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Maintenance of Mitotic Homeostasis in Somatic Cell Populations

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Recent theoretical and experimental studies have indicated that the rate of proliferative activity in somatic cell populations can be modulated by regulation of the transmembrane potential level (E_m). These studies have shown that such modulation is accomplished through control of DNA synthesis preparations, presumably through variations in the intracellular ionic conditions which accompany transmembrane potential changes. This capability for modulating mitotic activity appears to provide a potential mechanism for maintenance of mitotic homeostasis in somatic cell populations. The present paper, after a consideration of the primary kinetic requirement for homeostasis attainment, proceeds to develop two independent concepts of E_m-modulated homeostatic control: (1) "statistical" or "passive" homeostasis obtained by maintenance of a particular, constant E_m level at which proliferation rate equals death rate for the cells under study; and (2) "modulated" or "active" homeostasis obtained by local induction of cellular depolarization, from a high level where mitosis is blocked to a low level where mitosis is stimulated, by loss of intimate surface contact or chemical interaction with a dying cell. The problem of hormone-activated periodic or cyclic proliferative homeostasis is also interpreted in terms of E_m variation.

1. Introduction

Most primary organ and tissue systems of the adult animal body apparently maintain a reasonably constant cell number upon maturation, as is evidenced by the constancy of organ weight and volume measurements and the average-size constancy of the constituent cells. However, cytological studies have clearly revealed that, in most organs, mitotic activity proceeds on a continuing basis at various tissue-specific rates. It thus follows that these dividing cells must be replacing cells which have either died from normal statistical metabolic breakdown or functional trauma, or been removed from the proliferative

compartment by natural means (e.g. villus crypts, marrow). The relative constancy of organ cell mass and organ form under normal somatic conditions† suggests the operation *in vivo* of very exacting control mechanisms for guaranteeing the maintenance of mitotic homeostasis, failure of which would lead to total organ atrophy on the one hand, or to gross hyperplastic or malignant proliferation on the other. The underlying mechanism of such mitotic control is thus a subject of fundamental importance.

On the basis of a range of experimental evidence, I have proposed in recent papers that the intracellular ion hierarchy and its associated transmembrane potential difference (E_m) play a fundamental role in the control of DNA synthesis and mitosis in somatic cells (Cone, 1969, 1970; Cone & Tongier, 1970). This general theory of E_m-modulated mitotic control, along with certain aspects of the experimental evidence upon which it is based, suggest a particularly interesting explanation for the basis of mitotic homeostasis maintenance in somatic cell systems. The present paper, following a brief formalization of the basic requirement for maintenance of mitotic homeostasis *in vivo* and a consideration of the basic precepts of E_m control of mitosis, proceeds with the integration of these results to describe two possible systems whereby modulation of the cellular E_m level serves to effect precise mitotic homeostasis control. Although mitotic homeostasis in the body appears to result primarily from control of DNA synthesis through application of a G_1 mitotic block, populations of G_2-blocked cells also exist in a range of tissues and can serve as a pool of cells for mitotic homeostasis requirements. Aspects of G_2-mediated mitotic homeostasis have been developed by Gelfant (1969, 1970); this particular case of G_2 homeostasis control is not considered in the present study.

2. Mitotic Control Requirements for Cell Number Homeostasis *in vivo*

Three types of natural somatic cell proliferation systems in the adult (mammalian) body may be differentiated: (1) proliferation to replace dead cells (e.g. liver, lung, connective tissue); (2) proliferation to replace cells leaving the system, and which eventually die (e.g. basal cell layer of skin, hematopoietic system, villus crypts, lens epithelium); and (3) proliferation to produce a net (normal) increase in total cell number (e.g. estrogen induced mammary and uterine proliferation, lipoid cell proliferation). We shall be concerned principally with the first two types, which represent the primary

† This condition does not explicitly include the entirely normal expansions and contractions in the cell number of specific (target) organs, which occur periodically in response to particular physiological conditions (e.g. uterine and mammary proliferation in response to increased estrogen levels). The ensuing considerations will, however, cover this particular case of quasi-steady mitotic conditions.

steady state forms normally encountered, although the third form will also be considered as a special case.

With regard to the first type of proliferation, maintenance of a constant cell population (i.e. cell number), and hence organ mass (if subsequent replacement cells retain the same average size) requires that, on the average, for each cell which dies one must divide. Furthermore, maintenance of organ shape requires that replacement occur in the immediate vicinity of each cell death, but we shall merely assume for the present considerations that this important requirement for morphostasis is met by a uniform occurrence of cell death and replacement throughout the organ or tissue. Considerable experimental evidence shows that, following division, daughter cells *in vivo* remain indefinitely arrested or blocked in the G_1 period of the cell cycle (more properly called the G_0 period (Epifanova & Tershikh, 1969) in the arrested case) until stimulated or induced to divide (Cone, 1970), at which time DNA synthesis begins and the cell moves on through the S, G_2 and M periods. Let us assume that each cell of a given initial population of N_0 cells which is destined not to divide again and which therefore will reside its entire life in G_0, possesses on the average a lifetime denoted by $\bar{\mathscr{L}}$. Thus, on the average, if a given cell does not undergo division within a period of $\bar{\mathscr{L}}$ following its creation, it will die. There appears to be reasonable justification for hypothesizing the existence of such a parameter as $\bar{\mathscr{L}}$, as will become evident. For the present, however, it can be noted that since cells are dividing at a steady rate in adult somatic tissues, they must also be dying at a steady rate, and in view of the similar metabolic characteristics and environmental exposure conditions of constituent cells in a given tissue, it seems reasonable to expect that under such conditions genetically similar G_0 cells will possess characteristic lifetimes in the near vicinity of an average value $\bar{\mathscr{L}}$. (That is, the spread in the frequency distribution of $\mathscr{L}$ about $\bar{\mathscr{L}}$ is reasonably small.) The actual value of $\bar{\mathscr{L}}$ and the form of the associated $\mathscr{L}$ distribution may vary significantly of course from one cell type to another and from one location and physiological state to another in the body. For example, neurons of the central nervous system may remain viable in the G_0 state for the entire life of the animal. A technique for the experimental determination of $\bar{\mathscr{L}}$ is outlined in Appendix A.

This concept of an average lifetime $\bar{\mathscr{L}}$ for G_0 cells provides a convenient basis for defining the primary control requirement for mitotic homeostasis in replacement systems. Starting with a homogeneous, nonsynchronized population of N_0 cells at a given time ($t = 0$), all cells in this population which do not divide within an ensuing interval of $\bar{\mathscr{L}}$ (i.e. by $t = \bar{\mathscr{L}}$) will, by definition, die. We assume the death rate for those which do die to be uniform over the $\bar{\mathscr{L}}$ interval. However, to maintain population constancy, for each cell which dies one cell must divide, so that on the average exactly one-half of the original

population must have divided and one-half died in the $\bar{\mathscr{L}}$ interval.† Thus, denoting by N_d the number of cells dividing in the interval $\bar{\mathscr{L}}$, the primary requirement for mitotic replacement homeostasis becomes $(N_d/N_0)_{\bar{\mathscr{L}}} = 0{\cdot}5$. Consequently, the cell death rate and proliferation rate‡ of the population are identical and equal to $0{\cdot}5\ N_0/\bar{\mathscr{L}}$. The mitotic index is $(2\bar{\mathscr{L}})^{-1}$. This relation permits the experimental determination of $\bar{\mathscr{L}}$, as outlined in Appendix A.

For the case of systems where cell loss is due to transport of living cells out of a proliferative compartment instead of actual local cell death, the $\bar{\mathscr{L}}$ concept is still applicable if $\bar{\mathscr{L}}$ is redefined as the average time an emigrating cell remains in the proliferative compartment following its creation by mitosis. Thus, for maintenance of a constant population N_0 of cells in the proliferative compartment, exit of a cell from the compartment is entirely analogous kinetically to the condition of cell death in the simple replacement case considered above. For example, if a villus crypt consists (constantly) of N_0 total cells, and the average time an emigrating cell remains in the crypt prior to entering the villus surface is $\bar{\mathscr{L}}$, then the stem-cell proliferative rate in the crypt and the rate of cell efflux from the crypt are both equal to $0{\cdot}5\ N_0/\bar{\mathscr{L}}$. With such a definition of $\bar{\mathscr{L}}$ as above, the average cell cycle period $\bar{T}$ of the dividing stem cells is of the same order as $\bar{\mathscr{L}}$.

With the condition $(N_d/N_0)_{\bar{\mathscr{L}}} = 0{\cdot}5$ stating the basic requirement for mitotic homeostasis, the mechanism(s) by which N_d is regulated so as to satisfy this relation becomes a question of central importance. Considerable evidence exists indicating that the (G_1 or G_0) level of the cellular E_m is intimately involved in mitogenesis control (Cone, 1969, 1970; Cone & Tongier, 1970) and hence consideration of variations in E_m level as a basic mechanism for regulation of N_d is suggested. Consequently, we shall here digress briefly to

† This is the simplest mitotic scheme which satisfies the cell-number constancy requirement, but it is not the only possible pattern. For example, a much larger percentage than 50% of the "initial" population might die within $\bar{\mathscr{L}}$ if the remaining cells each contributed to several sequential divisions within the $\bar{\mathscr{L}}$ time span, i.e. $\bar{T} \ll \bar{\mathscr{L}}$, where $\bar{T}$ is the average generation cycle period of the dividing cells. In such a case, however, a majority of the initial cells would remain in G_0 for the full $\bar{\mathscr{L}}$ period, while the remainder would divide several times. If all of the initial cells remained in the same general environment (i.e. did not emigrate to areas where environmental influences were different), it would be expected that all would be influenced equally (in a statistical sense) and therefore exhibit similar mitotic characteristics. Hence the former condition discussed above, where $\bar{T}$ is of the same order as $\bar{\mathscr{L}}$, would appear to be more likely to exist in the case of the simple replacement homeostasis under present consideration. The latter condition would more likely apply in stem-cell systems where a continuous emigration of live cells from the proliferative compartment takes place, as will be discussed subsequently.

‡ The term "proliferation rate" (dN_d/dt) is used in this paper to denote the time rate of increase in population cell number due to mitosis. It is used in preference to the more common but somewhat ambiguous term "mitotic rate", which is often used to denote the rate of progress through only the mitotic M-period of the generation cycle. The mitotic index is merely the proliferation rate normalized with respect to N_0.

examine some pertinent theoretical and experimentally-determined aspects of the E_m level-mitotic activity relationship for subsequent application to the basic homeostasis problem.

3. Regulation of Somatic Cell Proliferation Rates by Variation of the E_m Level

The basic premise of mitotic control advanced by Cone (1969, 1970) suggests that the changes in ionic concentration levels and distributions which accompany different E_m levels in somatic cells might act to control DNA synthesis and hence mitosis. This proposition was based on a range of experimental observations indicating a positive correlation between E_m level and mitotic activity, wherein high E_m levels (e.g. -75 mv) appeared to block DNA synthesis and mitosis while low E_m levels (e.g. -10 mv) were accompanied by maximum proliferation rates.† In the latter paper cited above, a number of possible ways in which intracellular ionic conditions could influence preparations for DNA synthesis and synthesis itself was considered. Subsequently, an experimental study designed to check the validity of the overall theory using an arbitrarily chosen, but mitotically representative, cell line was carried out (Cone & Tongier, 1970). This study yielded conclusively positive results in support of the theory.

Of pertinent interest to the present considerations are certain of the experimental results of this validation study. Figure 1 illustrates experimental data showing the effects of (numerically) increasing E_m levels (simulated by use of specially-designed culture medium formulations) on the mitotic activity of naturally synchronized populations of CHO cells *in vitro* (see Cone & Tongier, 1970 for experimental details). This figure demonstrates some particularly interesting features. It is seen that at the low E_m levels (-10 to -45 mv), mitotic activity is essentially the same as for the normal *in vitro* level of -10 mv; i.e. up to an E_m level of -45 mv proliferation proceeds at the maximum possible rate, characteristic of growth conditions *in vitro*. In the intermediate to high E_m range (-45 to -75 mv), however, the fraction by which the given population increases in a given time interval decreases continuously with increasing E_m level, ultimately reaching zero at approximately $E_m = -75$ mv. Beyond this level ($E_m > E_m^*$), division is completely blocked.

The most direct and meaningful interpretation of these experimental variations is that the average length $\overline{T}$ of the cell cycle period, and also perhaps

† Since the potential of somatic cells is always negative relative to the bathing medium, reference only to the numerical value of the E_m level is made herein, unless explicitly noted otherwise. Thus "high E_m level" denotes a large negative value (e.g. -70 mv) while "low E_m level" denotes small negative values (e.g. -10 mv) of the transmembrane potential.

the spread of the mitotic wave (i.e. of the mitotic frequency distribution), increases continuously as the E_m level increases (due presumably to increases in G_1), as illustrated schematically in Fig. 2; consequently, fewer divisions

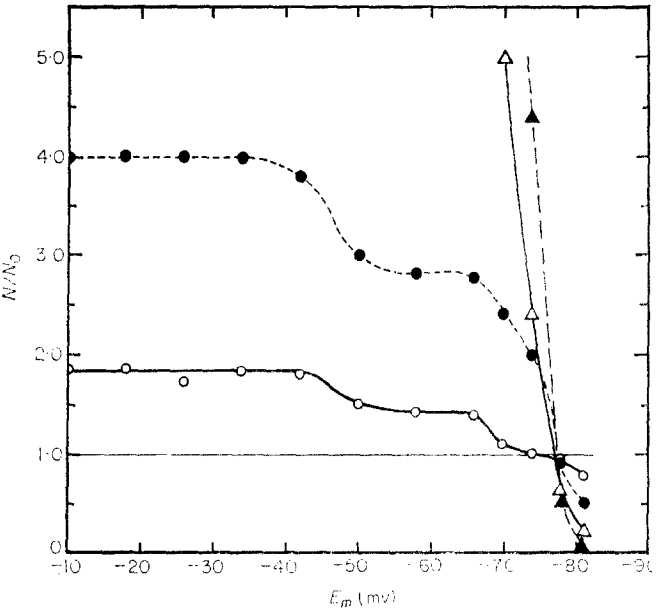

FIG. 1. Experimentally determined relationship between mitotic activity and E_m level in naturally synchronized CHO cells in monolayer cultures. E_m level was simulated by use of culture media having ionic concentrations adjusted to produce intracellular conditions approximating those normally accompanying the E_m level simulated. N/N_0; ratio of total viable cells N at given time to initial number N_0 in population at time zero (after Cone & Tongier, 1970). ———, 0 hr; ○———○, 24 hr; -●---●- 48 hr; △———△, 72 hr; -▲---▲-, 96 hr.

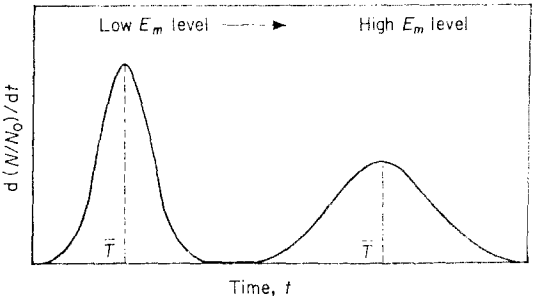

FIG. 2. Schematic illustration of the effect of low and high E_m levels on the average value and frequency distribution of the cell generation period T, as inferred from the data of Fig. 1 for naturally synchronized cell populations *in vitro*.

occur in a given time period as E_m increases (see Appendix B). Ultimately, the condition of complete blockage of DNA synthesis and division (corresponding to the G_0 condition *in vivo*) is reached at $E_m = -75$ mv. These *in vitro* data, if they are indicative in a general way of mitotic conditions *in vivo*, suggest the highly significant result that the average cycle period $\overline{T}$ and accompanying mitotic frequency distribution in somatic cell populations is a function of the basic E_m level existing in G_1, and ranges from a minimum value of $\overline{T} = \overline{T}'$ at the lowest E_m level (equivalent to free cell culture conditions *in vitro*) to a maximum as the condition of complete mitotic blockage (equivalent to the G_0 condition *in vivo*) is approached at $E_m = -75$ mv.

The variation in $\overline{T}$ with increasing E_m level appears to be primarily due to variation in the length of the G_1 period. Thus, while G_1 in its classic definition generally refers to the period of the *in vitro* cell cycle previous to DNA synthesis (over which the E_m level is approximately constant and minimum), this definition must now be expanded to include the variation of G_1 introduced by changes in the E_m level, i.e. $G_1 = G_1(E_m)$. The symbol G_0 is reserved herein for the condition of "permanent" mitotic blockage which accompanies maintenance of $|E_m| \geqq |E_m^*|$.

The precise metabolic factors which might underlie such a delay in mitotic activity at intermediate and high E_m levels are at present undetermined; a reasonable possibility, however, is that the activity of one or more enzymes associated with preparations for DNA synthesis is increasingly inhibited by the altered intracellular ionic condition as the E_m level increases, and the G_1 preparation period becomes increasingly extended. Ultimately, at $E_m = -75$ mv the ionic conditions reach a critical threshold above which DNA synthesis preparations become completely blocked. At this critical E_m level ($E_m = E_m^*$) the cell enters and remains in the arrested condition of the G_0 state.

The foregoing experimental results pertain to naturally synchronized cell populations. For non-synchronized natural populations of somatic cells *in vivo*, however, the same values of $\overline{T}$ and their associated frequency distributions would presumably apply for corresponding E_m levels, i.e. the T distribution of a given population of cells depends primarily on the E_m level, whether *in vitro* or *in vivo*. Of course, the E_m level will itself depend very much upon the aggregation state of the population (i.e. whether it is compacted *in vivo* or dispersed *in vitro*). Thus a proliferation rate of

$$dN/dt\,(E_m) = N_o \cdot (\ln 2) \cdot [\overline{T}(E_m)]^{-1} \cdot 2^{[t/\overline{T}(E_m)]}$$

would maintain over the E_m range from -10 mv (where the *in vitro* proliferation rate prevails) to -75 mv (where the cells enter G_0); here $\overline{T}$ is indicated as an explicit function of the E_m level. The variation $\overline{T}(E_m)$, therefore, provides

an effective mechanism for regulating cellular proliferation rates. While we have above cited effective ranges of E_m based upon experimental data for the CHO cell line, other cell types may possess somewhat different $\bar{T}(E_m)$ variations; however, the general features of the variations and the orders of the endpoint E_m levels E_m' and E_m^* should be reasonably similar to those found for the CHO cells.

4. Implications of E_m Control of Proliferation Rates for Mitotic Homeostasis *in vivo*

The primary implication of the foregoing experimental data for synchronized populations *in vitro* is that the average cell generation time $\bar{T}$, and also the associated frequency distribution of T about $\bar{T}$ (see Fig. 2), is a definite function of the basic (G_1) E_m level possessed by the particular cell type constituting the population. Applying this (empirical) principle to the case of natural, nonsynchronized cell populations *in vivo*, it follows that the required value of N_d/N_0 for mitotic homeostasis can be attained by appropriate modulation of $\bar{T}$ through maintenance of the proper E_m level.†

Since $\bar{T}$ and $\bar{\mathscr{L}}$ are in reality but average quantities associated with finite frequency distributions of the type illustrated in Fig. 2 (assumed for simplicity to be symmetrical Gaussian forms), for precise determination of mitotic homeostasis the finiteness of both these distributions (i.e. the statistical probability aspects of the problem) must be taken into account. The necessary relation between T and $\bar{\mathscr{L}}$ required to satisfy the homeostatic condition $(N_d/N_0)_{\bar{\mathscr{L}}} = 0{\cdot}5$, taking into account the distributions of T and $\mathscr{L}$, is illustrated in Fig. 3. The value of E_m maintained by the population must be such that the resulting $\bar{T}$ (and its associated distribution for the given population) is sufficiently less than $\bar{\mathscr{L}}$ that the area of the T-distribution *not* overlapping that of the $\mathscr{L}$ distribution (i.e. the shaded area in Fig. 3) has a value of exactly 0·5, thus producing a net fraction of dividing cells of 0·5. In the region of overlap (t_a to t_c), the *net* proliferation fraction in each Δt interval is equal to the local value of the T-distribution minus the local value of the $\mathscr{L}$-distribution (e.g. $t = t_b$). This result is merely equivalent to stating that in a common time interval Δt, the fraction of the population having values of $\mathscr{L}$ in this interval will die, thus

† The basic assumption here is that $\bar{T}$ and the associated distribution of T is a function only of the E_m level, and that the T-distributions which are obtained by simulation techniques *in vitro* for a given E_m level are equally representative of the distributions which exist *in vivo* at the same E_m level. Although a certain cell fraction may have a potential generation cycle time of T *in vivo* under ideal conditions (equivalent to that determined under *in vitro* conditions, say), its particular $\mathscr{L}$ under the existing conditions *in vivo* may be considerably less than this T value. Thus the cell fraction in this case will die before it is able to undergo division.

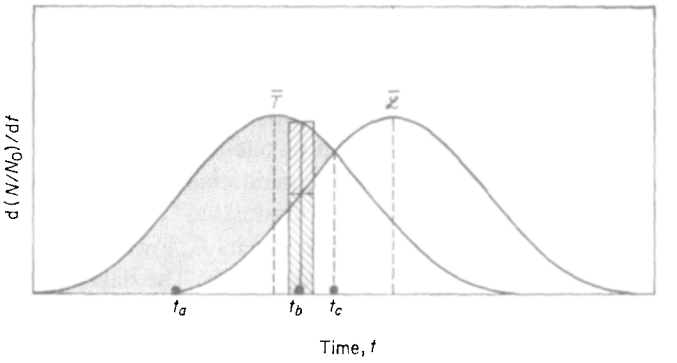

FIG. 3. Schematic illustration of the required relation between the distributions of T and $\mathscr{T}$ for statistical maintenance of mitotic homeostasis in somatic cell populations *in vivo*.

reducing the fraction which otherwise would have divided in the Δt interval (i.e. the fraction having T values within the Δt interval) by precisely this amount. (It is assumed here that cells which have $T = \mathscr{T}$ will die, not divide.) Since the fraction of cells dying at all values of $t > t_c$ exceeds the fraction which would otherwise have divided, all cells having $T > t_c$ will die. Hence, in the case of homeostasis, exactly one-half of the population will die.

Maintenance by a given cell population of an E_m level that will produce a $\overline{T}$ value permitting overlap of exactly one-half of its corresponding frequency distribution area with its own $\mathscr{T}$ distribution ($E_m = E_{m,h}$) will consequently allow continuous, automatic maintenance of mitotic homeostasis on a statistical basis. An outline for experimentally determining the variation of $\overline{T}$ (and its frequency distribution) with E_m level is presented in Appendix C.

In vivo populations of cells possessing a low E_m level equivalent to culture conditions, $E_m = E_m'$ ($\overline{T} = \overline{T}'$), will pass through a number of cycles in a time period $\overline{\mathscr{T}}$, the number depending upon the relative magnitudes of $\overline{T}$ and $\overline{\mathscr{T}}$ for the particular cells and conditions under consideration. Thus the existence of conditions which would decrease E_m from its homeostatic value of $E_{m,h}$ to E_m' would lead to a continuous, maximum increase in cell population with time. At a sufficiently high E_m level, $|E_m| \geqq |E_m^*|$, N_d/N_0 becomes zero and the cells assume the fully blocked condition of G_0. At this E_m level the population will die out in a period $\overline{\mathscr{T}}$ if the E_m^* level is continuously maintained. Thus transient variation of the E_m level by various factors can lead to an effective control of overall population number in any cell system. Modulation of the E_m level could override the basic homeostatic balance when a net increase or decrease of population is temporarily required for any reason.

The existence of the level E_m^* at which all mitosis is arrested, offers a second potential means for mitotic homeostatic control. If, instead of maintaining a

constant E_m level of $E_{m,h}$ where the condition $(N_d/N_0)_{\bar{\mathscr{P}}} = 0{\cdot}5$ is statistically maintained, each cell of the population maintains a level of E_m* or greater, but is positively induced to depolarize to a sufficiently lower value for a period long enough to enter division upon death (or emigration) of an adjacent cell, the same basic condition of one-for-one cell replication will be satisfied and mitotic homeostasis will be maintained. Homeostatic regulation by such a mechanism might be termed "modulated" or "active" homeostasis. Presumably, only the adjacent cell which had its E_m lowered sufficiently by its break in contact with the dead cell would divide. The data of Fig. 1 suggest that, provided a suitable means for effecting the local change in E_m level is available (e.g. loss of surface contact by adjacent cell death), such a mechanism would be quite effective; the lowered E_m level would have to be maintained for a sufficient length of time, however, for mitotic activation to take place in at least one cell. From the indication of Fig. 1, even slight depolarization below E_m^*, if maintained long enough, would serve to induce division, provided $\bar{\mathscr{P}}$ was not exceeded. Also, from the data of Fig. 1, the difference between $E_{m,h}$ and E_m* may be very small in an operational sense, so that the two basic mechanisms of maintaining homeostasis would be difficult to differentiate in actual practice.

5. Some Operational Aspects of an E_m-modulated Homeostatic Mechanism

In view of the dependency of these potential homeostatic mechanisms on the E_m level, the means whereby primary regulation of the E_m level might be effected are of basic importance. A discussion of the theoretical basis of E_m regulation in somatic cells and possible means whereby regulation of the E_m level might be achieved in *in vivo* cell systems has been presented in a previous paper (Cone, 1970). For present purposes, mention will be made of only two such means.

First, in the case of "statistical" maintenance of homeostasis (Fig. 3), it is necessary that all cells maintain a rather precise average E_m level of $E_{m,h}$. Such a requirement can presumably be met by an exacting surface specificity of the cell wherein the homeostatic E_m level would be a natural consequence of the particular surface polymer structure and its interaction with that of neighboring cells. With close maintenance of this E_m level ($E_{m,h}$), some 50% of any given population would, statistically, divide within the $\bar{\mathscr{P}}$ interval. The required value of $E_{m,h}$ would of course depend on the magnitude of $\bar{\mathscr{P}}$, thus leading to very specific interactions and mutual accommodations of the metabolic circuits involved in determining these two parameters.

Second, in regard to the local cell-for-cell replacement mechanism of "modulated" homeostasis maintenance, it would seem as already mentioned

that cell surface contact would be a major factor in the E_m level control. Since contact phenomena appear to exert a pronounced influence on the E_m level of somatic cells (Cone, 1970), the obvious disruption of surface contact which would accompany a local cell death could result in a temporarily lowered E_m level with consequent stimulation of DNA synthesis and division. Conceivably, enzymes and other products released by decomposition of the dead cell could also act upon adjacent cells to stimulate DNA synthesis. In any event, for mitotic balance the initiating mechanism must result in mitotic stimulation of only one ajdacent cell, on the average; presumably this would be the cell having the greatest effective area of surface contact with the cell which died, or the cell which was most immediately affected by the local death.

In addition to these two basic modes of steady homeostasis maintenance, tissue-specific modulation of the E_m level by hormonal action could serve as an additional fine-control means for ensuring long-term overall mitotic balance by adequate shifts of the E_m level for sufficient intervals of time (Figs 1 and 2) to attain the desired increase or decrease in total population. Such hormonal action could be mediated through feedback circuits with the target tissue. In the case of quasi-steady homeostasis mentioned earlier, hormonal modulation has been clearly established as the basic control factor; presumably this particular hormonal influence is mediated through transient changes in the cellular E_m level.

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APPENDIX A

Experimental Determination of $\bar{\mathscr{T}}$ for Somatic Cells

The mitotic index, MI (i.e. the fraction of a given population of cells entering mitosis per specified time interval) is given in terms of $\bar{\mathscr{T}}$ as $\mathrm{MI} = (2\bar{\mathscr{T}})^{-1}$. Thus $\bar{\mathscr{T}} = (2.\mathrm{MI})^{-1}$. The mitotic index is conveniently determined by use of colchicine or a similar agent to block in metaphase all cells entering mitosis during a prescribed period of time, fixing and staining the tissue (or culture), and determining the fraction of metaphase cells. If, for example, the MI were found to equal 0·03 for a 24-hour period for a particular tissue ($\mathrm{MI} = 0{\cdot}00125\mathscr{T}^{-1}$), $\bar{\mathscr{T}}$ would be 400 hours if the tissue were in mitotic homeostasis.

APPENDIX B

Variation of $\overline{T}$ with E_m Level as Determined by *in vitro* Data

Although the data presented in Fig. 1 are for naturally synchronized cell cultures, where increase in cell number takes place in waves or approximate steps rather than continuously, the conventional exponential proliferation formula $N(t) = N_0 \cdot 2^{t/\overline{T}(E_m)}$ is still applicable at integral values of $t/\overline{T}$ and ultimately becomes an exact representation for all times as synchronization is lost in the culture, provided appreciable cell death is not occurring simultaneously. Since cell death was negligible over the lower E_m and t(time-increment) ranges covered in Fig. 1, the variation of N/N_0 is essentially that due to mitotic activity alone. Thus, according to the above equation, the experimental decrease in values of N/N_0 with increasing E_m level for a constant t period signifies an increasing value of $\overline{T}$ as E_m increases, up to the level of mitotic blockage.

APPENDIX C

Experimental Determination of the $\overline{T}$ Frequency Distribution *in vitro*

The frequency distribution of the generation cycle period T can be estimated experimentally for all desired values of E_m by use of naturally synchronized cultures and simulation media. By continuously monitoring the occurrence of divisions within a given initial population of naturally synchronized cells (say by use of time lapse cinemicrography), the frequency distribution $(\mathrm{d}(N/N_0)/\mathrm{d}t$ vs. $t)$ for T, and hence the value of $\overline{T}$,can be determined for the population over the range of E_m values.