ASSESSMENT OF THE SYMMETRY OF STEM-CELL MITOSES

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ABSTRACT A model of Paneth-cell renewal in the small intestinal epithelium is used to estimate the probability that epithelial stem-cell mitoses are symmetric in the sense that they produce two cells of the same type. I found that counts of the number of Paneth cells per crypt (Paneth cells are terminally differentiated cells derived from small intestinal epithelial stem cells) support a model in which most, if not all, stem-cell mitoses are symmetric.

INTRODUCTION

The continuous production of new cells is a key feature of many vertebrate tissues (e.g., the intestinal epithelium). In these tissues there are cells, known as stem cells, with the potential to produce both new stem cells and other cells that differentiate (Leblond and Cheng, 1976). The pluripotentiality of the stem cell raises an important question; namely, when a stem cell divides is there a predictable pattern in the type of cells produced? The most frequently discussed possibilities are that stem-cell mitoses are either asymmetric (i.e., the daughter cells are different one from the other; fig. 1; Cairns, 1975; Potten, 1978; see also Leblond and Cheng, 1976), random (Marques-Pereira and Leblond, 1965), or symmetric in the sense of producing only either two stem cells or two differentiating cells (Leblond and Cheng, 1976; Leblond et al. 1967).

The lining of the small intestine, the small intestinal epithelium, is a good model system in which to investigate the issue of the symmetry of stem-cell mitoses. The epithelial stem-cell pool (found in structures known as the crypts of Lieberkühn) produces four differentiated cell types, one of which is the Paneth cell type (Cheng and Leblond, 1974*b*; Leblond and Cheng, 1976; Bjerknes and Cheng, 1981*a*).

Several features of the Paneth cell population render it useful for investigating the symmetry of stem-cell mitoses; Paneth cells are derived from the common epithelial stem-cell pool (Cheng and Leblond, 1974*b*; Leblond and Cheng, 1976; Bjerknes and Cheng, 1981*a*), Paneth cells do not divide (Cheng, Merzel, and Leblond, 1969; Cheng and Leblond, 1974*b*; Bjerknes and Cheng, 1981*a*), only small numbers of Paneth cells are present in each crypt (Cheng and Leblond, 1974*a*; Bjerknes and Cheng, 1981*a*), and they remain in the crypt until their death (Cheng and Leblond, 1974*b*; Bjerknes and Cheng, 1981*a*). Given these features of the Paneth cell population it is clear that the distribution of the number of Paneth cells in a crypt will be influenced by the pattern of stem-cell mitoses. For example, in the extreme, if all stem-cell mitoses were symmetric, then Paneth cells would be produced in pairs. If Paneth cells were produced in pairs, and if all Paneth cells had similar lifespans, then the number of Paneth cells in a crypt would have a higher probability of being an even number (e.g., 2, 4, 6, etc.) than would otherwise be the case.

With this in mind, I derived a model of Paneth-cell renewal in which newly differentiated Paneth cells arrive in clusters of one or two cells (depending upon the symmetry of the mitosis of the precursor stem cell). Once they have arrived, Paneth cells behave independently. They each live a period of time that is a random function and then they die. The form of the model is such that it predicts the distribution of the number of Paneth cells in crypts. By fitting the model to data derived from counts of the number of Paneth cells per crypt, I derived a maximum likelihood estimate of the proportion of the Paneth cell's immediate precursor, the stem cells, whose mitoses were symmetric.

METHODS

Experimental Methods

Duodenum was collected from three adult male Swiss albino mice. The tissue was embedded in Epon and 200–250 serial 1-μm sections were prepared from the tissue from each animal (iron hematoxylin and safranine O staining). The number of Paneth cells in whole crypts from each animal was counted. Thus, a sample distribution for the number of Paneth cells contained in a crypt was gathered. Estimates of the symmetry of stem-cell mitoses were then obtained from application of the model described below.

The Model

My goal in this section is to produce a model that provides a reasonable description of Paneth-cell renewal and hence of the distribution of the number of Paneth cells to be found in a crypt. Since the main issue of this paper is symmetry of stem cell mitoses, and since the degree of symmetry is a parameter of the model developed, I also present methods for deriving estimates of the probability of symmetry from the data.

The symmetry of stem-cell mitoses determines whether Paneth cells differentiate in groups of one or two cells. Whatever their composition, I

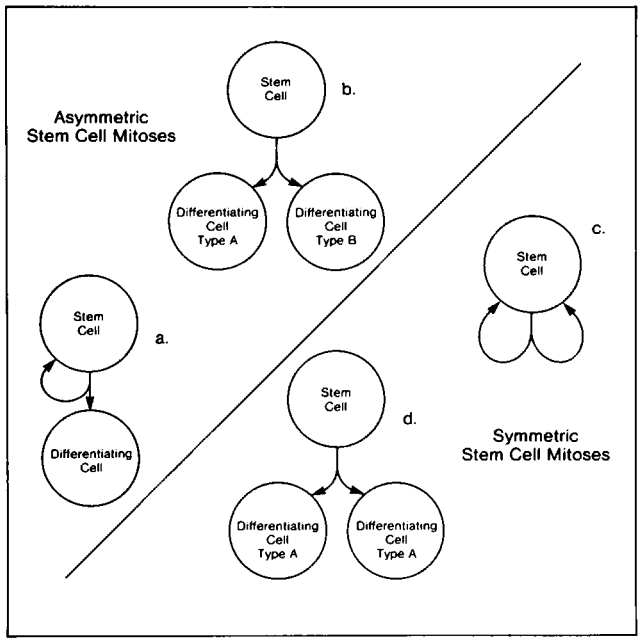

FIGURE 1 Schematic diagrams of two hypothetical classes of stem-cell mitoses. (*a*) and (*b*), asymmetric stem-cell mitoses. (*c*) and (*d*), symmetric stem-cell mitoses. (*a*), stem-cell mitosis producing a stem cell and a differentiating cell. Cells with this behavior are often referred to as exhibiting differential mitosis. This model has had some appeal in studies of renewing systems because it is thought to assure a balance between maintenance of the stem cell pool and production of differentiating cells. (*b*), asymmetric stem-cell mitosis producing two different types of differentiating cells (e.g., a Paneth cell and a columnar cell). (*c*), symmetric stem-cell mitosis producing two stem cells. (*d*), symmetric stem-cell mitosis producing two differentiating cells of the same type (e.g., two Paneth cells or two columnar cells).

call each group an arrival. Thus, an arrival may be composed of either one or two new Paneth cells. With this definition of an arrival, I may write a simple equation for $N(t)$, a random variable representing the number of Paneth cells in a crypt at time t given that there were no Paneth cells in a crypt at time 0,

$$N(t) = \sum_{i=0}^{G(t)} \{Y(t)\}_i \tag{1}$$

where $G(t)$ is a random variable representing the number of arrivals in the interval $(0, t)$, and the $\{Y(t)\}_i$ are independent identically distributed (for fixed t) random variables representing the number of Paneth cells that survive until time t from arrivals at arbitrary points in $(0, t)$. Thus, the number of Paneth cells in a crypt at time t is determined by summing the number of surviving Paneth cells from the $G(t)$ arrivals in the interval. It follows that $N(t)$ is the sum of a random number of random variables, which means, by definition, that $N(t)$ is a compound stochastic process.

The probability generating function (PGF) for an arbitrary discrete random variable, $W(t)$, is by definition (see any standard text on probability), $\Sigma_{k=0}^{\infty} z^k P\{W(t) = k\}$. Thus, by definition, the PGF for the probability density describing $N(t)$, $n_k(t)$ is

$$\phi(z, t) = \sum_{k=0}^{\infty} z^k n_k(t) \tag{2}$$

where $n_k(t) = P\{N(t) = k\}$. It then follows by a standard theorem of probability that since $\phi(z, t)$ describes a compound stochastic process,

$$\phi(z, t) = \psi(\varphi(z, t)) \tag{3}$$

where $\psi(z, t)$ and $\varphi(z, t)$ are the PGF for the processes defining $G(t)$ and $Y(t)$ respectively.

I will proceed by making the assumption that the lifetimes of Paneth cells are independent and identically distributed random variables, X, with probability density $f(t)$. To express this in another way, if one chose a new Paneth cell at random, the distribution of the probability of possible lifetimes for that cell would be described by $f(t)$ independent of the actual lifetimes of all other Paneth cells. I will have repeated need for the following identities involving $f(t)$. The probability that a Paneth cell dies at or before age t is

$$P\{X \le t\} = F(t) = \int_0^t f(x)\mathrm{d}x\,, \tag{4}$$

which represents the accumulation of the probability of death over the interval from 0 to t. Similarly, the probability that a Paneth cell lives longer than age t is

$$P\{X > t\} = 1 - F(t) = \int_t^{\infty} f(x)\mathrm{d}x\,. \tag{5}$$

If we now concentrate on an arrival that occurs at time x and consists of j Paneth cells, then the conditional probability that k Paneth cells survive ($k < j$) until time t ($x < t$) is

$$P\left\{\begin{array}{l|l} k \text{ cells survive} & \text{arrival occurs at} \\ \text{until time } t & \text{time } x \text{ and consists} \\ \text{from an arrival} & \text{of } j \text{ cells} \end{array}\right\}$$

$$= \left[\binom{j}{k}[1 - F(t - x)]^k\right][F(t - x)]^{j-k}, \tag{6}$$

where the first bracketed term on the right-hand side represents the probability that k cells will survive for at least the period $(t - x)$ multiplied by the number of ways of selecting k out of j cells, and the second bracketed term represents the probability that the other $j - k$ cells die in the interval. Now I will proceed to remove the conditions. First, let's allow the arrival to occur at any point in $(0, t)$. In this case we will need to know the probability that the arrival occurs at time x in the interval. I call this probability $h(x)$. Integrating over possible arrival times (i.e., by the law of total probability), it follows that

$$P\left\{\begin{array}{l|l} k \text{ cells survive} & \text{arrival consists} \\ \text{until time } t & \text{of } j \text{ cells} \\ \text{from an arrival} & \end{array}\right\}$$

$$= \int_0^t h(x)\left\{\binom{j}{k}[1 - F(t - x)]^k\right\}[F(t - x)]^{j-k}\mathrm{d}x. \tag{7}$$

To remove the final restriction, that the arrival consists of j cells, I require knowledge of another probability density, $i_j(x)$, which describes the probability that the arrival at time x consists of j Paneth cells. With $i_j(x)$ defined I am now able (by the law of total probability) to write the general form for the probability density function $Y_k(t) = P\{Y(t) = k\}$ which describes the conditional probability of having k Paneth cells alive at time t given exactly one arrival at a random point in the interval $(0, t)$,

$$y_k(t) = \sum_{j=k}^{\infty} \int_0^t i_j(x)h(x) \cdot \left\{\binom{j}{k}[1 - F(t - x)]^k\right\}[F(t - x)]^{j-k}\mathrm{d}x\,. \tag{8}$$

If I now assume that $G(t)$, the number of arrivals in the interval $(0, t)$, is described by a Poisson process (an assumption which I will justify in a moment) with arrival rate λ, then the probability that an arbitrary arrival occurs at time x in $(0, t)$ will be uniformly distributed over $(0, t)$. Hence,

$h(x) = 1/t$. For the Paneth cell population, any given arrival will be composed of either one or two cells (corresponding to an asymmetric or symmetric stem-cell mitosis respectively). I next assume that the probability that a given arrival consists of two cells is stationary with probability α (i.e., $i_2(x) = \alpha$). Accordingly, the probability that an arrival consists of one cell is $1 - \alpha$ (i.e., $i_1(x) = 1 - \alpha$). With these assumptions, $y_0(t)$, $y_1(t)$, and $y_2(t)$ may be written more explicitly (for $k > 2$, $y_k(t) = 0$) by substituting the appropriate terms into Eq. 8,

$$y_0(t) = 1 - y_1(t) - y_2(t) \tag{9}$$

$$\begin{aligned} y_1(t) &= \frac{1-\alpha}{t}\int_0^t [1 - F(t-x)]\,dx \\ &\quad + \frac{\alpha}{t}\int_0^t 2F(t-x)[1 - F(t-x)]\,dx \qquad (10) \\ &= \frac{1-\alpha}{t}\int_0^t [1 - F(x)]\,dx \\ &\quad + \frac{\alpha}{t}\int_0^t 2F(x)[1 - F(x)]\,dx \qquad \text{(by substitution)} \end{aligned}$$

$$y_2(t) = \frac{\alpha}{t}\int_0^t [1 - F(x)]^2\,dx\,. \tag{11}$$

At this point it will be useful to present arguments that, as a first approximation, the arrival of newly differentiated Paneth cells may reasonably be viewed as a homogeneous Poisson process.

To qualify as a homogeneous Poisson process, the phenomenon under consideration must exhibit the following properties: (*a*) The probability of an event must be independent of all past events. (*b*) Events do not occur at predetermined times and the probability of an event must be constant over time. (*c*) The probability of an event in a sufficiently short interval is roughly proportional to the length of the interval. (*d*) The probability of more than one event during an interval is negligible in a sufficiently short interval.

I now argue that, from the vantage point of the Paneth cell population, the event of stem cell differentiation into Paneth cell may be viewed, to a first approximation, as a Poisson process.

On the average there are perhaps 15 stem cells per crypt (Bjerknes and Cheng, 1981*b*). Each stem cell undergoes cell division about twice a day. From this background of stem cell activity are derived an average of about eight Paneth cells (duodenal crypts contain on average about eight Paneth cells; see Results) every 3 wk (Paneth cells have an average lifetime of ~3 wk; Bjerknes and Cheng, 1981*a*). This means, assuming for the moment that stem-cell mitoses are symmetric (i.e., $\alpha = 1$) and hence that Paneth cells are produced in pairs, that a pair of Paneth cells is produced on average about once every five days. Hence, on the order of 1 in 150 stem-cell mitoses yields Paneth cells; the upshot of which is that the event of stem-cell mitosis yielding Paneth cells is a relatively rare event. It follows that assumptions *c* and *d* are probably reasonable first approximations. Assumption *b* is justified in part by the fact that in the adult, epithelial renewal approximates a steady state. Furthermore, no evidence has been found for circadian or other rhythms in the pattern of stem-cell mitoses in crypts. The epithelial stem cells appear to operate as an asynchronously dividing population. Assumption *a*, independence of history, is the most difficult to justify. However, the relative rarity of the events and the absence of any experimental evidence for feedback from the Paneth cell population onto Paneth cell production make the assumption a reasonable first approximation.

To restate the model thus far, the number of stem cells that have differentiated into Paneth cells in an interval of duration t, $G(t)$, may be approximated by a Poisson process and hence, $P\{k \text{ arrivals in } (0, t)\} = \exp(-\lambda t)(\lambda t)^k/k!$, where λ is the mean rate of stem cell differentiation into Paneth cells. Each of these $G(t)$ arrivals contributes $Y(t)$ Paneth cells to the total with the probabilities presented in Eqs. 9–11.

Since $G(t)$ is a Poisson random variable, $N(t)$ is a compound Poisson process. This means that from Eq. 3 and from the fact that $\psi(z, t) = \exp[\lambda t(z - 1)]$, I may readily write the form of the PGF for $N(t)$,

$$\phi(z, t) = \psi[\varphi(z, t)] = \exp\{\lambda t[\varphi(z, t) - 1]\}, \tag{12}$$

where $\psi(z, t)$ and $\varphi(z, t)$ are the PGF for $G(t)$ and $Y(t)$ respectively. By definition of a probability generating function, $\varphi(z, t) = \sum_{n=0}^{\infty} z^n y_n(t) = y_0(t) + zy_1(t) + z^2y_2(t)$. Hence, after substituting $\varphi(z, t)$ into Eq. 12, using Eq. 9, and then taking the limit as $t \to \infty$, I derive $\phi(z)$

$$\begin{aligned} \lim_{t\to\infty} \phi(z, t) &= \phi(z) \\ &= \lim_{t\to\infty} \exp[\lambda t y_1(t)(z - 1) < \exp[\lambda t y_2(t)(z^2 - 1)], \end{aligned} \tag{13}$$

which is the PGF for the stationary (i.e., steady state) process describing the number of Paneth cells in a crypt. Now, consider the limit as $t \to \infty$ of $\lambda t y_1(t)$ and $\lambda t y_2(t)$. Substituting Eq. 10 into $\lambda t y_1(t)$, and recalling that for any nonnegative random variable X with cumulative distribution $F(x) = P\{X \le x\}$, $E[X] = \int_0^\infty [1 - F(x)]dx$ where $E[\,]$ is the expectation operator (see Eqs. 18–19 below for more detailed explanation), I derive

$$\begin{aligned} q_1 = \lim_{t\to\infty} \lambda t y_1(t) &= \lambda(1-\alpha)\int_0^\infty [1 - F(x)]\,dx \\ &\quad + 2\lambda\alpha\int_0^\infty F(x)\,[1 - F(x)]\,dx \\ &= \lambda(1 - 3\alpha)E[X] \\ &\quad + 2\lambda\alpha\int_0^\infty [1 - F^2(x)]\,dx \\ &= \lambda(1 - 3\alpha)E[X] + 2\lambda\alpha\,E[2F(X)X] \\ &= \frac{m}{1+\alpha} - \frac{3\alpha m}{1+\alpha} + \frac{2\alpha m E[2F(X)X]}{(1+\alpha)E[X]} \\ &\quad \text{(by substituting } m = \lambda(1+\alpha)E[X]) \\ &= \frac{m}{1+\alpha}(1 - 3\alpha + 2\alpha\gamma) \\ &\quad \left(\text{by substituting } \gamma = \frac{E[2F(X)X]}{E[X]}\right). \end{aligned} \tag{14}$$

Similarly,

$$\begin{aligned} q_2 = \lim_{t\to\infty} \lambda t y_2(t) &= 2\lambda\alpha E[X] - \lambda\alpha E[2F(X)X] \\ &= \frac{\alpha m}{1+\alpha}(2 - \gamma). \end{aligned} \tag{15}$$

Combining Eqs. 13–15, I arrive at the final form for the PGF of the stationary process describing the number of Paneth cells in a crypt,

$$\phi(z) = \exp[q_1(z - 1)] \exp[q_2(z^2 - 1)]\,. \tag{16}$$

Observe that the first exponential term on the right-hand side of Eq. 16 is equivalent to the PGF of a Poisson process with rate q_1. Similarly, the second exponential term is equivalent to the PGF of a bulk Poisson process in which arrivals occur in groups of two cells with arrival rate q_2 (as may readily be proven from first principles). Since the product of two probability generating functions is equal to the probability generating function of the convolution of the original probability densities (another

standard result from probability theory), I may invert $\phi(z)$ by inspection to obtain n_k, the stationary probability density function for the number of Paneth cells in a crypt;

$$n_k = \sum_{r=0}^{\lfloor k/2 \rfloor} \left[\frac{q_2^r}{r!} e^{-q_2}\right]\left[\frac{q_1^{k-2r}}{(k-2r)!} e^{-q_1}\right]; \tag{17}$$

where $\lfloor k/2 \rfloor$ is the greatest integer in $k/2$.

Interpretation of the Model Parameters

In this section I will attempt to interpret those parameters whose meaning is not immediately obvious from the model. I will begin with the parameter $m = \lambda(1 + \alpha)E[X]$, which was introduced in Eq. 14. This parameter corresponds to the mean or expected number of Paneth cells in a crypt, $E[N]$. This may be demonstrated as follows (after noting that the mean or expected value of a random variable may be derived from the derivative of the PGF of the variable evaluated at $z = 1$),

$$\begin{aligned}
E[N] &= \frac{d\phi(z)}{dz}\bigg|_{z=1} \\
&= q_1\phi(z) + 2zq_2\phi(z)\bigg|_{z=1} \\
&= q_1 + 2q_2 \\
&= \frac{m}{1+\alpha}(1 - 3\alpha + 2\alpha\gamma) + 2\frac{\alpha m}{1+\alpha}(2 - \gamma) \\
&= \lambda E[X](1 - 3\alpha + 2\alpha\gamma) + 2\alpha\lambda E[X](2 - \gamma) \\
&= \lambda(1 + \alpha)E[X] \\
&= m
\end{aligned}$$

as stated.

The next parameter $q_2 = \alpha m(2 - \gamma)/(1 + \alpha)$ (introduced in Eq. 15), represents the expected number of Paneth cells present that are derived from arrivals consisting of two cells, both of which survive long enough to be scored. Similarly, $q_1 = m(1 - 3\alpha + 2\alpha\gamma)/(1 + \alpha)$ (from Eq. 14) is the expected number of Paneth cells present that are operationally single. By operationally single I mean that either these cells were derived from arrivals composed of single cells or from arrivals originally composed of two cells either one of which died before the crypt was scored.

It would also be of use at this point to interpret some of the intermediate terms that appear in Eqs. 14 and 15. In particular, I will interpret the terms $E[X]$, $2\{E[2F(X)X] - E[X]\}$, and $\{2E[X] - E[2F(X)X]\}$.

The meaning of $E[X]$ follows immediately from the definition of the expectation operator. $E[X]$ is the mean or expected Paneth cell lifetime.

The meaning of $2\{E[2F(X)X] - E[X]\}$ is more subtle. Notice that it may be possible for one member of a pair of Paneth cells (originating from an arrival composed of two cells) to die before the other. This would leave the surviving Paneth cell as the only contributing member to the total number of Paneth cells in a crypt. I will demonstrate that $2\{E[2F(X)X] - E[X]\}$ represents the mean or expected time spent as single cells (whose sibling has died) by Paneth cells originally derived from arrivals composed of two cells. First, let me determine the expected time that a specific member of an arrival (originally composed of two cells) spends as a single cell given that the other cell dies time a after arrival. Thus, I wish to compute $E[X - a|X > a]$. By definition of conditional expectation,

$$E[X - a|X > a] = \frac{\int_a^\infty (x - a)f(x)\,dx}{\int_a^\infty f(x)\,dx}$$

$$= \frac{\int_a^\infty xf(x)\,dx - a[1 - F(a)]}{1 - F(a)}$$

$$= \frac{E[X] - \int_0^a xf(x)\,dx - a[1 - F(a)]}{1 - F(a)}.$$

Then, by multiplying by the probability that one cell dies at age a while the other lives longer than a, integrating over all possible values of a, and multiplying by two to account for the possibility that either cell may die first, I have for arbitrary t,

$$\begin{aligned}
E[X - t|X > t] &= 2\int_0^\infty f(a)\,[1 - F(a)]E[X - a|X > a]\,da \\
&= 2\int_0^\infty f(a)E[X] - f(a)\int_0^a xf(x)\,dx \\
&\quad - af(a)[1 - F(a)]\,da \\
&= 2\int_0^\infty f(a)E[X]\,da \\
&\quad - 2\int_0^\infty f(a)\int_0^a xf(x)\,dx\,da \\
&\quad - 2\int_0^\infty af(a)[1 - F(a)]\,da \\
&= 2E[X] - 2\int_0^\infty xf(x)\int_x^\infty f(a)\,da\,dx \\
&\quad - 2\int_0^\infty af(a)[1 - F(a)]\,da \\
&= 2E[X] - 2\int_0^\infty xf(x)[1 - F(x)]\,dx \\
&\quad - 2\int_0^\infty xf(x)[1 - F(x)]\,dx \\
&= 2E[X] - 4E[X] + 2E[2F(X)X] \\
&= 2\{E[2F(X)X] - E[X]\}
\end{aligned}$$

as stated.

Similar arguments lead to the conclusion that $\{2E[X] - E[2F(X)X]\}$ is the expected time that a pair of newly arrived Paneth cells spends as a pair (i.e., the average time spent by a pair before one cell, the other, or both cells die).

The final uninterpreted parameter, which first appeared in Eq. 14, is $\gamma = E[2F(X)X]/E[X]$. In a sense, γ gives a measure of the relative spread of the lifetime distribution of Paneth cells. Before discussing this further, it will be useful to investigate the range of possible values for γ. By definition of expectation,

$$\begin{aligned}
E[X] &= \int_0^\infty xf(x)dx \\
&= \int_0^\infty [1 - F(x)]dx,
\end{aligned} \tag{18}$$

as may be demonstrated either by integration by parts or by substituting Eq. 5 into the last term and reversing the order of integration. Recalling that $dF(x)/dx = f(x)$, where $F(x)$ is a cumulative distribution and $f(x)$ is the corresponding probability density, and noticing that $F^2(x)$, like $F(x)$, is a distribution function (because it ranges in value between 0 and 1 and is nondecreasing), it may then be seen that

$$\begin{aligned}
E[2F(X)X] &= \int_0^\infty 2xf(x)F(x)dx \\
&= \int_0^\infty [1 - F^2(x)]dx.
\end{aligned} \tag{19}$$

Now we are in a position to find the limits on γ. Since the distribution function $F(x)$ is restricted to values in the range of 0 to 1, $0 \le F(x) \le 1$, it follows that $[1 - F^2(x)] \ge [1 - F(x)]$, which in turn implies that

$$E[2F(X)X] = \int_0^\infty [1 - F^2(x)]dx \ge \int_0^\infty [1 - F(x)]dx = E[X]$$

and hence that $\gamma = E[2F(X)X]/E[X] \ge 1$. An upper limit to γ is also readily found by noting that, by the mean value theorem for integrals,

$$E[2F(X)X] = \int_0^\infty 2xf(x)F(x)dx = 2F(c)\int_0^\infty xf(x)dx \le 2E[X],$$

where c is a constant in the interval $(0, \infty)$. Thus,

$$1 \le \gamma \le 2 . \tag{20}$$

Returning to the issue of variability of Paneth cell lifetimes as reflected in γ, if all Paneth cells had identical lifetimes, then $\gamma = 1$ (one way to see this is to observe that in this case, $f(x)$ could be interpreted as the Dirac delta function while $F(x)$ would be a step function with the step at $E[X]$). Values of $\gamma > 1$ would then represent populations of Paneth cells with increasing lifetime variability.

As a final note to the discussion of the model, the reader may have noticed that I have not explicitly incorporated into the model variation in time of Paneth cell maturation to the point that Paneth cells are recognizable as such. This could readily be explicitly incorporated. However, it is not necessary for the estimation of the parameter of interest, α. This is because any variability in the time to recognizable differentiation will convolve with the lifetime distribution of the cells and hence is already implicitly incorporated into the model. We need only reinterpret the lifetime functions defined above as recognizable lifetime functions.

Estimation of the Model Parameters

In this section I derive maximum likelihood estimators for the parameters m, γ, and α. The likelihood function for a model yields the probability (or likelihood) of obtaining a set of data given a specified model with specified parameters. It may then be argued that the most reasonable estimate for the value of the parameters in a given model will be those for which the likelihood function is maximized.

By definition, the likelihood function of the model is

$$L(f_0, f_1, f_2, \ldots, f_v | m, \gamma, \alpha) = \prod_{k=0}^{v} (n_k)^{f_k}, \tag{21}$$

where f_k represents the number of crypts observed containing $k = 0, 1, \ldots, v$ Paneth cells. To derive maximum likelihood estimates of the model parameters, one would differentiate Eq. 21 with respect to each parameter, set each resulting equation equal to 0 (the point at which the first derivative of a function is equal to 0 is an extrenum of the function), and then solve for the parameter. By these means it may be readily shown that the maximum likelihood estimator for the expected number of Paneth cells in a crypt, $m = \lambda(1 + \alpha)E[X]$ is

$$\hat{m} = \sum_{k=0}^{v} kf_k \Big/ \sum_{k=0}^{v} f_k . \tag{22}$$

with $\hat{m}$ established, estimates for α may be derived by maximizing Eq. 21 (for example, by using computer optimization routines). However, another useful estimator for α may be derived by noting (I am grateful to Dr. J. Templeton for this suggestion) that the probability of an even number of Paneth cells in a crypt, $P\{\text{even number}\} = \frac{1}{2}[\phi(1) + \phi(-1)]$, and hence that

$$P\{\text{even number}\} = \tfrac{1}{2} + \tfrac{1}{2}\exp(-2q_1) , \tag{23}$$

using Eq. 16. From this I may derive the likelihood of having M and R crypts containing an even and odd number of Paneth cells respectively:

$$\begin{aligned} L(M, R | \alpha, \gamma) &= \binom{M+R}{M} [P\{\text{even number}\}]^M \\ &\quad [P\{\text{odd number}\}]^R \\ &= \binom{M+R}{M} [\tfrac{1}{2} + \tfrac{1}{2}\exp(-2q_1)]^M \\ &\quad [\tfrac{1}{2} - \tfrac{1}{2}\exp(-2q_1)]^R . \end{aligned} \tag{24}$$

The advantage of Eq. 24 over Eq. 21 is that direct analytical estimates for α may be derived from Eq. 24 whereas Eq. 21 results in far more complex derivatives. To find the maximum of the likelihood $L(M, R|\alpha, \gamma)$, I took the partial derivative of $\log L(M, R|\alpha, \gamma)$ with respect to α (note that $L(M, R|\alpha, \gamma)$ and $\log L(M, R|\alpha, \gamma)$ will have the same maximum since $L(M, R|\alpha, \gamma)$ is positive), equated the result to zero and solved for α. Thus, I derived a maximum likelihood estimate for α, $\hat{\alpha}$,

$$\hat{\alpha} = \frac{2m + \log[(M - R)/(M + R)]}{6m - 4m\gamma - \log[(M - R)/(M + R)]} . \tag{25}$$

Inspection of Eq. 25 makes it clear that since $0 \le \alpha \le 1$, and since $1 \le \gamma \le 2$ (by Eq. 20),

$$1 \le \gamma \le 1 - \frac{\log[(M - R)/(M + R)]}{2m} \le 2, \; M > R . \tag{26}$$

Substituting Eq. 26 into Eq. 25, I derive the range for the maximum likelihood estimator of α,

$$\frac{2m + \log[(M - R)/(M + R)]}{2m - \log[(M - R)/(M + R)]} \le \hat{\alpha} \le 1 . \tag{27}$$

This is an important result as it indicates the limitations of the methods used; I will not be able to obtain a unique best estimate for α. The best I will be able to do is provide a lower limit to the amount of symmetry. This deserves further comment. The problem stems from the fact that the data provide no direct independent estimate of γ, which is a measure of the relative variability in Paneth cell lifetimes. The model is such that the effects of a decrease in symmetry may be countered by a decrease in the variability of Paneth cell lifetimes. This follows from the comments made after Eq. 16 where it was indicated that the total number of Paneth cells results from the convolution of the number of Paneth cells that are effectively single with the number of Paneth cells that are paired. It is the latter population of cells that is crucial as they provide the excess parity bias (excess over what would be expected from a simple Poisson distribution). A decrease in α would result in a smaller proportion of paired Paneth cells arriving and hence would decrease the parity bias. However, if at the same time γ was also decreased, the decreased variability in Paneth cell lifetimes would offset the effects of the change in α by resulting in a relative increase in the proportion of time that these paired cells remained paired and hence contributed to the parity bias. Thus the effects of a change in α may be countered, within limits, by a corresponding change in γ. In the extreme of identical Paneth cell lifetimes (i.e., if $\gamma = 1$) we would reach the lower limit of the estimate for α consistent with the data. However, as is indicated in the discussion, it is unlikely that Paneth cells have identical lifetimes and hence the lower limit to α, as determined here, is conservative.

Thus, I used Eqs. 22, 26, and 27 to derive maximum likelihood estimates of m, γ, and α, respectively. The goodness of the fit of the model to the data was determined by the chi-squared test.

RESULTS

The distribution of the number of Paneth cells per crypt is shown in Fig. 2. The results were: mean number of Paneth cells per crypt = $\hat{m} = 8.53$, number of crypts containing an even number of Paneth cells = $M = 106$, and number of crypts containing an odd number of Paneth cells = $R = 35$. Substituting these results into Eq. 26 yields $1 \leq \hat{\gamma} \leq 1.0402$. Substituting this range of values for $\hat{\gamma}$ into Eq. 25 (i.e., by Eq. 27) yields a maximum likelihood estimate for the range of α (the probability that a Paneth-cell-producing-stem-cell mitosis is symmetric) of $0.92 \leq \hat{\alpha} \leq 1.0$ (note that similar results were obtained by maximizing Eq. 21 with computer-based optimization routines). I also determined an estimate of an interval in which the true value of α would be found with 95% confidence given that $\gamma = 1$ and $m = 8.53$. This range was $0.88 \leq \hat{\alpha} \leq 0.95$. Hence, a more conservative estimate of the probable range for α is $0.88 \leq \hat{\alpha} \leq 1.0$. There was no significant difference between the fitted model and the data (Fig. 2 *a*; χ^2, $P > 0.1$). I also tested a model in which $\alpha = 0$ (i.e., all Paneth cell producing stem-cell mitoses are asymmetric). The fit is poor (Fig. 2 *b*) and may be rejected with high statistical confidence (χ^2, $P < 0.001$).

DISCUSSION

A clear parity bias was observed in counts of the number of Paneth cells per crypt (106 out of 141 crypts contained an even number of Paneth cells). When the model of Paneth-cell renewal developed in this paper was applied to these results, I found that the results were consistent with a range for α of $0.92 \leq \alpha \leq 1.0$ where α is the probability that any given stem-cell mitosis is symmetric (from Eq. 27). In other words, 92%–100% of all Paneth-cell-producing-stem-cell mitoses produced two Paneth cells.

Results from previous experiments make it likely that the range of α is narrower than these results indicate. Previous studies of Paneth-cell renewal have found that while the mean Paneth cell lifetime is ~3 wk, degenerating Paneth cells were observed that were no more than 16 d old (Bjerknes and Cheng, 1981*a*). This suggests that the Paneth-cell-lifetime distribution is relatively wide. The model developed here is sensitive to variability in Paneth cell lifetimes, but the data allow only an estimate of the range of variability through the parameter γ (γ is a parameter sensitive to the variability inherent in the Paneth-cell-lifetime distribution; as the relative spread of the lifetime distribution increases, so does γ). The range of values for γ consistent with the results was $1 \leq \gamma \leq 1.04$. The lower limit to γ, 1, would correspond to a situation in which all Paneth cells had identical lifetimes. The evidence cited above indicates that this is not the case. Thus, I would argue that γ must be >1 and that therefore $\alpha > 0.92$, and probably ~1. Hence, it is likely that most if not all Paneth-cell–producing stem-cell mitoses are symmetric.

There are, of course, plausible explanations for the

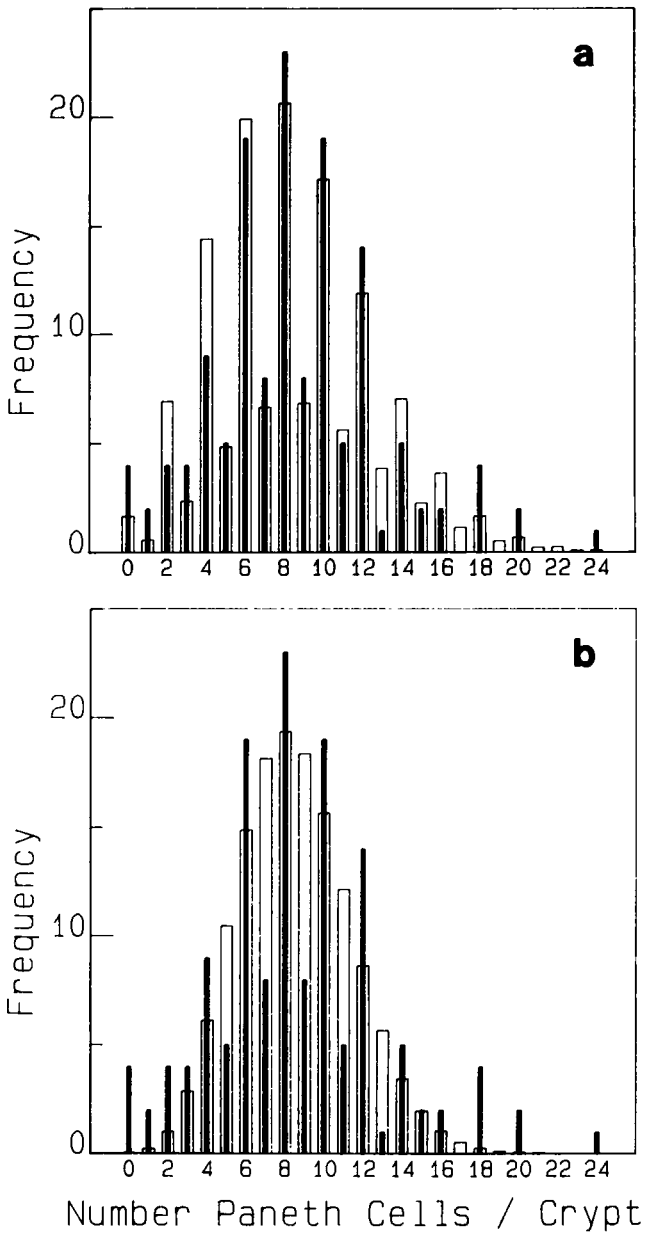

FIGURE 2 Observed (solid bars) and expected (open bars) distributions of the number of Paneth cells in mouse duodenal crypts. Note the high probability of crypts containing even numbers of Paneth cells (106/141 crypts). (*a*), the expected distribution is derived from a fit of the model of Paneth cell renewal described in the text. The values of the model parameters used in this fit were: $\alpha = 1.0$ (i.e., all Paneth-cell-producing stem-cell mitoses are symmetric and hence produce Paneth cells two at a time); $\gamma = 1.0402$; and $m = 8.53$. The fit was not significantly different from the data (χ^2, $P > 0.1$). (*b*), the fitted model had $\alpha = 0.0$ (i.e., all Paneth-cell-producing stem-cell mitoses are asymmetric and hence produce Paneth cells one at a time) and $m = 8.53$. With these parameters, the model is equivalent to a Poisson process with rate m. The fitted distribution was significantly different from the observed results (χ^2, $P < 0.001$).

observed parity bias in the number of Paneth cells per crypt other than symmetric stem-cell mitoses. The most likely alternative is the existence of an undifferentiated but committed Paneth-cell progenitor (e.g., a cell type intermediate between the stem and Paneth cells) that divides symmetrically. Another plausible alternative would be the existence of some mechanism forcing correlations between Paneth cell producing stem cells. To my knowledge, no evidence exists at present that would support either alternative. It would also be worthwhile to note that if the transition from the stem cell state to the differential state is not coincident with stem cell mitosis then all stem cell mitoses must be symmetric.

In conclusion, the results are consistent with a model of Paneth-cell renewal in which every Paneth-cell–producing stem-cell mitosis is symmetric. The implications of this conclusion for the issue of the symmetry of stem-cell mitoses in general are obvious but await further study.

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