A SIMPLE MODEL OF A STEADY STATE
DIFFERENTIATING CELL SYSTEM

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ABSTRACT
A simple theoretical model is hypothesized to describe the steady state behavior of a differentiating cell system as exemplified by blood cells. The cell system consists of several morphologically distinguishable cell classes which develop sequentially. Each cell class except the last one is mitotically capable. Mitosis is assumed to be either heteromorphogenic, homomorphogenic, or asymmetric. Some algebraic equations are derived which are conservation equations describing the flux of cells from one class to another. The theoretical considerations have been applied to some experimental observations in humans concerning neutrophil production, particularly in reference to relative cell numbers and mitotic fractions of the myeloblast, promyelocyte, and myelocyte cell classes. These observations are utilized to help determine the values of the parameters which characterize the model. Among these parameters are the generation times of the various cell classes, and the predicted values of the generation times are found to be in excellent agreement with observed grain-count halving times. However, the predicted mitotic times are in disagreement with their observed values.

INTRODUCTION
A certain amount of quantitative data has by now been accumulated regarding the kinetics and the life cycle of blood cells. Most of the information concerns the erythrocyte and the most common granulocyte, the neutrophil. Therefore, it has become feasible to study these data from a theoretical point of view, particularly the cell density function which depends on two variables, the time and a cell maturity measure (Rubinow, 1968). The possibility of using the cell density function to study population dynamics is not a new one (Scherbaum and Rasch, 1957; von Foerster, 1959), but the particular application to a differentiating cell system such as occurs in hemopoiesis does not appear to have been made as yet.

By a differentiating cell system we mean one that comprises many morphologically distinguishable cell classes. Another feature of such a system is that the cells in at least one of these distinguishable classes are capable of undergoing mitosis. Thirdly, when a cell of a given class divides, the daughter cells may be either like or unlike the parent cell. In the next section, we formulate a theoretical model of a differentiating cell system.

Variability from cell to cell of a given type and from one organism to another is of course axiomatic in biology and medicine. To utilize the concept of the cell density function presented herein presupposes a population of cells which are identical in their temporal development and maturation characteristics. In applying the model to human or mammalian cell systems, the model may be thought of as representing a population of identical "average" cells.

A number of simplifying assumptions are made which have the effect of making the mathematical
analysis quite trivial, although an attempt is made to preserve the essential biological features of the cell system. Thus, although all cells are homogeneous with regard to maturation, cells are assumed to be different in regard to the outcome of mitosis. Mitosis takes place more than once at different maturation levels in the course of a cell's development. In addition, the system is assumed to be in a steady state, and no cell deaths occur in the course of the maturation process.

It is shown how application of the boundary conditions at the beginning of each generation leads to a set of equations which are purely algebraic. The equations are expressed in terms of the relative fractions of cells representing different outcomes of mitosis, and certain cell density constants. These equations are conservation laws for the flux of cells from each generation to the succeeding one. The cell density constants may also be related to the relative frequencies of different cell classes and the generation and mitotic times associated with these classes. These relations are commonly utilized in the subject of cell kinetics, and it is hoped that their presentation in the context of an explicit model of a differentiating cell population will help explicate their use.

In the last section of the paper, these equations are applied to observed data concerning the neutrophil precursors found in human bone marrow aspirates. The data consist of the relative populations of dividing precursor classes and the fractions in mitosis (Killman et al., 1962). In order to make the mathematical results of the second section applicable to these data, some further simplifying assumptions must be made in order to reduce the number of unknown parameters so that the values of the parameters are determinable by the data. The principal assumption is that the mitotic times are equal in the mitotically capable cell classes (myeloblast, promyeloocyte, and myelocyte), although there is some evidence that mitotic time increases somewhat with maturation, at least in the case of erythropoietic cells (Rondanelli, Gorini, and Pecorari, 1959). It is then possible to derive the values of important parameters of the system such as relative fractions of cells undergoing different kinds of mitosis, and the generation times associated with the different cell classes. The latter are compared with other observations made of cells labeled with tritiated thymidine, namely grain-count halving times of proliferative cell classes (Cronkite et al., 1960).

These grain-count halving times are crude measures of generation times. The reasonable agreement of these observed quantities and their theoretically predicted values tends to support the general features of the model of the differentiating cell system. However, there remains a serious discrepancy between the observed and predicted mitotic times.

THE STEADY STATE
DIFFERENTIATING SYSTEM

We shall formulate a theoretical model of a differentiating cell system in the particular context of the production process of the neutrophil, a granulocytic white blood cell. It is generally considered that there are six morphological classes of precursor cells which develop sequentially in the bone marrow and which culminate in the development of a mature neutrophil. Of these classes, only the first three are proliferative classes with members which are capable of division. They are the following: 1. myeloblast, 2. promyelocyte, 3. myelocyte. The final three classes are known to be merely different maturation stages of a nonproliferative cell. They are called metamyelocyte (or juvenile), band, and segmented (or polymorphonuclear) forms, according to Diggs, Sturm, and Bell (1956). Normally, only the band and segmented forms leave the marrow to enter the blood. For the purpose of understanding the proliferative process, we may combine the final three classes into a single class which we shall call the “nonproliferating granulocyte” class.

There is some belief that a myeloblast originates from a “stem” cell, or ultimate precursor cell, but such a cell has not been positively identified. We shall allow for the possibility that there may be a steady flux of newborn myeloblasts originating from stem cells.

When a proliferative cell undergoes mitosis, its daughter cells may or may not be distinguishable from it. If a daughter cell is distinguishable from its mother, we shall assume that it belongs to the succeeding cell class. Consequently, there is no return flux of cells from one cell class to a class which precedes it. There are three possible types of mitosis that have been postulated (Osgood, 1957). In the language of the Brookhaven investigators (Killman et al., 1963) they are as follows: (a) heteromorphogenic mitosis, both daughter cells are alike and distinguishable from
The main reason for the utility of introducing the area under the curve of unit age is just the total population \( N(t) \) replaced by age, density per unit maturation interval. With \( s \) is because of this property that time \( t \) is given by the integral \( \int_{t_i}^{t} \). Cells between maturity levels \( t \). Therefore, the population of a cohort of cells in the maturation interval \( \mu \) to \( s + 1 \). The quantity \( n(\mu, s) \) represents the number of cells at a given maturity level \( \mu \) at time \( t \). Therefore, the population of a cohort of cells between maturity levels \( \mu_1 \) and \( \mu_2 \) at any time \( t \) is given by the integral \( \int_{\mu_1}^{\mu_2} n(\mu, t) \, d\mu \). It is because of this property that \( n \) is called the cell density per unit maturation interval. With \( \mu \) replaced by age, \( n \) is called the cell density per unit age. In particular, the total population \( N(t) \) is just the area under the curve of \( n(\mu, t) \) (Fig. 1).

The main reason for the utility of introducing \( n \) is that, given the initial state of a cell population and the manner of cell birth and death (described mathematically), the behavior of the cell population for all future times may be mathematically predicted, by solving the differential equation which governs \( n(\mu, t) \).

In introducing \( n(\mu, t) \) to describe neutrophil production, we are tacitly assuming that neutrophils are a single population of cells which are homogeneous with respect to the maturation process, but for which mitosis may occur more than once at different maturation levels. Furthermore, there are different outcomes of cell division among the cells, whenever it takes place. The cell classes will be considered as defined by successive maturation intervals of this single population. We assume further that the neutrophil production system is in a steady state, so that \( n \) is time-independent and is a function of \( \mu \) alone, \( n(\mu) \). Let \( \mu \) be measured in units of time so that it is identical with age of the cell measured from the moment when myeloblast birth takes place. We designate \( \mu = T_1 \) as the age at which the first cell division takes place. Thus, \( T_1 \) is the generation time of the first generation. Let \( T_2 \) and \( T_3 \) be the generation times of succeeding generations which occur at larger maturation levels, i.e., the next maturation levels at which mitosis take place are \( T_1 + T_2 \) and \( T_1 + T_2 + T_3 \). Finally, let us assume that no cell death takes place during the maturation process. It follows directly from the differential equation governing \( n(\mu) \) that \( n(\mu) \) is a constant within the maturation interval assigned to a given generation. In addition, there is a discontinuity in \( n \) at every maturation level at which cell division occurs. Therefore \( n(\mu) \) has the form shown in Fig. 2. In the figure, we have indicated a hypo-
Theoretical (morphological) distinction between a cell class defined by the maturity interval labeled 5, and a less mature class defined by the maturity interval labeled 4, even though no cell division occurs when cells mature from class 4 to class 5.

We shall tentatively identify the generation whose generation time is $T_1$ as being identical with the morphologically identifiable cell class called the myeloblast. This assumption is not essential to our analysis but is motivated, together with our other assumptions, by the desire to postulate the simplest possible theoretical scheme consistent with the known observations.

In fact, it is not known whether any given cell class comprises one or more generations or whether the class begins or terminates at a junction of two generations. Let the subscript 1 generally denote the myeloblast class. Similarly, we assume that the subscript 2 denotes the promyelocyte class, subscript 3 denotes the myelocyte class, and subscript 4 denotes the nonproliferating granulocyte class.

It is important to bear in mind that in this representation scheme a cell may actually undergo several divisions before it changes its identity from one type to a succeeding type. As long as the time for such successive divisions within a given cell class is the same and the maturation levels of each generation belonging to the given cell class are indistinguishable from one another, the cell class may be validly represented as comprising only a single generation. This representation has the effect of "telescoping" or superposing all the generations comprising a single cell class onto a single generation because it identifies cells only by their state of maturation. This assumption is not inconsistent with the experiments of Boll and Kuhn (1965) who observed the evolution of a promyelocyte in vitro through three successive myelocyte generations into some metamyelocytes. The myelocytic generation cycles observed were approximately equal and thus support the concept of equal generation cycles within a given cell class.

For a cell class which is capable of mitosis because of the uncertainty as to the result of mitosis, we shall permit all possible proposed types of mitosis. Thus, let $q$ be the fraction of cells of mitotic age which undergo heteromorphogenic mitosis, $r$ be the fraction of cells of mitotic age which undergo homomorphogenic mitosis, and $p$ be the fraction of cells of mitotic age which undergo asymmetric mitosis. A moment's reflection shows that if two cells undergo asymmetric mitosis, then the result is indistinguishable from that of two cells undergoing mitosis, one of which is heteromorphogenic and the other homomorphogenic. Consequently, it is not necessary to utilize the parameter $p$, even though actual cell divisions may occur which are of asymmetric type. Mathematically, if $p$ were used in the boundary conditions, it could be made to disappear by means of the transformation $r' = r + \frac{p}{2}$ and $q' = q + \frac{p}{2}$.

For the sake of greater generality in the class of models we propose to investigate, we shall introduce a fourth possibility, namely that a cell which belongs to a mitotically capable cell class does not undergo mitosis when it becomes of mitotic age. Such a possibility is consistent, again, with the above mentioned observations of Boll and Kuhn. They observed that a daughter cell of a myelocyte underwent mitosis, but that its sister cell did not. Furthermore, the resting time for the nondividing cell was the same duration as the generation time of the dividing cell: the daughter cells became metamyelocytes at the same time.
time. We shall let $t$ be the fraction of cells of mitotic age which do not undergo mitosis. These $t$ type or resting cells do not necessarily have to have the same life-time as the generation time of a dividing cell, although we assume here that they do, on the basis of the fragmentary information we have just quoted.

At the boundary between two cell classes, the rate at which cells are being born at age zero in a given class equals the flux of cells from the preceding cell class due to aging or heteromorphogenic mitosis, plus the flux of cells due to homomorphogenic division in the given class. In the more general case of a steady state population in which cell death is occurring, the rate at which cells are born $n(o)$ equals the newborn cell flux from the exterior designated by $s_0$, plus the newborn cell flux from cells of age $T$ undergoing division, $2n(T)$ (see Fig. 1). If only a fraction $r$ of the cells of age $T$ are undergoing homomorphogenic mitosis, the quantity $2n(T)$ is replaced by $2rn(T)$. Thus, $n(o)$ and $n(T)$ would be related by the following expression,

$$n(o) = s_0 + 2rn(T).$$

However, if the cell population is not subject to cell death or disappearance as we have assumed for neutrophil production, then $n(\mu)$ is a constant and the additional condition applies that $n(o) = n(T)$. Thus, for the myeloblast class of our model, with the constant value of $n$ designated by $n_1$, equation (1) becomes

$$n_1 = s_0 + 2rn_1. \quad (2)$$

Here $s_0$ is the cell flux per unit time from the stem cells, and $2rn_1$ represents the birth rate of cells of age zero as a result of homomorphogenic mitosis of myeloblast cells at time $T_1$. Because $n(\mu)$ is discontinuous at $T_1$ we signify, by placing $+$ or $-$ after $T_1$, whether we are referring to the value of $\mu$ just after $(+)$ or just before $(-) T_1$.

The number of new promyelocytes per unit time $n_2$ which appear at age $T_1$ is given by the expression

$$n_2 = (2q_1 + t_1)n_1 + 2s_1n_1. \quad (3)$$

Here $2q_1n_1$ is the cell flux per unit time due to heteromorphogenic mitosis of myeloblasts, and $t_1n_1$ is the cell flux per unit time due to aging of resting myeloblasts. These fluxes are the contributions from cells of age $T_1$. The last term in equation (3) represents the birth rate of promyelocytes resulting from homomorphogenic mitosis of promyelocyte cells at age $T_1 + T_2$.

Similarly,

$$n_3 = (2q_2 + t_2)n_2 + 2s_2n_2, \quad (4)$$

and

$$n_4 = (2q_3 + t_3)n_3, \quad (5)$$

where $n_4$ represents the first nonproliferative cell class. If, as for erythropoiesis $n_4$ represented a proliferative class, then an equation similar to (4) would hold for it. By definition, the fraction $q_i, r_i,$ and $t_i$ must be positive and add up to unity:

$$q_i + r_i + t_i = 1, \quad i = 1, 2, 3. \quad (6)$$

Equations (2–6) constitute 7 equations involving 14 unknown parameters, so that 7 additional relations are needed in order to be able to determine all the parameters. Even if $s_0$ and $n_1$ are observed, however, these equations are not sufficient to uniquely determine the $q_i, r_i,$ and $t_i$. Nevertheless, the fact that a solution is required to exist for which these parameters are positive numbers whose magnitude is not greater than unity, may in itself be very informative. In fact, the $r_i$ must satisfy the inequality $r_i \leq \frac{1}{2}$, as otherwise no solution of equations (2–6) is possible: $r_i > \frac{1}{2}$ implies that the steady state hypothesis is violated and that the cell class is in an exponential growth phase. For example, equation (2) may be rewritten as $r_i = \frac{1}{2} - \frac{1}{2} s_0/n_1$, and since $s_0$ and $n_1$ cannot be negative numbers, $r_i \leq \frac{1}{2}$.

In practice, these equations may be combined with observation of the total number of cells of each class that are observed in bone marrow aspirates. In view of the assumption that the boundaries separating the cell classes are the same as the boundaries separating the generations,

$$N_i = n_iT_i, \quad i = 1, 2, 3, \quad (7)$$

where $N_i$ is the total population of the $i^{th}$ cell class, and $T_i$ for $i = 1, 2, 3$ is the mean generation time of the $i^{th}$ cell class. By $T_4$ is meant the mean life-time or transit time of nonproliferating granulocytes. It is a trivial matter to generalize these
relations so that a given cell class is made up of fractional parts of generation cycles, but, in the absence of any experimental information regarding this matter, it is simplest to utilize equations (7). These relations do not supply any new information regarding \( n_i \) unless the\( T_i \) can be determined by some independent observations. If the mitotic periods \( T_{M_i} \) are known, they can be combined with observation of the fractions of cells in mitosis \( f_{M_i} \) to yield three additional relations,

\[
f_{M_i} = \frac{n_i(1-t_i)T_{M_i}}{N_i}, \quad i = 1, 2, 3. \tag{8}
\]

Alternatively, equations (8) may be utilized to determine \( T_{M_i} \) if the other parameters are known.

If the fraction of cells of the \( i \)-th class in DNA synthesis \( f_{L_i} \) is observed in a pulse labeling experiment, then

\[
f_{L_i} = \frac{n_i(1-t_i)T_{S_i}}{N_i}, \quad i = 1, 2, 3. \tag{9}
\]

where \( T_{S_i} \) is the DNA synthesis period in the \( i \)-th cell class. It follows that the values of \( T_{S_i} \) are required if these relations are to help determine \( n_i \). Note that equations (8) and (9) may also aid in the determination of the relative numbers of \( t \) type cells.

An alternative form of equations (2–5) which is instructive and more convenient for some purposes may be obtained if we combine these equations with (6) to eliminate \( q_i \). Thus,

\[
n_i (1 - 2r_i) = s_0, \tag{10}
\]

\[
n_i (1 - 2r_i) = s_0 + n_i (1 - t_i), \tag{11}
\]

\[
n_i (1 - 2r_i) = s_0 + n_i (1 - t_i) + n_i (1 - t_2), \tag{12}
\]

\[
n_i = s_0 + n_i (1 - t_i) + n_i (1 - t_2) + n_i (1 - t_3). \tag{13}
\]

These relations are in the nature of conservation equations for the flux of cells from one class to another. It is obvious how these equations may be extended to any number of proliferative classes. Note that \( n_i (1 - t_i) \) represents the “birth rate” of cells in a given class due to mitosis. On the other hand, \( n_i \) represents the “production rate” of cells in a given class, that is, birth rate as a result of mitosis within the class and influx from the preceding class. These rates are equal only if there are no resting cells. Equation (13) states that the production rate of cells in the first non-proliferative class equals the net birth rate of all the preceding proliferative classes. This relation has been recognized previously (Killman et al., 1963) and used to test whether or not there is “ineffective” poiesis. Similarly, very general considerations of fluxes between proliferating compartments have been presented previously (Cronkite et al., 1965). Here, the introduction of the description of the cells in terms of maturity level via the cell density function, as well as the details of the different mitotic schemes, serves to place more stringent requirements on the behavior of the proliferating system. It follows from these relations that if there are no resting cells \( (t_i = 0) \), then

\[
n_{i+1} \geq n_i, \quad i = 1, 2, 3. \tag{14}
\]

Fig. 2 illustrates schematically the appearance of \( n \) for this case.

**THE NEUTROPHIL SERIES IN HUMANS**

Killman et al. (1962) observed the averages of differential counts of 500 granulocytic marrow cells in each of 6 humans. The results of their observations are presented in Table I.

We have applied the results of the second section to the above data, with its concomitant assumptions regarding the underlying processes taking place. Specifically, we utilize equations (6–8) and (10–13). In order for these equations

### Table I

**Averages of Differential Counts of 500 Bone Marrow Granulocytes in Each of Six Humans, Observed by Killman et al. (1962)**

<table>
<thead>
<tr>
<th>Class</th>
<th>( i )</th>
<th>( N_i )</th>
<th>( f_{M_i} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myeloblast</td>
<td>1</td>
<td>1</td>
<td>0.0240</td>
</tr>
<tr>
<td>Promyelocyte</td>
<td>2</td>
<td>3.37</td>
<td>0.0146</td>
</tr>
<tr>
<td>Myelocyte</td>
<td>3</td>
<td>16.30</td>
<td>0.0109</td>
</tr>
<tr>
<td>Nonproliferating</td>
<td>4</td>
<td>36.14</td>
<td></td>
</tr>
</tbody>
</table>

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TABLE II

Derived values of $n_i$, $q_i$, $r_i$, $T_{Mi}$, and $T_i$

The last column gives experimental values of the grain-count halving time (Cronkite et al., 1960), which may be very approximately equated with the generation time.

<table>
<thead>
<tr>
<th>Class</th>
<th>$i$</th>
<th>$t_i$</th>
<th>$n_i/n_1$</th>
<th>$q_i$</th>
<th>$r_i$</th>
<th>$T_{Mi}$ (hr)</th>
<th>$T_i$ (hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myeloblast</td>
<td>1</td>
<td>0.0400/hr</td>
<td>1</td>
<td>0.500</td>
<td>0.500</td>
<td>0.623</td>
<td>25.0</td>
</tr>
<tr>
<td>Promyelocyte</td>
<td>2</td>
<td>0.0800</td>
<td>2.00</td>
<td>0.750</td>
<td>0.250</td>
<td>0.623</td>
<td>42.1</td>
</tr>
<tr>
<td>Myelocyte</td>
<td>3</td>
<td>0.288</td>
<td>7.12</td>
<td>0.710</td>
<td>0.290</td>
<td>0.623</td>
<td>56.6</td>
</tr>
<tr>
<td>Nonproliferating granulocyte</td>
<td>4</td>
<td>0.406</td>
<td>10.1</td>
<td></td>
<td></td>
<td>89*</td>
<td></td>
</tr>
</tbody>
</table>

* The value $T_i = 89$ hr for nonproliferating marrow granulocytes was assumed, taken as equal to the observed mean transit time of nondividing neutrophil precursors (Cronkite et al., 1961, 1960).

TABLE III

Idealized integral values of $n_i/n_1$ as inferred from Table II

The values of $q_i$ and $r_i$ are a consequence of these values and equations (10-13) with $s_i$ and $t_i$ equal to zero, $i = 1, 2, 3$.

<table>
<thead>
<tr>
<th>Class</th>
<th>$i$</th>
<th>$n_i/n_1$</th>
<th>$q_i$</th>
<th>$r_i$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myeloblast</td>
<td>1</td>
<td>1</td>
<td>½</td>
<td>½</td>
</tr>
<tr>
<td>Promyelocyte</td>
<td>2</td>
<td>2</td>
<td>¾</td>
<td>¼</td>
</tr>
<tr>
<td>Myelocyte</td>
<td>3</td>
<td>7</td>
<td>¾</td>
<td>¾</td>
</tr>
<tr>
<td>Nonproliferating granulocyte</td>
<td>4</td>
<td>10</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

In addition, in a separate observation of one human, Cronkite et al. (1960) measured the grain-count halving time of each of the mitotically capable neutrophil precursors. These measurements can be approximately equated with generation times, if we ignore in particular the error introduced by labeled cell influx into one class from the preceding class, and the error caused by daughter cells of lightly labeled mothers falling below the threshold of observation of labeled cells, and consequently being counted as unlabeled cells. These observations are listed in the last column of Table II. The agreement between these measurements and the derived values of $T_i$ must be considered surprisingly good, in view of the many simplifying assumptions that have been made in the derivation.
The calculated mitotic time of 0.623 hr does not agree with the observed value of 1.47 ± 0.33 hr for the average duration of 93 myelocytic and promyelocytic mitoses in vitro (Boll and Kuhn, 1965). The discrepancy between the predicted and observed mitotic times represents a very serious disagreement between the model and experience. Additional experiments and information about mitotic times and other kinetic parameters of the neutrophil production process are needed to clarify this matter and permit the testing of theoretical models which are less simplistic than the one presented here.

The values of the ratios \( n_i/n_1 \) listed in Table II suggest, perhaps fortuitously, that they are precisely integers. Such values could be suggestive of a simple mitotic scheme, although it is premature to speculate on this matter. If the ratios do assume the suggested integral values, then the \( q \) and \( r \) values are rational fractions as shown in Table III. Based on these numbers, a possible proliferation scheme is shown in Fig. 3 as if asymmetric mitosis actually takes place some of the time. We reiterate that these data can not determine whether asymmetric mitosis actually does take place, or whether the same results are obtained by a combination of homomorphogenic and heteromorphogenic mitosis.

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