INTERNATIONAL

Review of Cytology

EDITED BY

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ASSISTANT EDITOR
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VOLUME 70

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ACADEMIC PRESS *A Subsidiary of Harcourt Brace Jovanovich, Publishers*
New York London Toronto Sydney San Francisco

ACADEMIC PRESS, INC.
111 Fifth Avenue, New York, New York 10003

United Kingdom Edition published by
ACADEMIC PRESS, INC (LONDON) LTD.
24/28 Oval Road, London NW1 7DX

LIBRARY OF CONGRESS CATALOG CARD NUMBER: 52–5203

ISBN 0–12–364470–4

PRINTED IN THE UNITED STATES OF AMERICA

81 82 83 84 9 8 7 6 5 4 3 2 1

Contents

Morphological and Biochemical Aspects of Adhesiveness and Dissociation of Cancer Cells

HIDEO HAYASHI AND YASUJI ISHIMARU

The Cells of the Gastric Mucosa

HERBERT F. HELANDER

Ultrastructure and Biology of Female Gametophyte in Flowering Plants

R. N. KAPIL AND A. K. BHATNAGAR

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INTERNATIONAL REVIEW OF CYTOLOGY, VOL. 70

Cycling ⇄ Noncycling Cell Transitions in Tissue Aging, Immunological Surveillance, Transformation, and Tumor Growth

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I. Introduction

In a previous report (Gelfant, 1977), we presented a model for cell and tissue proliferation based upon the idea that cycling cells can arrest at three different

ISBN 0-12-364470-4

points in the cell cycle: in early G_1 (blocked by a G_0 barrier); in late G_1 (by a G_1 block); and in late G_2 (by a G_2 block). The model describes four major categories of cells: cycling cells, noncycling G_0-blocked cells, noncycling G_1-blocked cells, and noncycling G_2-blocked cells. These represent the potential proliferating pool in cells in culture and in tissues and tumors *in vivo*. The particular proliferative needs of a tissue or tumor are brought about by specific noncycling⇄cycling cell transitions.

The present article extends the details and the significance of this model and uses it to explain and to interrelate the problems of tissue aging, immunological surveillance, transformation, and tumor growth.

II. Background: Cycling and Noncycling Cells

A. Explanation of Cycling and Noncycling Cells

The scheme presented in Fig. 1 is based upon the idea of three inherent arrest points in the cell cycle: a G_1-block located at the G_1/S border; a G_2-block located

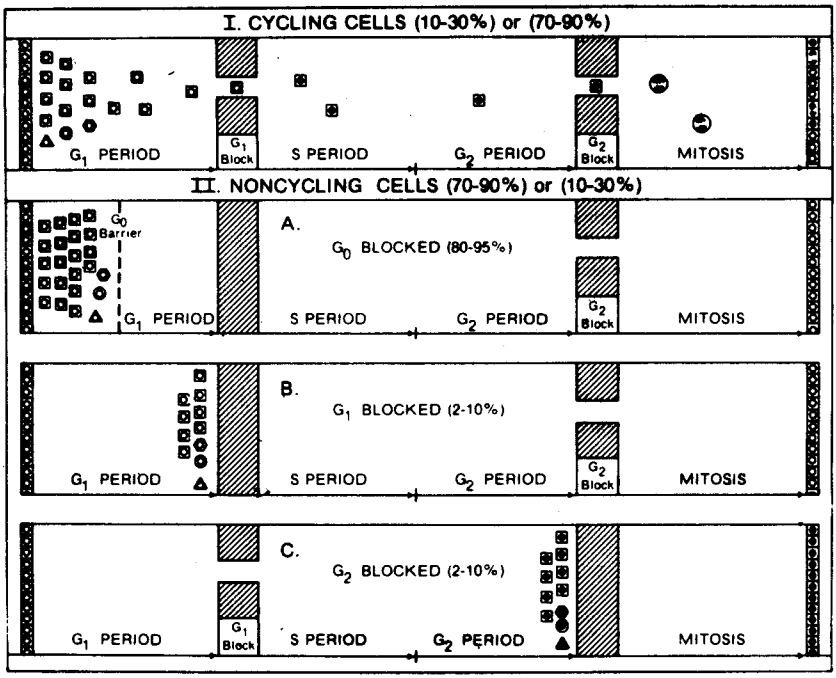


FIG. 1. Tissue and tumor proliferative ecosystem (modified after Gelfant, 1977).

at the G_2/M transition point; and a G_0 barrier (dashed line) arrest mechanism located early in the G_1 period of the cell cycle. In relation to these inherent cell cycle arrest points, there are four categories of cycling and noncycling cells; cells of the same type existing within a tissue or a tumor as a complex heterogeneous proliferative ecosystem. The main import of this concept is that it redefines the histological definition of a tissue, and it emphasizes the inherent cellular heterogeneity of individual tissues and individual tumors (i.e., that tissue and tumor cells of the same type are subdivided into separate and distinct categories in terms of their cell cycle proliferative states).

1. *Four Major Categories*

In the first category, cycling cells are actively proliferating; they are asychronously moving through the cell cycle, $G_1 \rightarrow S \rightarrow G_2 \rightarrow M$; where G_1 and G_2 are pre- and post-DNA synthesis gap periods, S is the period of nuclear DNA synthesis in interphase; and M is the period of motosis—which produces two daughter cells. The G_1 and G_2 blocks in these cells are depicted as being partially open—implying various physiological states of expansion or retraction. (Also, there is no G_0 barrier arrest mechanism in cycling cells.) Squares with unshaded circles represent cells in the G_1 period of interphase with nuclear DNA contents of 2C; the shaded circles indicate synthesis of DNA. During the S period, cells have intermediate nuclear DNA contents between 2C and 4C; cells in G_2 have 4C DNA contents. Cells in mitosis are depicted in anaphase configurations. Cycling cells can be identified in S or in M by cytochemical–autoradiographic–microscopic techniques. See column of cells on right representing cycling cells as they would appear within a tissue, for example, within a single layer of basal epidermis.

The potential proliferating pool in a tissue or a tumor is composed of three categories of noncycling cells arrested at different points in relation to the G_1 and the G_2 cell cycle blocks and the G_0 barrier.

Noncycling G_0-blocked cells are arrested early in G_1 by a G_0 barrier. These cells have 2C nuclear DNA contents, and they are located at a distance in time from the S period. For conceptual uniformity, the G_1 block is depicted as being closed for G_0-blocked cells (also, opening of the G_0 barrier implies concomitant opening of the G_1 block).

The second noncycling category, G_1-blocked cells, arrest late in the G_1 period and are located at the G_1/S border (nuclear DNA contents, 2C).

The third noncycling category, G_2-blocked cells, arrest late in the G_2 period and are located at the G_2/M border (nuclear DNA contents, 4C).

In general, noncycling G_0-blocked cells have been demonstrated by a variety of cell kinetic growth fraction techniques in a wide variety of tissues and tumors (Gelfant, 1977). Since noncycling cells are not moving through S or through M, since they cannot be distinguished from cycling cells in the G_1 or in the G_2

periods on the basis of nuclear DNA contents, and since noncycling G_0- and G_1-blocked cells also cannot be distinguished from one another on the basis of nuclear DNA contents (see columns of cells on the right, depicted as squares with shaded and unshaded circles in the diagrams in Fig. 1), special cell kinetic growth fraction techniques and specific procedures and experimental designs must be used to identify and distinguish all four categories of cycling and noncycling cells as they exist within the same tissue or tumor (see Section III).

Figure 1 provides an estimate of the relative proportions and fluctuations of the four categories of cycling and noncycling cells. Under normal circumstances only about 10–30% of the cells in a tissue or a tumor are in the cycling state. Most of a tissue or tumor proliferative pool resides in the noncycling state (70–90%). And of the three categories of noncycling cells, most (80–95%) reside in the G_0-blocked state; tissues and tumors also contain small proportions of noncycling G_1- and G_2-blocked cells (2–10%).

2. *Subpopulations*

The concept in Fig. 1 also implies that there are additional subpopulations of cells within the major categories—which are qualitatively different from each other in the sense that they may be selectively and independently activated; or in the sense that they may be in different temporal states. Some examples of specific and selective activation of subpopulations of noncycling G_2-blocked cells come from studies of mouse ear epidermis *in vitro* in which there are separate sugar, sodium, and potassium ion-responding subpopulations (Gelfant, 1966); also, noncycling G_2-blocked Ehrlich ascites tumor cells can be specifically activated to enter mitosis by antilymphocytic serum (DeCosse and Gelfant, 1968). And both noncycling G_1- and G_2-blocked mouse liver cells can be specifically activated to enter S or to enter M by injection of lead acetate *in vivo* (Choie and Richter, 1978). Figure 1 also depicts subpopulations of G_0-blocked cells which are in different temporal states of arrest—and when released by different stimuli, they enter S after variable G_0 delay periods; and also shown are subpopulations of cycling cells representing cells moving through the cell cycle at much slower or faster speeds. All of these subpopulations are depicted as *triangles, circles,* and *hexagonal* cells in each of the major categories in Fig. 1. With regard to tumors, we speculate that the system of subpopulations of noncycling G_1- and G_2-blocked tumor cells may have specific metastatic capabilities.

B. Tissues and Tumors as Proliferative Ecosystems

An ecosystem is defined as a system formed by the interaction of a community of organisms with their environment—which confers adaptive value to the system. By analogy and as speculation, Fig. 1 introduces the concept of tissues and tumors as proliferative ecosystems. It is proposed that tissues and tumors main-

tain an adaptive system of cell proliferation—with the use of the four major categories of cycling cells, noncycling G_0-, G_1-, and G_2-blocked cells and their subpopulations to service the actual and the potential proliferative needs of the tissue or tumor. Because of their arrest points in the cell cycle (at the G_1/S and at the G_2/M borders), noncycling G_1- and G_2-blocked cells provide tissues with a fast-acting renewal capacity, for when released by appropriate stimuli, these cells enter the cycling S and M periods without delay—in comparison to the slower acting delayed reentry of released G_0-blocked cells. The fact that noncycling cells can arrest at different temporal and biochemical points in the G_1 and in the G_2 gap periods of the cell cycle [recent evidence indicates that neoplastic cells can also arrest in the S period (Darzynkiewicz *et al.*, 1979)], and the fact that most cells reside in the noncycling state offers the tactical advantage of quiescence (at different points in interphase) over the turmoil involved in the continuous synthesis of the genetic and the mitotic machinery necessary for chromosome replication, chromosome movement, and cytoplasmic cleavage (i.e., the cycling state). In terms of tumor survival and resistance to therapy, the quiescent state provides an additional advantage because most chemotherapy acts only on cells in the cycling state (specifically on cells in S and in M). Physiological subpopulations within the major categories of noncycling cells would provide an additional adaptive dimension to the proliferative ecosystem of the tissue or the tumor. Such cells capable of being released to the cycling state only by very specific or unusual stimuli serve as another restrictive system to secure proliferative quiescence. For further support of our concept of tissues and tumors as proliferative ecosystems and for the adaptive significance of noncycling cells as described above, see publications entitled, "Mechanisms Underlying the Differential Sensitivity of Proliferating and Resting Cells to External Factors" (Epifanova, 1977), "The Survival Value of the Dormant State in Neoplastic and Normal Cell Populations" (Clarkson, 1974), and "The Biological Essence of Resting Cells in Cell Populations" (Lerman, 1978).

III. Procedures for Demonstrating the Existence of Noncycling G_0-, G_1-, and G_2-Blocked Cells in the Same Tissue

A. Monitor Cells Entering M and S at Hourly Intervals after Stimulating Quiescent Tissues

As depicted in Fig. 2, if one stimulates a quiescent or experimentally suppressed tissue and monitors cells entering M and S in autoradiographs at hourly intervals, one observes a prompt and transient increase in the number of mitoses, representing release of G_2-blocked cells into M; there is also a prompt and transient increase in the number of labeled nuclei within the first few hours,

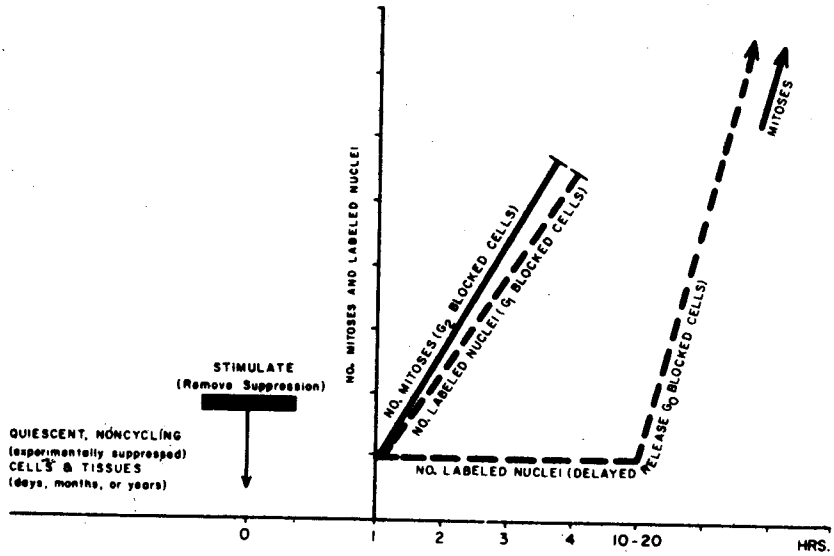


FIG. 2. Procedure for demonstrating the existence of noncycling G_0-, G_1-, and G_2-blocked cells in the same tissue (after Gelfant, 1977).

representing release of G_1-blocked cells into S. Then after a delay of about 10 to 20 hours, one observes a second, much larger increase in the number of labeled nuclei, representing G_0-blocked cells entering S after a delay; this is followed by a comparable and subsequent increase in the number of mitoses as depicted in Fig. 2. It should be emphasized that there is very little or no DNA labeling or mitotic activity in quiescent noncycling cells and tissues. Also, cells may remain in the noncycling state for months or years.

Examples of quiescent, noncycling (experimentally suppressed) cells and tissues are adult liver, kidney, salivary glands, hormone-depleted or nutritionally starved tissues, *in vivo;* density or media depleted stationary cell cultures, *in vitro*. Quiescent tissues and cell cultures can be stimulated by regenerative stimulation such as partial hepatectomy, partial nephrectomy; wounding; hormone resupply; refeeding, *in vivo;* or by replating or media change of cell cultures, *in vitro*.

In a previous report (Gelfant, 1977), we presented three tables of examples of noncycling G_0-, G_1-, and G_2-blocked cells. Noncycling G_0-blocked cells have been demonstrated in all tissues and tumors and cell culture systems both *in vivo* and *in vitro*. Noncycling G_2-blocked cells have been found in a wide variety of animal, plant, and tumor tissues both *in vivo* and *in vitro*. The number of examples of noncycling G_1-blocked cells is small because most workers do not ordinarily monitor DNA synthesis immediately after stimulation; also, the increase in the number of cells entering S from the G_1-blocked state is much less

and relatively transient when compared to the subsequent delayed increase in G_0-blocked cells entering DNA synthesis (as shown in Fig. 2). Nevertheless, there are reports demonstrating the existence of noncycling G_1-blocked cells in tissues such as epidermis, tongue, kidney epithelium, liver, mammary gland, capillary endothelial cells, hemopoietic cells, ascites tumor cells, *in vivo;* and hemopoietic cells, *in vitro*.

B. Combined Cytophotometric-Autoradiographic and Unlabeled Mitoses Procedures

The following is an outline of another general procedure for demonstrating and distinguishing all four categories of cycling and noncycling cells within the same tissue *in vivo* or *in vitro* (after Gelfant, 1966).

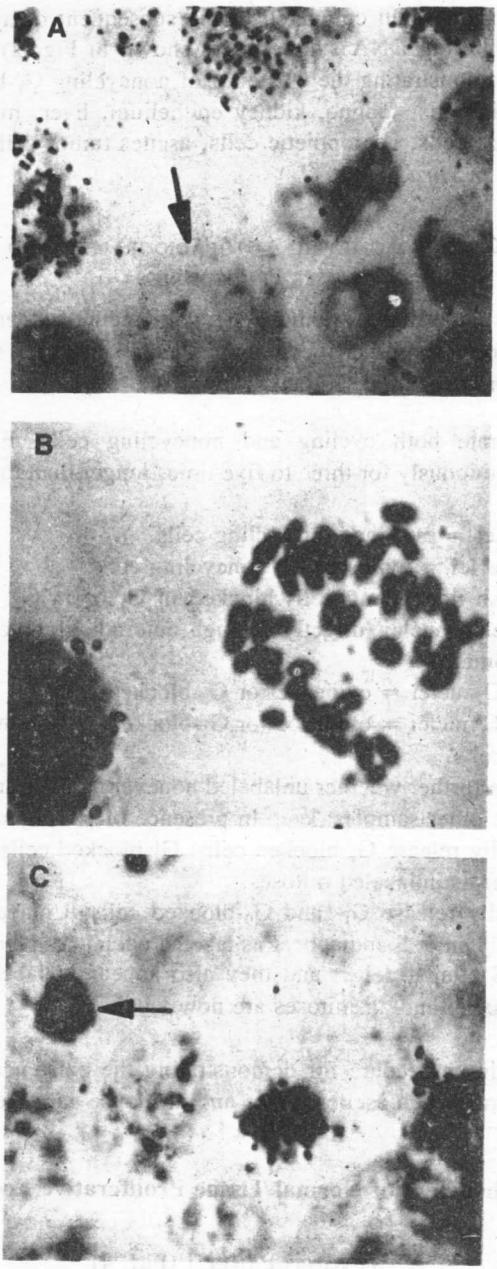
1. To demonstrate both cycling and noncycling cells: Expose cells to [^{3}H]thymidine continuously for three to five times longer than the particular cell generation time.
   a. Labeled nuclei = evidence for cycling cells.
   b. Unlabeled nuclei = evidence for noncycling cells.
2. To distinguish noncycling cells blocked in G_1 or in G_2: Measure DNA contents of unlabeled nuclei (directly through autoradiographic emulsion with Feulgen cytophotometry).
   a. Unlabeled 4C nuclei = evidence for G_2-blocked cells.
   b. Unlabeled 2C nuclei = evidence for G_1-blocked cells and/or evidence for G_0-blocked cells.
3. To determine further whether unlabeled noncycling cells are G_1, G_0, or G_2 blocked: Stimulate other samples; keep in presence of [^{3}H]thymidine.
   a. Experimentally release G_2-blocked cells: G_2-blocked cells promptly enter M and appear as unlabeled mitoses.
   b. Experimentally release G_1- and G_0-blocked cells: Unlabeled G_1-blocked cells promptly enter S and appear as labeled nuclei. Unlabeled G_0-blocked cells enter S after a delay; and they also appear as labeled nuclei. (All interphase nuclei and all mitoses are now labeled.)

Figure 3 uses this procedure for demonstrating the existence of noncycling G_2-blocked cells in Ehrlich ascites tumor and in mouse ear epidermis *in vivo*.

IV. Establishment of Normal Tissue Proliferative Ecosystems

A. Synopsis Panel I (Fig. 4)

Panel I (Fig. 4) depicts the origin and the cell cycle point of arrest (in relation to the G_1 and G_2 cell cycle blocks and the G_0 barrier) of the three major categories


A
B
C

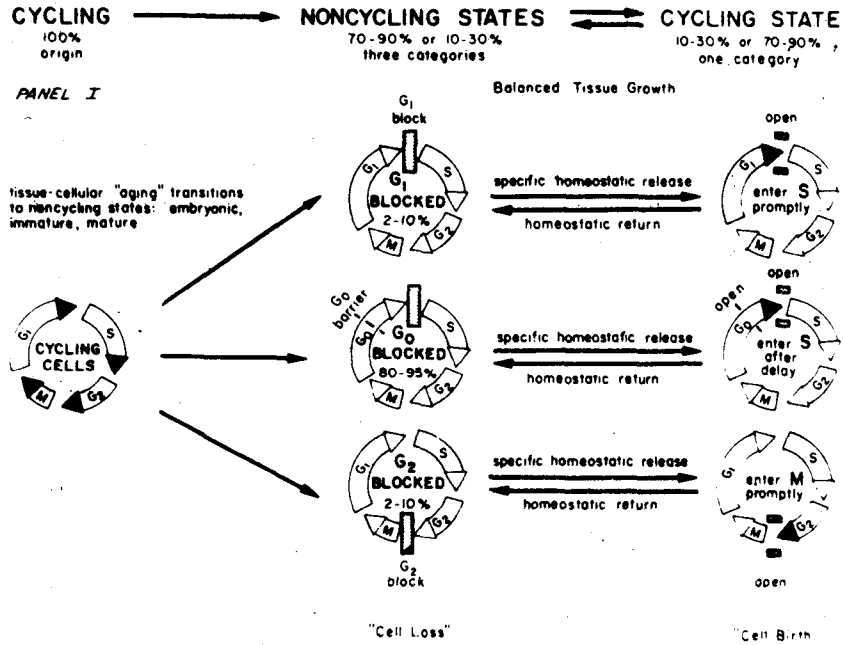


FIG. 4. Establishment of a normal tissue proliferative ecosystem.

of noncycling G_1-, G_0-, and G_2-blocked cells as each category converts from the cycling state to the noncycling state (termed cellular "aging" transitions) in different tissues during different periods of chronological development of the entire organism. Balanced tissue growth is a result of noncycling⇄cycling state transitions involving specific homeostatic release and return of each of the three

FIG. 3. Combined cytophotometric-autoradiographic and unlabeled mitoses procedures for demonstrating the existence of noncycling G_2-blocked cells. G_2-blocked cells appear as unlabeled interphase nuclei with 4C DNA contents and as unlabeled mitoses in autoradiographs—having been exposed to [^{3}H]thymidine for long periods of time prior to stimulation. (A) Ehrlich ascites tumor cells (mouse peritoneal cavity) exposed to continuous administration of [^{3}H]thymidine for 96 hours (five times longer than EAT cell cycle-generation time). Combined DNA Feulgen stain cytophotometry-autoradiography techniques. Unlabeled nucleus (arrow) contains 4C DNA content—thus, demonstrating the existence of noncycling G_2-blocked tumor cells. (B) Unlabeled mitosis Ehrlich ascites tumor—representing release of unlabeled noncycling G_2-blocked cell shown in (A); released into mitosis by antilymphocytic serum in the presence of and after 48 hours of continuous administration of [^{3}H]thymidine. Similar results were obtained by injecting other immunosuppressants, hydrocortisone and azathioprine (DeCosse and Gelfant, 1968). (C) Unlabeled mitosis (arrow) mouse ear epidermis *in vivo*. Demonstrates existence of noncycling G_2-blocked epidermal cell, released into mitosis by wounding, in the presence of and after prior continuous administration of [^{3}H]thymidine for 5 days. Similar results were obtained after 6 months of prior continuous administration of [^{3}H]thymidine (Pederson and Gelfant, 1970).

major categories of noncycling cells. Released noncycling G_1- and G_2-blocked cells enter S or M promptly—because they had arrested or had been blocked at the G_1/S or at the G_2/M transition points (and thus, serve tissues as fast-acting renewal systems). Released G_0-blocked cells enter S after a delay in time—because they arrest in early G_1—having been held in the noncycling state by the G_0 barrier. Because most noncycling cells come to rest in the G_0-blocked state, the overall growth characteristics of a tissue are primarily due to noncycling $G_0 \rightleftarrows$cycling cell transitions. For a review of the concept of a tissue as a proliferative ecosystem, see Section II, B.

B. Commentary Panel I

Tissue cellular "aging" transitions to noncycling states: In a previous report (Gelfant and Smith, 1972), we defined tissue cellular aging as, "Aging on a cellular level is described as a progressive conversion of cycling to noncycling cells in tissues capable of proliferation." Embryonic aging transitions: Some tissues complete their cellular aging transitions to the noncycling G_1-, G_0-, and G_2-blocked states during embryogenesis, for example, pancreas, lens, tongue muscle. Immature aging transitions: Other tissues complete their cellular aging transitions to the noncycling states during adolescence, i.e., before completion of maximum growth of the entire organism, for example, liver, kidney, bone. Mature aging transitions to the noncycling states: These take place during animal senescence in tissues such as epidermis and epithelium of the gastrointestinal tract. The following quotation from Pardee (1974) also supports our depiction of cellular aging transitions to the noncycling states: "Most animal cells *in vivo* exist in a nonproliferating state in which they remain viable and metabolically active. They arose from proliferating cells whose metabolic patterns were switched to quiescence at some time during differentiation."

Balanced tissue growth: When overall tissue cell birth exceeds cell loss, cycling cells move into the noncycling state. When cell loss due to trauma or to disease exceeds cell birth, noncycling cells move into and remain in the cycling state until repair, size, and balanced tissue growth is achieved; for example, renewal and repair of liver, kidney, epidermis, and other tissues *in vivo* (Cameron, 1971). And in restoration of hematopoietic equilibrium after hemorrhage: "Normal hematopoiesis is tightly regulated so that production of new cells exactly balances cell loss due to senescence and other causes. The rate of production can be increased in response to increased cell loss (e.g., hemorrhage), but once the imbalance is corrected, hematopoietic equilibrium is restored at the original level" (Clarkson and Rubinow, 1977). In general, the growth fraction of an unperturbed tissue, i.e., the ratio of cycling to noncycling cells for each tissue depends upon its function and its particular proliferative state.

In Panel I, homeostatic release and return from noncycling states are con-