1984). Damage to the villus might even send stimulatory signals to the crypt (coeliac disease; Rijke *et al.* 1976). However, our modelling exercise showed that the feedback concept should not be taken for granted. Indeed the crypt could be a self-organizing system with modulation imposed by the environment (hormones, growth factors, nutrients). Thus the question of regulation of this tissue in our mind remains open.

**Limitations of the model**

There are a number of limitations inherent in the model. First, we considered the damage process only in a simplistic way. In particular the process of irreversible loss from proliferation (premature maturation) was not considered in any great detail. In this model these cells were considered to loose their proliferative status right after irradiation (on day 0). The possibility of cells undergoing one or two divisions before reaching this stage was not considered. Had this been taken into account the simulated LI would have maintained higher values on day 1 and 2.

Secondly, the definition of clonogens and stem cells needs further discussion. It is unclear which model cells should be compared with the experimental clonogenic cells. Clonogenic cells cannot be counted directly in a given crypt using a specific marker, but rather they have to be assayed after imposing an additional irradiation damage on the system in split dose experiment. Thus the numbers shown in Figure 1c represent an indirect estimate of a property which is linked to stemcellness. This it is necessarily an arbitrary decision when comparing clonogenic cell numbers to a particular model cell stage. Model A cells recover during 4 days as do the experimental clonogenic cells. But the A cells do not over compensate and their number is small (16). In contrast, the sum of A and T1 cells overcompensate; in control circumstances their number is nearer the experimental clonogenic number of 32 but the recovery in Figure 5c is faster than suggested by the data given in Figure 1c. Taylor *et al.* (1991) observed a more rapid recovery in their data.

Thirdly, when many crypts die, fission of crypts is a process that helps maintain gut function (Cairnie 1976), particularly at later times. This process should also be included into a more comprehensive model. Loeffler & Großmann (1991) have suggested that crypt fission may be linked to a stochastic growth of stem cells, and if the number of crypt stem cells exceeds a certain threshold, the crypt will split. Qualitatively this implies that crypt fission should not occur before the stem cell number has recovered after damage. Consequently, overcompensation in stem cell numbers should be correlated with more frequent fission. Available data seem consistent with this time sequence (Cairnie 1976).

**Suggestions for future studies**

It would be interesting in the future to focus on some principal aspects of epithelial organization, such as which features of the tissue are inherently programmed to develop and evolve and which features are variable and subject to control. It would be essential to show that an irreversible age structure exists among proliferating cells and that the capacity for cell division is limited to only a few rounds of cell division for most of these cells. In particular, more detail on the pedigree is warranted, e.g. whether or not it is largely symmetrical and how many divisions are involved. Furthermore, the identification and measurement of control factors, their location, and dispersal would give us additional information about the regulatory mechanism. This implies a search for diffusible (e.g. via gap junctions) growth factors within the tissue, similar to the morphogens controlling developmental processes or the growth factors known for haematopoiesis.

The model raises the question of whether there is biological evidence for local regulatory processes. At present such evidence is limited, as is the evidence for feedback control from the villus claimed by other models. The most interesting observation in this respect is that the gap junctions allow for the exchange of small molecules between crypt columnar cells (Bjerknes, Cheng & Erlandsen 1985). It is remarkable that the exhange rate, i.e. diffusion velocity, is highest in cells located in the lower half of the crypt just above the Paneth cells, where we would expect the most intensive exchange of information in order to control cell division and migration.

With respect to further modelling tasks the following directions appear most relevant. The simulation of regeneration after cytotoxic drugs should be undertaken more extensively to see whether the processes proposed here can further be supported. Model calculations would surely be improved by incorporating a flexible crypt geometry after irradiation. A three-dimensional model simulating single cells and their migration is necessary taking into account the spatial changes after perturbations.

Finally, it should be mentioned that the model as presented is a simple version of a similar model used for describing the control processes in haematopoiesis (Wichmann & Loeffler 1985) in which haematopoietic stem cells and erythro/granulopoietic regulation are described. The existence of three control loops which included, autoregulation of stem cells, feedback of transit cells on stem cells and feedback of mature cells on transit cells was postulated. It is, of course, possible that the crypt control system is simpler, implying only a control of the first type. The more complicated regulation in haematopoiesis might be related to the differences in development time from stem cells to mature cells (haematopoiesis: 14 days; crypt: 2 days) and the length of the cell lineage (haematopoiesis over 15 transit divisions, crypt 4–6). However, such comparisons are speculative.

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## REFERENCES

BJERKNESS M, CHENG H, ERLANDSEN S. (1985) Functional gap junctions in mouse small intestinal crypts. *Anat. Rec.* **212**, 364.

BRITTON NF, WRIGHT NA, MURRAY JD. (1982) A mathematical model for cell population kinetics in the intestine. *J. Theor. Biol.* **98**, 531.

CAIRNIE AB. (1976) Homeostasis in the small intestine. In: Cairnie AB, Lala PK, Osmond DG, eds. *Stem-cells of Renewing Cell Populations.* New York: Academic Press, 67.

CHWALINKSI S, POTTEN CS. (1986) Radiation induced mitotic delay: duration, dose and cell position dependence in the crypts of the small intestine in the mouse. *Int. J. Radiat. Biol.* **49**, 809.

HENDRY JH, POTTEN CS, CHADWICK C, BIANCHI M. (1982) Cell death (apoptosis) in the mouse small intestine after low doses: effects of dose-rate, 14.7 MeV neutrons, and 600 MeV (maximum energy) neutrons. *Int. J. Radiat. Biol.* **42**, 611.

HENDRY JH, POTTEN CS, GHAFFOR A, MOORE JV, ROBERTS SA, WILLIAMS PC. (1989) The response of murine intestinal crypts to short-range promethium-147 irradiation: deductions concerning clonogenic cell numbers and positions. *Radiat. Res.* **118**, 364.

IJIRI K, POTTEN CS. (1983) Response of intestinal cells of differing topographical and hierarchical status to ten cytotoxic drugs and five sources of radiation. *Br. J. Cancer,* **47**, 175.

IJIRI K, POTTEN CS. (1987) Further studies on the response of intestinal crypt cells to various cytotoxic drugs. *Br. J. Cancer,* **55**, 113.

KAUR P, POTTEN CS. (1986a) Circadian variation in migration velocity in small intestinal epithelium. *Cell Tissue Kinet.* **19**, 59.

KAUR P, POTTEN CS. (1986b) Cell migration velocities in the crypts after cytotoxic insult are not dependent on mitotic activity. *Cell Tissue Kinet.* **19**, 601.

KICHERER G. (1983) *Modellstudie der Strahlenwirkung im Gastrointestinalen Zellsystem.* Dissertation Stuttgart.

LOEFFLER M, GROßMANN B. (1991) A stochastic branching model with formation of subunits applied to the growth of intestinal crypts. *J. Theoret. Biol.* **150**, 175.

LOEFFLER M, POTTEN CS, PAULUS U, GLATZER J, CHWALINSKI S. (1988) Intestinal crypt proliferation II. Computer modelling of mitotic index data provides further evidence for lateral and vertical cell migration in the absence of mitotic activity. *Cell Tissue Kinet.* **21**, 247.

LOEFFLIER M, STEIN R, WICHMANN HE, POTTEN CS, KAUR P, CHWALINSKI S. (1986) Intestinal cell proliferation. I. A comprehensive model of steady-state proliferation in the crypt. *Cell Tissue Kinet.* **19**, 627.

MEINZER HP, BAUR HJ, SANDBLAD B, RODRIGUES L. (1990) Mögliche Mechanismen zur Proliferations- und Differentiationskontrolle. In: Giani G, Repges R, eds. *Biometrie und Informatik – neue Wege zur Erkenntnisgewinnung in der Medizin, Medizinische Informatik und Statistik.* Berlin: Springer-Verlag, 71.

POTTEN CS. (1977) Extreme sensitivity of some intestinal crypt cells to X and gamma irradiation. *Nature,* **269**, 518.

POTTEN CS. (1986) Cell cycles in cell hierarchies. *Int. J. Radiat. Biol.* **49**, 257.

POTTEN CS. (1990) A comprehensive study of the radiobiological response of the murine (BDF1) small intestine. *Int. J. Radiat. Biol.* **58**, 925.

POTTEN CS. (1991a) The role of stem cells in the regeneration of intestinal crypts after cytotoxic exposure. Chemically induced cell proliferation. In: Butterworth BE, Slaga TJ, Farland W, McClain M, eds. *Implications for Risk Assessment.* New York: John Wiley, 155.

POTTEN CS. (1991b) Regeneration in epithelial proliferation units as exemplified in the small intestine. *CIBI Foundation Publ.* 160: Chichester: John Wiley, 64.

POTTEN CS, HENDRY JH. (1983) Stem cells in murine small intestine. In: Potten CS, ed. *Stem Cells: Their Identification and Characterisation.* Edinburgh: Churchill Livingstone, 155.

POTTEN CS, HENDRY JH. (1985) The microcolony assay in mouse small intestine. In Potten CS, Hendry JH, eds. *Cell Clones: Manual of Mammalian Cell Techniques.* Edinburgh: Churchill Livingstone, 50.

POTTEN CS, LOEFFLER M. (1990) Stem cells: attributes, cycles, spirals, pitfalls and uncertainties. *Development,* **110**, 1001.

POTTEN CS, CHWALINSKI S, SWINDELL R, PALMER M. (1982) The spatial organisation of the hierarchical proliferative cells of the small intestine into clusters of 'synchronised' cells. *Cell Tissue Kinet.* **15**, 351.

POTTEN CS, HENDRY JH, MOORE JV. (1987) New estimates of the number of clonogenic cells in crypts of murine small intestine. *Virchows Arch.* [*B*] **53**, 227.

POTTEN CS, OWEN G, ROBERTS S. (1990) The temporal and spatial changes in cell proliferation within the irradiated crypts of the murine small intestine. *Int. J. Radiat. Biol.* **57**, 185.

POTTEN CS, TAYLOR Y, HENDRY JH. (1988) The doubling time of regenerating clonogenic cells in the crypts of the irradiated mouse small intestine. *Int. J. Radiat. Biol.* **54**, 1041.

RIJKE RPC, HANSON WR, PLAISIER HM, OSBORNE JW. (1976) The effect of ischemic villus cell damage on crypt cell proliferation in the small intestine. *Gastroenterology,* **71**, 786.

RIJKE RPC, VAN DER MEER-FIEGEN W, GALJAARD H. (1974) Effect of villus length on cell proliferation and migration in small intestinal epithelium. *Cell Tissue Kinet.* **7**, 577.

SATO F, MURAMATSU S, TSUCHIHASHI S, SHIROGAL AH. (1972) Radiation effects on cell populations in the intestinal epithelium of mice and its theory. *Cell Tissue Kinet.* **5**, 227.

TAYLOR JM, WITHERS HR, HU Z. (1988) A comparison of mathematical models for regeneration in acutely responding tissues. *Int. J. Radiat. Oncol. Biol. Phys.* **15**, 1389.

TAYLOR JM, WITHERS HR, MASON KA, DAVIS CA. (1991) Repopulation of mouse jejunal crypt cells. *Radiother. Oncol.* **20**, 181.

TSUBOUCHI S, POTTEN CS. (1985) Recruitment of cells in the small intestine into rapid cell cycle by small doses of external gamma or internal beta-radiation. *Int. J. Radiat. Biol.* **48**, 361.

TUCKER SL, HENDRY JH. (1990) Predicted dose-latency relationships for early- and late-responding hierarchical tissues. *Int. J. Radiat. Biol.* **57**, 163.

WICHMANN HE, LOEFFLER M. (1985) *Mathematical Modelling of Cell Proliferation.* Vol I. **1**, 31. Florida: CRC-press.

WRIGHT N, ALISON M. (1984) *The Biology of Epithelial Cell Populations.* Vol. 2. Oxford: Clarendon Press, 537.

WRIGHT N, AL-NAFUSSI A. (1982) Kinetics of villus cell populations in the mouse small intestine. II. Negative feedback control after death of proliferative cells. *Cell Tissue Kinet.* **15**, 610.

WRIGHT N, CARTER I, IRWIN M. (1989). The measurement of villus cell population size in the mouse small intestine in normal and abnormal states: a comparison of absolute measurements with morphometric extimators in sectioned immersion-fixed material. *Cell Tissue Kinet.* **22**, 425.

YAKOVLEV AY, ZORIN AV. (1988) Computer simulation in cell radiobiology. *Lecture Notes in Biomathematics*, Vol. 74. Berlin: Springer Verlag, 1.