#1245

J. theor. Biol. (1991) **150**, 175–191

A Stochastic Branching Model with Formation of Subunits Applied to the Growth of Intestinal Crypts

MARKUS LOEFFLER† AND BERND GROSSMANN‡

† *Medizinische Klinik I, Abteilung Biometrie, LFI-Ebene* 5, *Josef-Stelzmann-Str.* 9, *D*-5000 *Köln* 41 *and*
‡ *Institut für Theoretische Physik, Philosophenweg* 19, *D*-6900 *Heidelberg, Germany*

(*Received on* 15 *May* 1990, *Accepted in revised form on* 13 *September* 1990)

The intestinal epithelium is one of the most rapidly regenerating tissues in mammals. Cell production takes place in the intestinal crypts which contain about 250 cells. Only a minority of 1–60 proliferating cells are able to maintain a crypt over a long period of time. However, so far attempts to identify these stem cells were unsuccessful. Therefore, little is known about their cellular growth and selfmaintenance properties. On the other hand, the crypts appear to exhibit a life cycle which starts by fission of existing crypts and ends by fission or extinction. Data on these processes have recently become available. Here, we demonstrate how these data on the life cycle of the macroscopic crypt structure can be used to derive a quantitative model of the microscopic process of stem cell growth.

The model assumptions are: (1) stem cells undergo a time independent supracritical Markovian branching process (Galton-Watson process); (2) a crypt divides if the number of stem cells exceeds a given threshold and the stem cells are distributed to both daughter crypts according to binomial statistics; (3) the size of the crypt is proportional to the stem cell number. This model combining two different stochastic branching processes describes a new class of processes whose stationary stability and asymptotic behavior are examined. This model should be applicable to various growth processes with formation of subunits (e.g. population growth with formation of colonies in biology, ecology and sociology). Comparison with crypt data shows that intestinal stem cells have a probability of over 0·8 of dividing asymmetrically and that the threshold number should be 8 or larger.

Introduction

The intestinal epithelium is one of the most rapidly regenerating tissues in mammals. It is a one cell layer thick arrangement of cells covering the inner lining of the gastro-intestinal system. Cell production takes place in the intestinal crypts which are imbedded in the wall of the gut. From here cells migrate onto the surface of the villi which protrude into the lumen of the gut. Here cells are finally discarded after having performed their function of absorbing and digesting food (for details see Potten & Hendry, 1983; Wright & Alison, 1984).

Crypts in the small intestine of mice contain about 250 cells of which 150 are proliferating. Only a minority of proliferating cells are able to maintain the epithelium over a long period of time. For these cells the concept of selfmaintaining intestinal stem cells was postulated (Cairnie *et al.*, 1965; Potten & Hendry, 1983).

0022-5193/91/100175+17 $03.00/0

So far however, attempts to identify and count the stem cells on the grounds of histology or genetics were unsuccessful. Little is known about the growth properties of the cells. Their number may range between 1 and 60 (Potten, 1990).

Crypts are dynamic structures. Their life cycle starts by a longitudinal fission of existing crypts (Cairnie & Millen, 1975) and ends by fission or extinction. This process is schematically displayed in Figure 1. On the left a section of a normal crypt is shown. Crypt doubling occurs by a longitudinal fission of a crypt starting at the bottom (the presumable stem cell location) and continuing upwards (middle). Sections displaying this morphologically can be seen (Cairnie & Millen, 1975). At the end of this process two independent crypts are generated. Crypts disappear by shrinking in length and circumference until they are integrated into the surface lining (right) (Potten, 1990). Neither do they leave a sign of their former existance nor can they reliably be identified in the process of extinction. It is assumed that this extinction occurs only if a crypt has lost all selfmaintaining stem cells. It is believed that a crypt can stay alive as long as it has at least one active stem cell (Potten & Hendry, 1983; Potten *et al.*, 1987; Potten, 1990).

Data on the life cycle of crypts in the normal unperturbed circumstances have recently become available. They are summarized in Table 1.

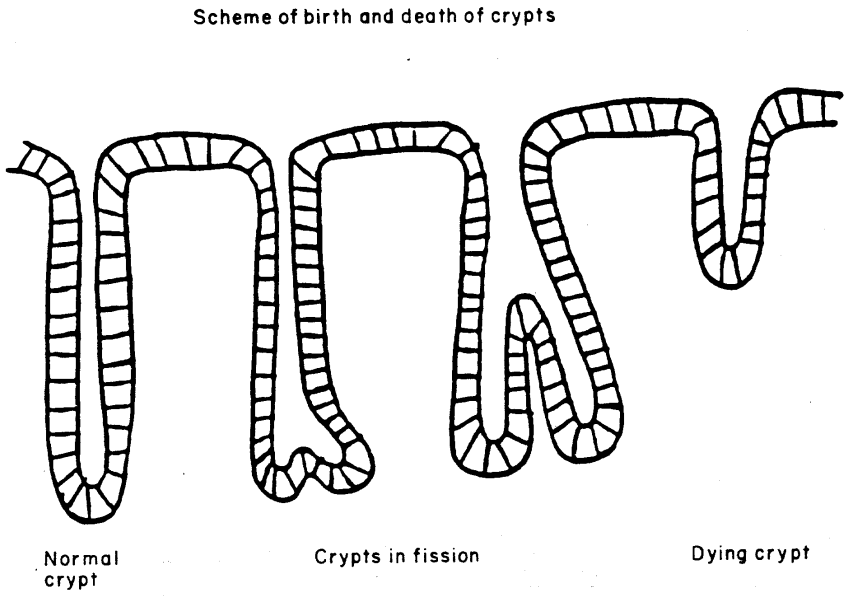

FIG. 1. Schematic representation of crypts in various states of their life cycle (longitudinal section). (a), Normal crypt; (b) and (c), crypts in fission; (d), dying crypt.

In a previous model of the steady-state we showed that the spatial and functional organization of the crypt can be explained if one assumes that the number of mature progeny produced per stem cell and time unit is constant (Loeffler *et al.*, 1986; Potten & Loeffler, 1987). This implies a proportionality of crypt size and stem cell number. Based on this assumption crypt size distributions have recently been measured by Totafurno *et al.* (1987). Figure 2 gives a schematic reproduction of

TABLE 1

Data on the life cycle of murine intestinal crypts

Parameter		Value
Doubling time of crypts	(T)	100 day†
		700 day‡
Fission rate	(ϕ)	0·006–0·0013 day^{-1}†§
Extinction rate	(Ω)	$\ll$ 0·001 day^{-1}†
Duration of stem cell cycle time		~1 day

† Derived from data obtained by Totafurno *et al.* (1987).

‡ Potten (1989, personal communication) found that the number of crypts in the small intestine of mice approximately doubles between week 11 and week 111 of age. In this period 1·5% of all crypts were found in the fission process.

§ Obtained by the relation: incidence = prevalence of crypts in fission/duration of fission. The data are: Totafurno *et al.* (1987): 0·006 (experiment 1), 0·0048 (experiment 2); Potten (1989, personal communication): 0·0013.

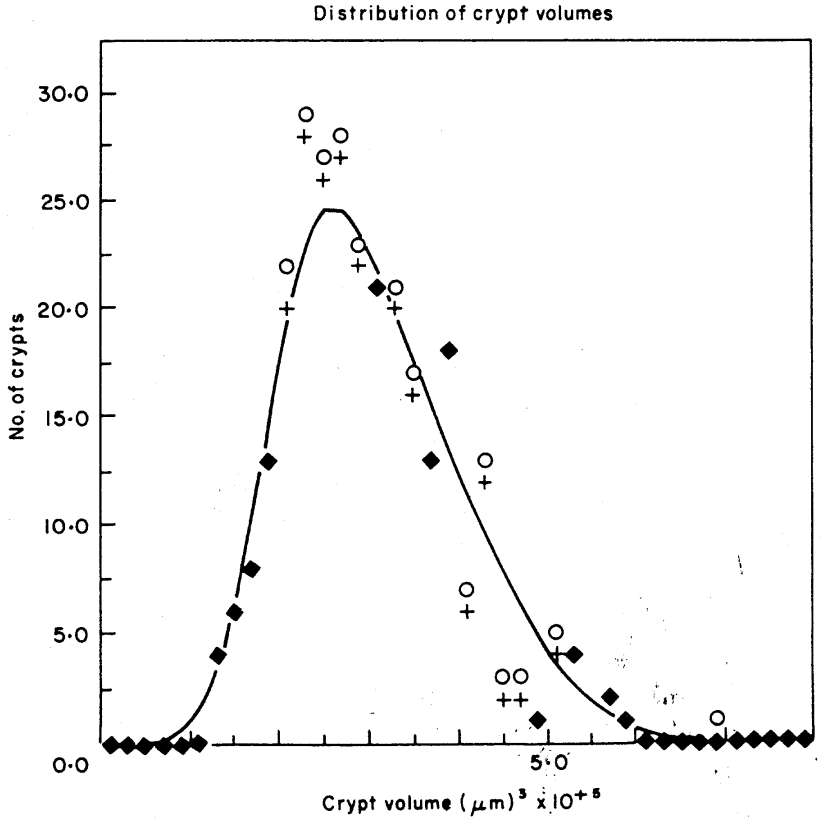

FIG. 2. Crypt size distribution as obtained by Totafurno *et al.* (1987) (modified reproduction). $n \approx 291$; $C_V \sim 0{\cdot}25$.

their findings. The distribution shows a coefficient of variation of about 0·25. The mode of the distribution is about half of the maximum. Small crypts are very rare. In addition bigger crypts are more frequently found in the fission process. Similar results were found by Potten (unpublished data) when he measured the circumference of crypts in transverse sections. He also found a coefficient of variation of about 0·25.

The basic data can be summarized as follows:

(1) The number of crypts increases continuously with age with a doubling time of several hundred days;
(2) The crypt fission rate can be estimated to be between 0·0013 and 0·006 day^{-1};
(3) The crypt extinction rate can be estimated to be considerably lower than 0·001 day^{-1};
(4) Small crypts are very rare and the mode of the crypt size distribution is about $\frac{1}{2}$ of the size of the biggest crypts.

Objective of the Modelling

It is the objective of this paper to demonstrate a model that can quantitatively explain the life cycle of crypts and the size distributions on the basis of growth characteristics of individual stem cells.

This implies that one can relate the dynamics of the macroscopic crypt structure to the microscopic cellular process of stem cell selfmaintainance and differentiation. At each division a stem cell divides into two daughter cells which can become stem cells again or differentiating cells (with mandatory maturation after a fixed number of transient cell divisions). It will be assumed that out of one stem cell either none (with probability q) or one (r) or both (p) of the daughter cells can become stem cells. It is assumed that the decision between these options is random and independent of the previous development (Markov property) and the probabilities are constant in time (homogeneity property). With this concept of the cellular growth process in mind it is the particular aim of the modelling to (1) give an average stationary probability distribution of stem cells per crypt, (2) to calculate the total number of crypts, and (3) to calculate the stationary extinction and fission rates in terms of probabilities. This requires the investigation of a new type of stochastic branching model which takes the formation and disappearance of the crypts into account. Rather than looking merely at the population of cells one has to consider also the population of subunits (crypts).

Remarks on Properties of Conventional Galton–Watson Processes

Stochastic branching processes have found wide application in biology (Jagers, 1975). One of the first applications of stochastic processes to the analysis of stem cell systems was given by Vogel *et al.* (1968, 1969). They investigated the haemopoietic stem cells and assumed a Galton–Watson Process (GWP) with p, q and r being independent of the size and the age of the system. Let $m := 2p + r$ denote the mean offspring of one cell per generation and S_n the number of cells at generation

n (discrete integer time steps). Then the expected value $E(S_n)$ grows exponentially

$$E(S_n) = S_0 \,.\, m^n, \tag{1a}$$

and one can calculate the probability for the extinction of the entire system. The asymptotic extinction probability at some time in the future is

$$\Omega_\infty = P(\lim_{n\to\infty} S_n = 0) = \min\,[1, (q/p)^{S_0}]. \tag{1b}$$

These formulae indicate that the system dies out with certainty if $p \leq q$ i.e. $m \leq 1$ (subcritical). Only for $p > q (m > 1)$ is there a finite probability for the system to survive in which case it grows exponentially (supracritical). A stable steady-state does not exist.

This lack of stability led to the development of controlled GWPs in which the number of offsprings depends in a convergent fashion (for $n \to \infty$) on the size S_n of the system (Kesten & Stigum, 1967; Fujimagari, 1976, 1980; Höpfner, 1985*a*, *b*; 1986; Klebaner, 1983, 1984*a*, *b*, 1985). However, again under fairly general assumptions it was shown that no stable steady-state could be maintained. The systems either die out or grow (at least logistically) (Klebaner, 1984). With respect to our particular question these results are interesting, because they imply that biological systems operating on such simple rules must grow supracritically at the cellular level in order to survive. As the data indicate this is the case for the intestine.

All these models, however, cannot be readily applied to our problem for two reasons. First, they do not take segregation of the system into subunits into account. Second, they are considering the total population (including all individuals that have ever existed dead and alive) while in our biological system one has to look at conditional probabilities, i.e. we have to look only at crypts which are alive at any given moment. Therefore, the previous concepts cannot be applied.

A Model of a GWP with Threshold Dependent Segregation into Subunits

The following model assumptions are introduced:

Assumption 1 (*Cell division*):
If the number S of stem cells in a crypt is below a critical value S_f stem cells grow according to a supracritical time independent Markov process of the Galton-Watson type with probabilities p, r, and q producing 2, 1, and 0 new stem cells $(p+q+r=1)$.

Assumption 2 (*Crypt division*):
If the number S of stem cells in a crypt exceeds S_f two new crypts are formed and the stem cells are distributed according to binomial statistics with a probability v [i.e. $B(v, S)$]. With respect to the old crypt this is equivalent with the view that either 0 or 1 stem cells remain. (For practical reasons we will assume $v = \frac{1}{2}$ later on.)

These two assumptions enable us to write down the transition probability $P_{ij}(v)$ for a crypt of size $S_n = i$ in generation n to one of size $S_{n+1} = j$ in generation $n+1$. It is given by

$$P_{ij}(v) = P(S_{n+1} = j | S_n = i)\,. \tag{2a}$$

$$= \begin{cases} P_{ij}^{*}(v) & \text{for} \quad i>0, j\geq 0 \\ 0 & \text{for} \quad i=0, j>0 \\ 1 & \text{for} \quad i=0, j=0, \end{cases} \tag{2b}$$

where $P_{ij}^{*}(v)$ stands for the i-fold convolution of $\{p_{ik}(v)\}$ at point j and

$$p_{ik}(v) = \begin{cases} q & \text{for} \quad i<S_f \quad \text{and} \quad k=0 \\ r & \text{for} \quad i<S_f \quad \text{and} \quad k=1 \\ p & \text{for} \quad i<S_f \quad \text{and} \quad k=2 \\ 1-v & \text{for} \quad i\geq S_f \quad \text{and} \quad k=0 \\ v & \text{for} \quad i\geq S_f \quad \text{and} \quad k=1 \\ 0 & \text{else} \end{cases} \tag{2c}$$

In order to obtain experimentally meaningful results one has to consider an additional assumption which leads to conditional probabilities.

Assumption 3 (*Extinction of subunits*):
Crypts with zero stem cells are considered extinct (absorbing state). They are eliminated from consideration.

Based on assumptions 1-3 one could start a computation of the problem which would have to take all possible outcomes of cellular growth and crypt segregation into account. The number of combinations however increases exponentially with n which makes the task numerically difficult. On the other hand, such a computation is not required because the experimental observation of the evolution of individual crypts over time is not possible. The experimental observables available are only expectation values averaged over certain time periods and populations of crypts. This justifies the following assumption.

Assumption 4 (*Effective crypt approximation*):
The evolution of a population of crypts (with all the possible combinatorics) can be approximated by consideration of an effective probability distribution for an average crypt and a separate counting of the total number of crypts.

This assumption has the advantage that the dynamics of the number of crypts and the cell distribution per (effective) crypt can be separated. It allows the use of a particular renormalization procedure for the calculation of the probability distributions. Let $P_{\text{eff}}(S_n)$ denote an appropriately defined effective probability distribution of stem cells in step n. Then one has to assume fission of those crypts with $S_n \geq S_f$ cells. The fraction of crypts which is added by this doubling process is given by

$$g = \sum_{i\geq S_f} P_{\text{eff}}(S_n=i) \Big/ \left\{ \sum_{i<S_f} P_{\text{eff}}(S_n=i) + 2 \cdot \sum_{i\geq S_f} P_{\text{eff}}(S_n=i) \right\}. \tag{3}$$

g can be used as a renormalization factor because it records the excess crypts originating from the fission process. Equations (2a-c) determine the transition from n to $n+1$. By the cell division and crypt fission processes one obtains a new distribution $P(S_{n+1})$ which is not properly normalized because the increase in crypt

numbers due to fission is not taken into account properly. One can return to a properly normalized effective probability distribution including crypt fission via a renormalization in which the fraction of crypts not having divided is weighted by a factor $(1-g)$ and the new crypts by a factor g yielding:

$$P_{\text{eff}}(S_{n+1}=i)=P(S_{n+1}=i\,|\,S_n>0)\,.\,(1-g)+P(S_{n+1}=i\,|\,S_n\geq S_f)\,.\,g. \qquad (4)$$

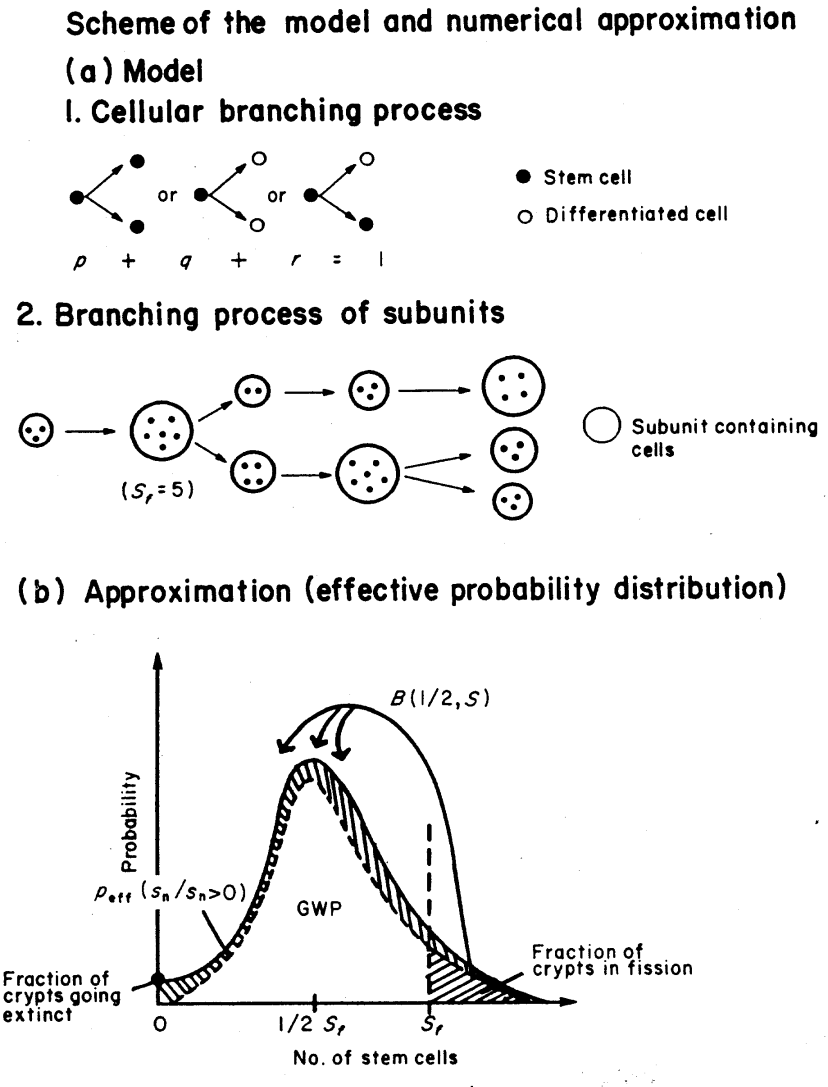

FIG. 3. (a) Describes the basic model assumptions 1 and 2: (1) The cellular branching process: schematic summary of the model concept. With probabilities p, r, and q the stem cells (full circles) produce 2, 1, and zero daughter stem cells; (2) The branching process describing the growth of subunits (crypts) containing stem cells (dots). Within a subunit the cells may grow until their number exceeds S_f. Then the subunits divide and the cells are distributed. (b) Approximative model of the evolution of the crypt population. By consecutive averaging over the daughter crypts one defines an effective average crypt and counts the number of crypts in the population separately. The dashed line describes the effective conditional distribution $P_{\text{eff}}\,(S_n\,|\,S_n>0)$. Due to the processes 1 and 2 a transition is generated leading to a not normalized distribution (full line). Due to fission one obtains two new crypts proportional to the shaded integrals. In order to obtain $P_{\text{eff}}(S_{n+1})$ one has to renormalize the distribution. Consequently one determines the extinction rate $P_{\text{eff}}(S_{n+1}=0)$ and obtains the conditional probability $P_{\text{eff}}(S_{n+1}\,|\,S_{n+1}>0)$.

With these quantities one can derive all other quantities of interest. Let C_n denote the number of crypts in step n. The average number of crypts is then given by

$$E(C_{n+1}) = E(C_n) \cdot \left[\sum_{i \geq 1} P(S_{n+1} = i \mid S_{n+1} \neq 0) \right]. \tag{5}$$

The fission probability (rate) is defined by

$$\phi := \sum_{i \geq S_f} P_{\text{eff}}(S_n = i \mid S_n \neq 0), \tag{6}$$

and the extinction probability (rate) is

$$\Omega := P_{\text{eff}}(S_{n+1} = 0 \mid S_n \neq 0). \tag{7}$$

In addition the elimination of dead crypts implies that one has to look at the conditional probability $P_{\text{eff}}(S_{n+1} = k \mid S_{n+1} \neq 0)$. Thus, the entire transition process from n to $n+1$ can be summarized as follows

$$P_{\text{eff}}(S_{n+1} = k \mid S_{n+1} \neq 0) = C \cdot \left\{ \sum_{j < S_f} P_{kj}(v) \cdot P_{\text{eff}}(S_n = j \mid S_n \neq 0) \right.$$

$$\left. + \sum_{j \geq S_f} [P_{kj}(v) + P_{kj}(1-v)] \cdot P_{\text{eff}}(S_n = j \mid S_n \neq 0) \right\}, \tag{8}$$

where C is an appropriate normalization factor for P_{eff} and P_{kj} is defined in (2). From here the iteration continues with eqns (3) and (4). Figure 3 gives a schematic summary of the model concept and the approximation strategy.

In order to compare the probability distribution of stem cells with macroscopic observables a fifth assumption holds.

Assumption 5 (*proportionality*):
The size of a crypt is proportional to the number of stem cells in it.

Numerical Procedure and Choice of Parameters

Assumptions 2 and 3 make an analytical treatment of the model difficult. To our knowledge theorems on conditional probabilities for controlled Galton-Watson processes do not exist nor do they for the type of process considered here. Only for simple GWPs is it known that a stationary conditional probability distribution exists in the subcritical case ($m < 1$). One can show (Jagers, 1975) that under these circumstances $\lim P(S_n = K \mid S_n > 0) = b_K$ exists and $\sum b_K = 1$. In the fission process the average number of offsprings m_f in a crypt is smaller than 1. As the qualitative behaviour is mostly determined by m_f we conjecture that similar statements about stationary solutions also hold for our case. The proof is left for further studies.

The question of whether a stationary probability distribution exists could not be answered analytically. However, assumption 4 makes a numerical calculation possible. It allows us to avoid the computation of the combinatorics of all possible configurations which grow exponentially with generation n. The separation of the problem into a determination of an appropriately renormalized effective distribution

and a determination of the crypt population enables an iterative procedure according to the above formulae. Iteration was stopped if two consecutive generations differed by not more than 10^{-6} (L_1-measure). Convergence of the iteration is generally fast (in the order of 100 iteration steps) and independent of the initial distribution [if $q > \delta > 0$, with δ sufficiently large (e.g. 10^{-3})].

There are only three free parameters in the model. One is the fission threshold S_f. Based on data it is assumed to range between 4 and 64. For numerical purposes values of 4, 8, 16, 32, and 64 are used. The second parameter is the doubling time T of the number of crypts. It relates to the microscopic parameters p, q, and r via the formula $(2p+r)^T = 2$ and $p+q+r=1$. For numerical purposes values of 12, 25, 50, 100, 200, and 400 generations are used for T (one generation corresponds to one cell cycle which is about 1 day). As a consequence it remains for one of the microscopic parameters to be chosen. We will usually chose the probability for asymmetric stem cell division r as the third parameter. It will be varied between 0 and the maximum value r_{max} which is given by

$$r_{max} = 2 - 2^{1/T}. \tag{9}$$

Typically $r = 0, 0{\cdot}6, 0{\cdot}9, 0{\cdot}97, 0{\cdot}99, r_{max}$ is chosen.

Model Results

STATIONARY PROBABILITY DISTRIBUTION

Numerical calculations of the effective probability distribution (P_{eff}) were undertaken. The procedure exhibited rapid convergence towards a stationary distribution which was (under minor restrictions) independent of initial conditions. However, shape and position of the distribution was found to depend strongly on the choice of the parameters S_f, r and T. This dependence is complex.

Figure 4 gives a number of examples for the distribution of stem cells per crypt. The distributions in the left three panels [(a), (c) and (e)] are obtained for the same threshold parameter S_f and the same doubling time T. It is apparent that variation of the asymmetric division r drastically influences the probability distributions. For low r (e.g. 0·6) the distributions are broad and crypts with very few and many stem cells are likely. The coefficient of variation exceeds 0·5. For very high r (>0·99) the distribution is shifted to the right and the mode approaches $1/2S_f$. Coefficients of variation become as small as 0·25. In these cases the probability to have crypts with few stem cells is very small. In the remaining three panels [(b), (d) and (f)] either the doubling time T or the threshold parameter S_f is changed. Shortening of T and reduction of S_f generally broaden the distribution relative to S_f.

Comparison of the effective probability distribution of stem cells with the size distribution of crypts (Fig. 2) suggests that the broad distributions associated with small r, high T and low S_f are qualitatively not consistent with the data. In particular distributions with a flat left-hand side do not exist for $S_f < 8$.

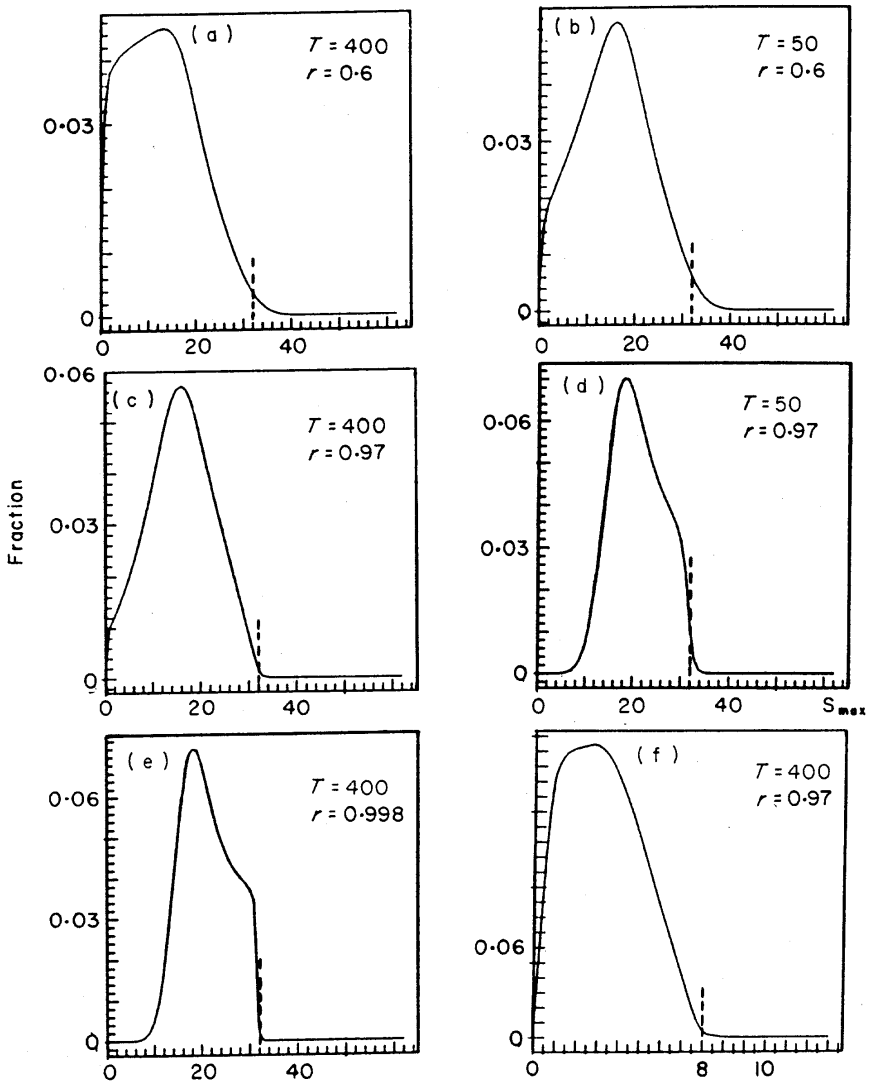

FIG. 4. Examples of effective stationary probability distributions obtained for various sets of parameters (discussion see text):

	T	S_f	r
(a)	400	32	0·6
(b)	50	32	0·6
(c)	400	32	0·97
(d)	50	32	0·97
(e)	400	32	0·998
(f)	400	8	0·97

EXTINCTION AND FISSION PROBABILITIES

While the comparison of stem cell distributions with crypt size distributions depends on the proportionality assumption the extinction (EP) and fission (FP) probabilities do not. One can therefore expect to gain a good quantitative insight by considering how EP and FP change with variation of the three essential model parameters. Extensive numerical investigations were undertaken to explore the

behaviour over a wide range of reasonable parameters. Two-dimensional projections of the results are displayed in Fig. 5 (fixed T) and Fig. 6 (fixed S_f) with variation of the other two parameters.

Figure 5(a) and (b) shows the extinction probabilities obtained from the stationary effective probability distribution. Each point in the diagram represents one calculation for a fixed combination of parameters. For example, the combination of $r = 0{\cdot}9$, $S_f = 32$ and $T = 50$ is associated with a probability of $7{\cdot}4 \times 10^{-5}$ that a given crypt dies due to loss of all its stem cells in the next generation (next cell division). The lines interpolate between model scenarios of equal values of r. It becomes apparent that for given T and r the EP declines monotonously with increasing S_f. This dependency is, however, weak if r is small ($<0{\cdot}6$) but strong for large r. In the first case EP may change by two orders of magnitude in the latter case by 10 or more.

One can relate these diagrams directly to experimental observations. As discussed above the data indicate that the extinction rate is realistically lower than 0·001/cell cycle (Table 1). Consequently all model scenarios above the dashed horizontal lines can be considered as unrealistic. Apparently only models with sufficiently large r and S_f can give EPs consistent with the data (scenarios below the dashed lines).

Figure 5(c) and (d) shows similar plots for the fission probabilities. The FP declines monotonously with increasing r and S_f, but now the dependency is stronger for small r than for large r. Interestingly, there appears to be an asymptotic value which depends only on T and not on the other parameters.

One can again relate these diagrams to experimental observations. As discussed above the data indicate that the fission probability is lower than 0·006/cycle but higher than 0·0013/cycle. Thus, only the scenarios which lie between these values are consistent with the data. For $T = 50$ none of the models fulfills the criterion. They all lie outside the range consistent with the data (dashed line).

Figure 6 shows a different projection of the parameter space with the threshold parameter S_f being fixed (16) and T and r as variables. Figure 6(a) shows the EP and Fig. 6(b) the FP. Again one finds that higher values in r are associated with lower EP and FP. However, EP is increasing with T (for given fixed r) and FP is decreasing with T. This dependency is weak for large T and one can conjecture asymptotic behaviour of EP and FP for large T.

Consistency with data can again be checked as before. Taking these consistency conditions together one can obtain some conservative lower estimates for r and S_f. Given that the crypt doubling time is not shorter than 100 stem cell cycles models consistent with the data on extinction and fission probabilities can only be obtained if $r \geq 0{\cdot}8$ and $S_f \geq 8$.

RELATIONSHIP BETWEEN CONVENTIONAL GWP AND GWP WITH THRESHOLD DEPENDENT FORMATION OF SUBUNITS

As the cellular (microscopic) growth processes in the conventional GWP and the GWP with threshold dependent segregation into subunits are basically the same (p, r, q) the question arises how one can compare the two.

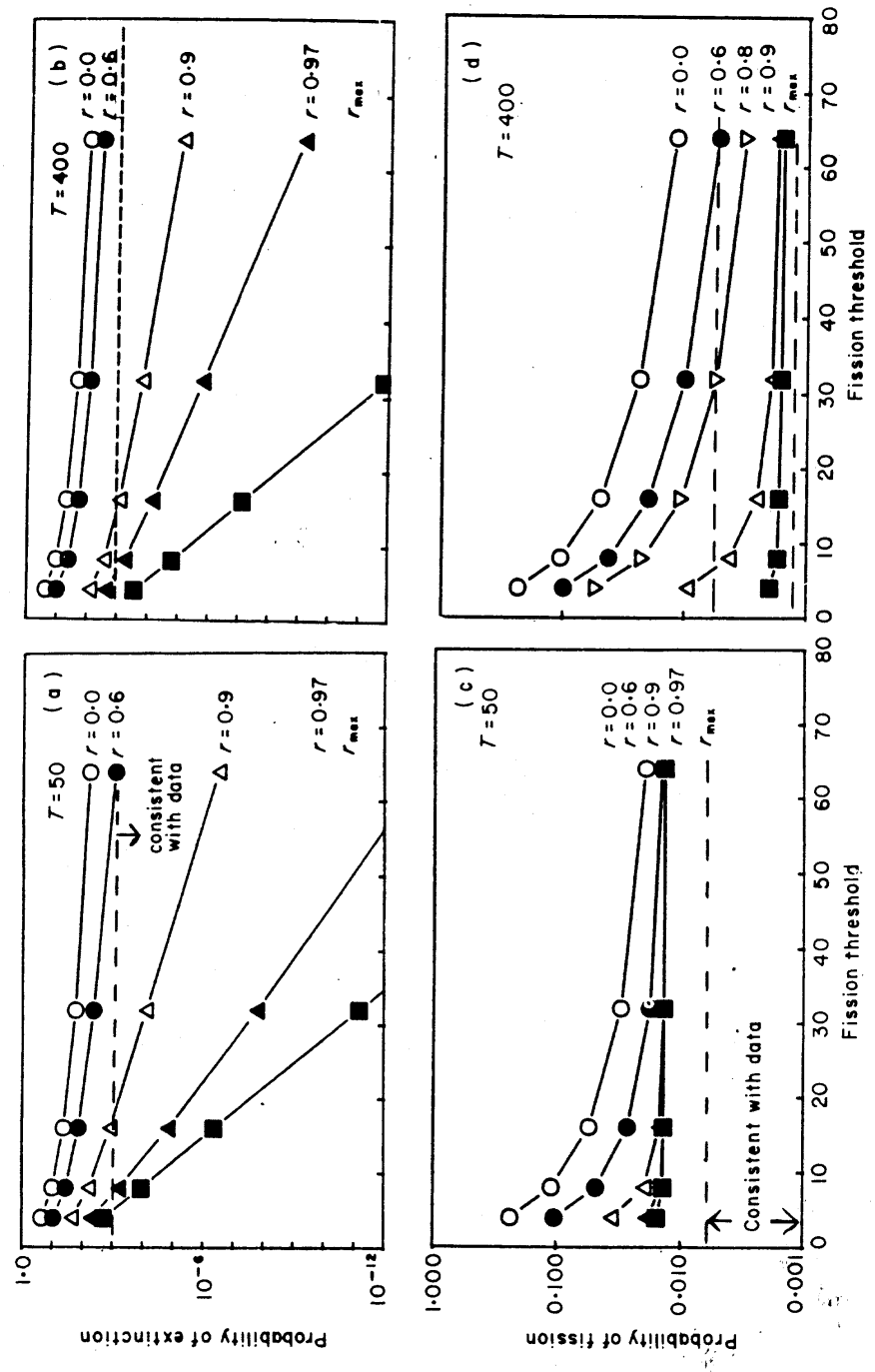

FIG. 5. Extinction and fission probabilities for fixed doubling times T (50 or 400) and variation of r and S_f. (a), Extinction ($T = 50$); (b), extinction ($T = 400$); (c), fission (50); (d), fission (400). Only models are consistent with experimental data on crypts whose extinction probability is $\ll 10^{-3}$ and whose fission probability ranges between 0·006 and 0·0013 (see table 1).

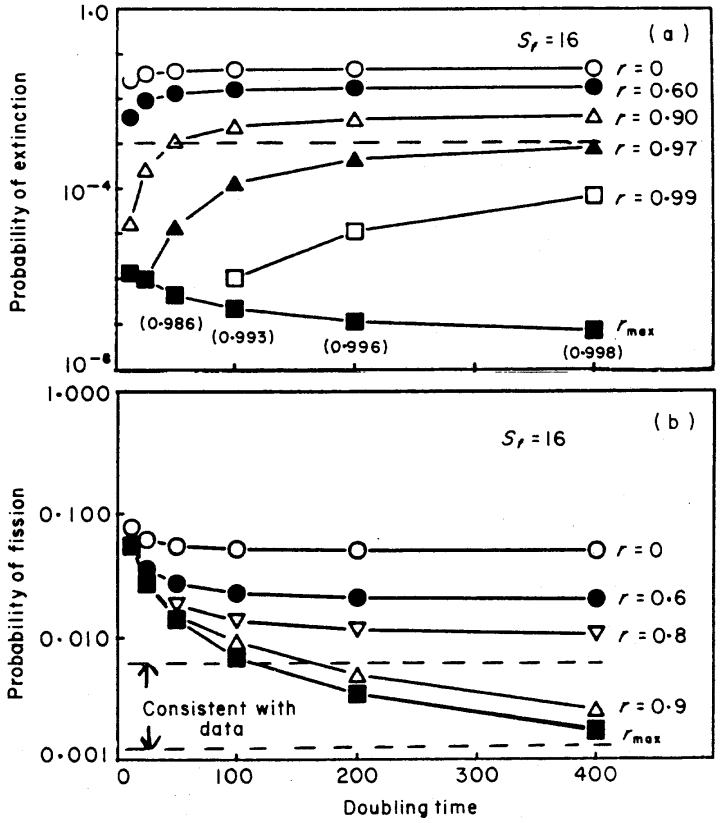

FIG. 6. Extinction (a) and fission (b) probabilities for fixed threshold ($S_f = 16$) and variation in T and r.

In a conventional GWP without segregation the probability of a population of size S to go extinct in one generation is given by $\Omega = q^S$ i.e. $\log(\Omega) = S \cdot \log(q)$ (Vogel *et al.*, 1969). The relationship of $\log(\Omega)$ and $\log(q)$ is linear.

Figure 7 shows analogous $\log(\Omega)$-$\log(q)$ plots for the GWP with segregation. Again each point describes a model scenario with one specified set of parameters (S_f fixed at 32). The free parameters were T and a parameter K defined as

$$K := p/(p-q), \tag{10a}$$

K is introduced to select model scenarios with an equal fraction of symmetric stem cell division (p) in the growth process ($p - q > 0$). One obtains

$$q = (2^{1/T} - 1)(K - 1) \tag{10b}$$

$$r = 1 + (1 - 2K)(2^{1/T} - 1). \tag{10c}$$

Thus, specification of T and K give unique values for q and r. Numerical calculations were undertaken for various K (2, 4, 10, 30, 100). The results are shown in Fig. 7. Lines connect values of models with equal K but varying T (12, 25, 50, 100, 200, 400 wherever possible). Apparently the relationships for equal K are linear and furthermore they appear to have the same slope irrespective of K. It is justified to assume a linear relationship of $\log(\Omega)$ and $\log(q)$ also for the GWP with segregation:

$$\log(\Omega) = S_{\text{eff}} \cdot \log(q) + C(K, S_f). \tag{11}$$

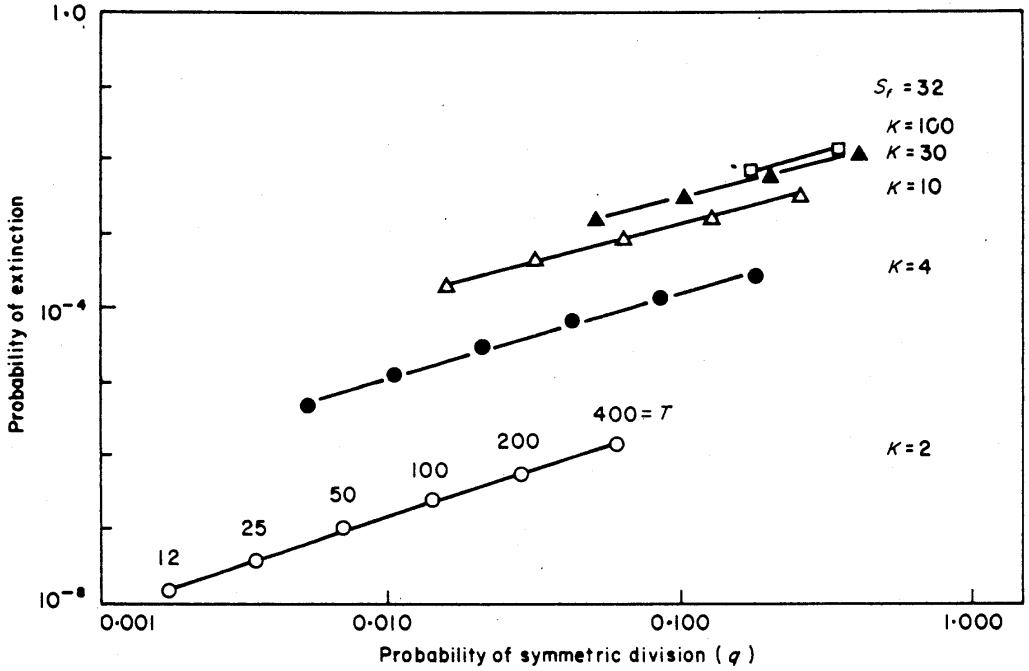

FIG. 7. Log (Ω)-log (q) diagram for fixed threshold S_f and variation in T and $K = p/(p-q)$. The slope of the curves can be interpreted as effective stem cell number.

This relationship implies that one can translate the GWP with segregation into a conventional GWP with an effective number of stem cells S_{eff}. Linear regression analysis reveals that S_{eff} is close to 1 (1·3–1·5). Apparently S_{eff} is much smaller than S_f. This can be understood as the extinction probability of a system with S cells is q^S. Given the small values for q [see (10b)] any power of q will rapidly converge to zero. Consequently the modified GWP considered here is behaving qualitatively like a conventional GWP with a small effective stem cell number. The quantitative differences depend on the choice of K and S_f and are reflected by the additive term $C(K, S_f)$. This term can be interpreted as the logarithm of the probability of having S_{eff} stem cells in the equilibrium configuration.

Therefore, one expects for small values of S_f that the curves for different K are more similar than for larger S_f. Indeed calculations show that the K-curves are very close in the case of $S_f = 4$ and spread over orders of magnitude for $S_f = 64$ (figures not shown).

Discussion

In this paper we have discussed properties of a new type of stochastic branching process. The basic process is a microscopic cellular renewal process of stem cells which is basically described by a conventional Galton-Watson Process. The new component is a threshold dependent segregation of the cells into subunits.

Biologically the investigation was undertaken to understand the birth and death process of macroscopic intestinal crypts and relate it back to the microscopic birth and death process of a few epithelial stem cells which are the constituents of the

crypt. Consideration of the segregation process allowed us to introduce a new (weaker) concept of stability. We found that even in the case of supracritical growth of the entire cell number the probability distributions (per effective crypt) are stationary. It is essential for this concept of stationarity to consider probabilities conditioned on non-extinction. This implies that irrespective of the actual number of subunits (crypts) present the distribution is invariant. Furthermore, this concept allows for a meaningful definition of a fission rate. The extinction properties of this process are different from those of a conventional GWP.

It is noteworthy that similar processes of cellular growth and fission have been analytically studied in some detail within the framework of deterministic models using partial differential equations (Diekmann *et al.*, 1983). However, since a stochasticity of the growth is not taken into account extinction is not possible in these models. Thus, these results cannot directly be related to our problem but appropriate modification of the PDE approach might be an interesting future perspective.

The particular system of intestinal crypts can quantitatively be explained by the stochastic branching model proposed. It was designed to explain four sets of data: the size distribution of crypts, the low extinction rate, the apparent fission rate and the growing number of crypts. We conclude that these phenomena can quantitatively be explained by a stochastic branching process of epithelial stem cells if the following conditions hold: (1) the probability for asymmetric stem cell division r is at least 0·8; (2) the number of stem cells doubles within a few hundred cell generations; (3) the threshold value S_f is at least 8. The high value of r is biologically remarkable. If one is considering individual crypts on short time scales one can assume with sufficient precision that the divisions are only asymmetric. This gives a justification for a previous model of our group in which the short-term steady-state of single crypts was described on the basis of the assumption of strictly asymmetric divisions of stem cells (Loeffler *et al.*, 1986; Potten & Loeffler, 1987). In contrast, some stochasticity is essential for the description of the long-term behaviour of individual crypts and for consideration of populations of crypts. The stochastic nature of the process would also predict that the sizes and fission times of adjacent crypts are only weakly correlated, a finding actually reported by Totafurno *et al.* (1987).

The biological basis for the asymmetric division is unclear. Several explanations are conceivable. Stem cells might be defined by their attachment to some supportive neighbour cell, or they might be determined by some internal marker attached to the mother chromosomes (Cairns, 1975). Discrimination of these possibilities is presently not possible. Biologically the most important conclusion is that the crypt behaviour can entirely be explained by the stem cell behaviour. This makes it clear that future experiments should centre around understanding these cells and their control in more detail.

A number of simplifying assumptions entered into the model. First, it is not likely that all stem cells exhibit the same cell cycle. A realistic model would have to consider a distribution of slowly and rapidly cycling cells (Potten, 1990). Second, the effective probability distributions are technically achieved by averaging over two daughter crypts. Further simulations showed that one can expect higher order

corrections for EP and FP of few percent if one takes more members of a crypt family into account. Third, and most important the assumptions on the segregation process itself may be too simplisitic. It can be doubted whether stem cells are independently allocated to the daughter crypts. Due to the spatial arrangement of cells in a ring at the bottom of the crypt this is rather unlikely. On the other hand, experimental observations suggest that the fission process usually tends to halve the crypt bottom and thereby the stem cell population. The binomial distribution $B(1/2, S)$ is characterized by a coefficient of variation that declines according to $1/\sqrt{S}$. Consequently, the binomial fission process tends to result in a near perfect halving process the larger S_f is chosen. Therefore, the process assumed appears nevertheless to be an acceptable description of the crypt fission process. In addition it is important to note that any asymmetric segregation would produce model scenarios with higher extinction and fission probabilities for given T, r and S_f. In this sense the values obtained for r can be considered as conservative lower estimates.

With respect to the biology of the intestinal crypt the present model allows a number of predictions to be made whose further experimental investigation is suggested. The first relates to the cellular growth process. The stem cells in the model are all indistinguishable from one another, each having the same growth possibilities. This implies that they are all equal competitors. Recently it was shown that one can label a single stem cell and all its offsprings by a particular label (e.g. Winton *et al.*, 1988). Our model predicts that in the long run only two situations can be found. Either this stem cell and its offsprings will entirely be lost from the crypt or they will populate the entire crypt. Long-term coexistence is unlikely. Thus, the model predicts that any crypt will ultimately converse to monoclonal phenotype. A more detailed examination of this effect and of the time scales involved in the conversion to monoclonal phenotype is in preparation.

A second prediction refers to the threshold dependent crypt fission. The model suggests three ways by which an increase in crypt numbers (i.e. crypt fission rates) may occur.

First, there could be a reduction in the fission threshold which would lead to more but smaller crypts. Secondly, there could be a reduction in r (with $p-q=$ constant, i.e. same T), leading to more crypts with a much broader spectrum in sizes (greater C_V). Thirdly, there could be an increase in the growth advantage of stem cells (increase in $p-q$, i.e. shortage in T), which would lead to more equally sized crypts. It should be interesting to find out whether these processes play a role in ontogenesis (presumably the first would), or the in recovery following damage or even in the onset of adenoma formation (presumably the second and third would). This should encourage experimental investigations relating the frequency and mode of crypt fission to the behaviour and characteristics of stem cells in the crypts under various circumstances.

Although exemplified for the intestinal crypt the process discussed here should in principle be applicable to other systems in biology, as well as ecology and sociology. The general phenomenon described is the formation of groups, cohorts, colonies, families, parties, subsystems etc on the basis of an underlying growth mechanism of individuals (cells, animals, societies).

We would like to thank C. S. Potten (Manchester), Ursula Paulus and Dirk Hasenclever (Koeln) for helpful discussion and various suggestions. Heiner Stroetmann (Koeln) was particularly helpful in performing some of the numerical calculations.

REFERENCES

CAIRNIE, A. B., LAMERTON, L. F. & STEEL, G. G. (1965). Cell proliferation studies in the intestinal epithelium of the rat. *Exp. Cell Res.* **39,** 539-553.

CAIRNIE, A. B. & MILLEN, B. H. (1975). Fission of crypts in the small intestine of the irradiated mouse. *Cell Tissue Kinet.* **8,** 189-196.

CAIRNS, J. (1975). Mutation selection and the natural history of cancer. *Nature, Lond.* **225,** 197-200.

DIEKMANN, O., LAUWERIER, H. A., ALDENBERG, T. & METZ, A. J. (1983). Growth fission and stable size distribution. *J. math. Biol.* **18,** 135-148.

FUJIMAGARI, T. (1976). Controlled Galton-Watson process and its asymptotic behavior. *Kodai Math. Sem. Rep.* **27,** 11-18.

FUJIMAGARI, T. (1980). On the extinction time distribution of a branching process in varying environments. *Adv. appl. Prob.* **12,** 350-366.

HÖPFNER, R. (1985*a*). A note on the probability of extinction in a class of population-size-dependent Galton-Watson processes. *J. appl. Prob.* **22,** 920-925.

HÖPFNER, R. (1985*b*). On some classes of population-size-dependent Galton-Watson processes. *J. appl. Prob.* **22,** 25-36.

HÖPFNER, R. (1986). Some results on population-size-dependent Galton-Watson processes. *J. appl. Prob.* **23,** 297-306.

JAGERS, P. (1975). *Branching Processes with Biological Applications.* New York: Wiley & Sons.

KESTEN, H. & STIGUM, B. P. (1967). Limit theorems for decomposable multidimensional Galton-Watson processes. *J. math. Anal. Applic.* **17,** 309-338.

KLEBANER, F. C. (1983). Population-size-dependent branching process with linear rate of growth. *J. appl. Prob.* **20,** 242-250.

KLEBANER, F. C. (1984*a*). On the population-size-dependent branching processes *Adv. appl. Prob.* **16,** 30-55.

KLEBANER, F. C. (1984*b*). Geometric rate of growth in population-size-dependent Galton-Watson processes. *J. Appl. Prob.* **21,** 40-49.

KLEBANER, F. C. (1985). A limit theorem for population-size-dependent Galton-Watson processes. *J. appl. Prob.* **22,** 48-57.

LOEFFLER, M., STEIN, R., WICHMANN, H. E., POTTEN, C. S., KAUR, P. & CHWALINSKI, S. (1986). Intestinal cell proliferation. I. A comprehensive model of steady state proliferation in th crypt. *Cell Tiss. Kinet.* **19,** 627-645.

POTTEN, C. S. (1990). The response of the murine small intestine to acute irradiation, exemplified by a 15 year study of BDF1 mice. *Int. J. Radiat. Biol.* (in press).

POTTEN, C. S. & HENDRY, J. H. (1983). Stem cells in murine small intestine. In: *Stem Cells: Their Identification and Characterisation* (Potten, C. S., ed.) pp. 155-159. Edinburgh: Churchill Livingstone.

POTTEN, C. S., HENDRY, J. H. & MOORE, J. V. (1987). Estimates of the number of clonogenic cells in crypts of murine small intestine. *Virchows. Arch.* **53,** 227-234.

POTTEN, C. S. & LOEFFLER, M. (1987). A comprehensive model of the crypt of the small intestine of the mouse provides insight into the mechanisms of cell migration and the proliferation hierarchy. *J. theor. Biol.* **127,** 381-391.

TOTAFURNO, J., BJERKNES, M. & CHENG, H. (1987). The crypt cycle. Crypt and villus production in the adult intestinal epithelium. *Biophys. J.* **52,** 279-294.

VOGEL, H., NIEWISCH, H. & MATIOLI, G. (1968). The self renewal probability of hemopoietic stem cells. *J. Cell. Physiol.* **72,** 221-228.

VOGEL, H., NIEWISCH, H. & MATIOLI, G. (1969). Stochastic development of stem cells. *J. theor. Biol.* **22,** 249-270.

WINTON, D. J., BLOUNT, M. A. & PONDER, B. A. J. (1988). A clonal marker induced by mutation in mouse intestinal epithelium. *Nature, Lond.* **333,** 463-466.

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