Mitotic and Functional Homeostasis:

_A Speculative Review_

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I. INTRODUCTION

One of the most important of all biologic problems concerns both the manner in which cells differentiate to form tissues and the manner in which, in such tissues, differentiation is maintained and mitotic activity is controlled. In recent years biologists have taken a number of new steps toward an understanding of this problem, and although much of what is now being written is admittedly hypothetical, it nevertheless seems worthwhile at this moment to attempt to review these new ideas and to place them in a logical context.

Perhaps the most significant of recent biologic discoveries in this field have been those that have provided new knowledge and understanding of the genetic manipulation of protein synthesis (see, for instance, Ref. 262), of the changes that occur in the expression of the genome when an embryonic cell differentiates (see, for instance, Refs. 165, 166), and of the homeostatic mechanisms by which both specialized function and mitotic activity are controlled in the differentiated cells of adult mammals (see, for instance, Refs. 73, 75). These 3 fields of research are evidently closely interrelated, and the conclusions that have emerged probably apply generally to all metazoan animals. However, in the present review consideration has been restricted to the situation that exists in the tissues of adult mammals and to the manner in which the expression of the genome in a differentiated cell may be controlled by mitotic and functional homeostatic mechanisms.

It appears self-evident that any conclusions regarding these mechanisms must have important implications in the problem of cancer, and indeed that little or no real understanding of the changes occurring during carcinogenesis is likely to be achieved without some considerable previous knowledge of the manner in which normal tissues maintain their organization. It seems reasonable to conclude that the main, and perhaps only, reason why cancer has remained such a mystery for so long is our lack of any adequate understanding of these normal mechanisms.

Received for publication February 4, 1965.

II. THEORIES OF GENE EXPRESSION

A. Genetic Control Mechanisms

The most important biologic discoveries of recent years have concerned the structure of the DNA molecule, and the most important hypotheses have concerned the possible manner in which genetic information may be coded in the base sequences of this molecule. This work is outside the scope of the present review except in so far as it forms the background for our understanding, first, of the manner in which instructions may emanate from the nucleus to induce the synthesis of specific types of proteins, and second, of the manner in which, from time to time, these instructions may be changed to take account of the changed conditions, whether inside or outside the cell. Regarding these 2 points, most of our information has been derived from studies of microorganisms; recent reviews are those of Monod et al. (262, 355, 356), whose conclusions have been discussed in a more general chemical context by Dean and Hinsheiwood (131). There are, however, strong indications that similar mechanisms must also exist in the differentiated cells of adult mammals, in which they may act as an essential part of homeostatic mechanisms.

As regards microorganisms, current hypotheses may be summarized as follows. Two main types of genes are recognized: the structural genes and the regulator genes. A structural gene, acting through the intermediary of an RNA messenger, is stimulated to initiate the synthesis of a particular protein. The stimulus to commence activity originates in a particular region of the gene called the "operator," and each operator may be in control of 1 or of several related structural genes. Those genes, whether 1 or many, that are under the control of a single operator are together known as an "operon." When an operon contains several structural genes, these are usually adjacent to each other so as to form a cluster on the chromosome (134).

Each operator, and therefore each operon, is under the

1 The following abbreviations are used: DNA, deoxyribonucleic acid; RNA, ribonucleic acid; ACTH, adrenocorticotropic; ATPase, adenosine triphosphatase; ATP, adenosine triphosphate.
negative control of a substance called a "repressor," which may be a protein. Each repressor, which acts only on a specific operator, is produced in response to the activity of a regulator gene, probably through the normal messenger RNA mechanism. It is because the repressor has the ability to react with particular substances, which in the case of microorganisms are metabolites of low molecular weight, that the whole system is so remarkably versatile in its response to the needs of the moment. A metabolite that reacts with a repressor in this way is called an "effector"; in the reaction the repressor is either inactivated, so that the relevant messenger RNA and proteins begin to be synthesized, or it is activated, so that the synthesis of messenger RNA and protein is inhibited. Thus in microorganisms the expression of the genome is determined by the type of metabolite available, and it is important to note that the reaction to a new situation is almost instantaneous. A reaction time of less than 15 sec has been established (262).

It is interesting to note that an effector of this type may not necessarily exert its influence on the repressor by binding at the same active site as that which binds the operator. A recent hypothesis suggests the possibility that a repressor may be an allosteric protein, which possesses 2 separate active sites (355). In this situation the activation or inactivation of the repressor is considered to depend on the molecular distortion caused when the effector becomes bound to its own site. Because of this an effector need not bear any obvious chemical relationship to the synthetic pathway it influences.

Although these conclusions and suggestions relate almost entirely to the reactions of unicellular organisms to substances, they are also obviously relevant to any consideration of cellular activity in metazoans. In spite of the fact that in the case of the metazoans the evidence is only slight, Monod and Jacob (356) have concluded that it may still be sufficient to "justify the conclusion that the rate of structural gene expression is controlled, in higher organisms as well as in bacteria and bacterial viruses, by closely similar mechanisms, involving regulator genes, aporepressors, operators and operons." Some of the still inadequate mammalian evidence relates to the control of the agouti hair pattern (43, 118) and to certain liver enzymes (315, 367, 392). The existence of typical messenger RNA in the mammal has also been demonstrated in association with the ribosomes in rat liver cytoplasm (300, 362).

However, apart from the existence of this evidence, it seems most improbable from what is already known of the remarkable uniformity of the biochemical organization in all living matter that the mechanisms of gene expression and gene control will prove to be fundamentally different in the cells of the various groups of organisms, even when these are as different as mammals and bacteria.

B. EMBRYONIC DIFFERENTIATION

In considering metazoan animals it is obvious that, apart from their multicellular nature, their outstanding peculiarity lies in the manner in which their component cells become differentiated and in the way in which these differentiated cells are grouped together to form tissues. It is extremely important to determine the nature of the process of differentiation and also the effect it must have on the expression and control of the genome.

The nucleus of a fertilized egg contains a gene complement that is capable of directing the construction of each and every part of the fully differentiated adult; in other words, the genome is totipotent. It is also evident, at least in some animals such as molluscs (408) and amphibians (164, 165), that certain cytoplasmic constituents that may originate in the cortex (126) of the newly fertilized egg are already so organized and distributed as to predetermine the positions of such major adult organs as brain, intestine, or muscles, and that as the egg undergoes repeated cleavage this basic pattern becomes even more firmly stabilized by the developing cell walls (133, 167). It is also evident that as development proceeds the cytoplasmic components themselves undergo increasing specialization and diversification.

Thus the cells in each region of the developing body are characterized by a particular type of cytoplasm, and it has been suggested that it is the contents of this which, by reacting with the genome, determine the type of differentiation that will occur. It is interesting that in the higher plants differentiation is also evidently determined by the situations in which the cells find themselves (460).

One of the methods by which cytoplasmic specialization may be induced, so that it in turn may determine the pattern of differentiation within a group of cells, is through the influence exerted by some neighboring preformed tissue. Thus it is commonly said that one tissue will induce the appearance of another (see Refs. 231, 430, 431). Such a tissue interaction may be envisaged as involving either a stimulus or an inhibition: in the sense of a stimulus it may be considered that the differentiating cells, following intrinsic genotypic instructions "select among, translate and amplify these instructions in response to extrinsic influences" (213); in the sense of an inhibition it is possible to regard the reaction as the outcome of the suppression of intrinsic genotypic instructions when the "products of differentiating tissues act as inhibitors of like activities in surrounding tissues" (50). Some indication of the complexity of the induction process is given by Saxén (430), who stresses that the inducing factors may act as triggers only and that the main part of the differentiation mechanism evidently lies inside the reacting cells.

This situation is immediately reminiscent of the effector-repressor-operator type of mechanism described in microorganisms. The simplest suggestion would be that in embryonic cells all the specialized genotypic instructions remain suppressed until, during differentiation, one specific repressor is inhibited by a particular effector. If this suggestion is valid, then, at least in the higher animals, it must also be accepted that this process involves the permanent suppression of all or most of the other specialized potentials of the genome (53, 166).

It is interesting to note that in the higher plants it is possible to stimulate a single fully differentiated cell to such a degree that it will give rise to a new plant (460), and this must indicate that within such a differentiated cell the genome is still potentially totipotent. However, there is at the moment no evidence at all that differentiated mammalian cells, with which this review is primarily con-
cerned, can act in this way. In such cells differentiation commonly appears to be irreversible.

From what has been said it is clear that differentiation is a process that, in mammals, takes place almost entirely in the embryo. Once organogenesis is complete all the cells of the body, except for instance the germ cells and the stem cells of the hemopoietic systems (see p. 1706), are specialized to such an extent that each must continue ever afterwards to breed true to type. It is with this type of differentiated cell that the present review is mainly concerned, and in such a cell it is the genetic potentialities that remain after differentiation that are of outstanding importance. It now appears that in most adult mammalian tissues the only remaining functional or potentially functional genes are those that dictate the synthesis of the enzymes needed for the general metabolic pathways, for mitotic activity, and for tissue function.

Unfortunately, in the literature it is common to find that only those cells that function in the manner typical of the tissue to which they belong are regarded as differentiated. Cells that belong to the tissue but have never functioned in the typical manner are described as undifferentiated, while cells that have ceased to be able to function in the typical manner are described as dedifferentiated. In fact, almost all the somatic cells of an adult mammal, whatever may be their appearance or function, must be regarded as fully differentiated in the sense that the power of expression of their genome has become severely and typically limited. For an excellent discussion of the meaning of the term “differentiation” see Weiss (513).

C. CELL DIVISION AND CELL FUNCTION

Except for a few highly specialized cells such as neurones and for a number of aging cells such as circulating erythrocytes and granulocytes, the tissue cells in an adult mammal are commonly capable of at least 3 basic programs of protein synthesis, of which 1 is essential while the others appear to be optional. The essential program ensures a continuous supply of the enzymes needed in such vital metabolic pathways as that of the tricarboxylic acid cycle (125, 169, 363). In this connection it is interesting to note that Davidson et al. (129) have shown that, when actinomycin D is added to connective tissue cells in vitro to block the synthesis of messenger RNA, such generalized enzymes as succinic dehydrogenase continue their action unaffected. This suggests that essential enzymes may not be subject to any immediate genetic control, which may indicate either that they have an unusually long functional life or that their synthesis is dictated at the ribosome level and needs no repeated genetic stimulus for its continuance.

By contrast, the other 2 possible programs of protein synthesis are highly specialized. These are, 1st, the synthesis of those enzymes on which mitosis depends, and 2nd, the synthesis of those enzymes that direct the specific activities typical of the tissue. It appears that, although cells are capable of undertaking these 2 types of syntheses, none are capable of undertaking them both simultaneously; the operation of one synthetic program always seems to exclude the operation of the other (see p. 1699). This is again reminiscent of the situation in microorganisms in which the commencement of 1 type of specialized synthetic activity may be accompanied by the cessation of another.

On theoretical grounds it has been thought for some time that an integral part of the control mechanism that determines whether a cell indulges in protein synthesis for mitosis or in protein synthesis for function may take the form of a diffusible chemical messenger (377), which may act as part of a negative feedback mechanism to suppress mitosis within the cells concerned (259, 348). In the language of the microbiologists such a substance might be considered to be an effector.

Recently 2 lines of research have converged on this problem. In the 1st, there has been an investigation of the possible functions of the histones, which not only are associated with chromosomes but also exist free in the cell. Stedman and Stedman (458) questioned whether they may be concerned in the tissue-specific control of gene expression, and Huang and Bonner (253) have shown that they are capable of suppressing RNA synthesis. The question still remains unanswered (see Refs. 5, 24, 504), but it is already clear that the histones are not as tissue specific as was originally thought and as would be necessary if they were to form an integral part of a tissue-specific control mechanism (272).

In the 2nd line of research, Bullough and Laurence (86, 87) have extracted from mouse epidermis a substance that inhibits epidermal mitosis and has the necessary characteristics to act as part of a negative feedback mechanism, and they have also obtained evidence indicating that similar tissue-specific mitotic inhibitors are associated with the hypodermis and the hair bulbs (82). In addition, tissue-specific mitotic inhibitors have been extracted by Saetren (425) from liver and kidney and by Rytömaa and Kiviniemi (423) from mature granulocytes. All these substances have similar physical properties, and Bullough (73) has proposed that they should be called chalones, a name that refers to their inhibitory actions. These actions are discussed in detail below; here it is necessary only to stress that they result in the suppression of synthesis for mitosis within the tissues in which they originate.

However, if the chalones do form part of the mechanism whereby a choice is made between possible synthetic programs, it must be emphasized that in some tissues the choice is not simply between 2 alternatives. In some tissues, such as striped muscles and nerves, there is evidently no choice at all, and at all times the cells continue synthesis for function. In other tissues, such as epidermis (44, 339, 347), the cells are at least potentially capable of more than 2 types of synthesis. The many varieties of epidermis can be classified into 3 major groups: surface epidermis, which produces the outer sheet of “soft” keratin; hair bulbs, which produce “hard” keratin; and sebaceous glands, which produce fatty sebum. In all normal situations the cells either undergo mitosis or produce their typical proteins, but in abnormal situations the various types of cells are found to be interchangeable (357). Thus, after the destruction of the skin surface any of these epidermal types may contribute cells towards the formation of a new and entirely typical surface epidermis; after the destruction of the sebaceous glands by methylcholanthrene new glands may be produced from the cells of
the outer follicle sheath (357, 358); and in certain circum-
stances the surface epidermis may even give rise to new
hair follicles, each of which will possess a sebaceous gland
(40, 42, 52, 350). Thus it appears that at least some of
the epidermal cells retain a certain residual versatility that
can be exploited in an emergency.

Other examples of this type of reaction are found in
liver cells (308) and in melanoblasts, which do not synthe-
size melanin until they receive an appropriate stimulus
(43). Indeed, as Willis (526) has emphasized, any study
of pathologic histology soon indicates what the various
cell types “can be or do in all manner of abnormal environ-
ments . . . and shows that great transformations of cellular
structure . . . are possible in most tissues.” It has been
suggested by Weiss (512, 513) that when a differentiated
cell changes its activities in this way the phenomenon
should be called modulation. In the case of epidermis it is
clear that such modulation occurs only as the result of
some drastic change in the cellular environment, which
must in turn cause a significant intracellular change.

III. MITOTIC HOMEOSTASIS IN ADULT TISSUES

A. Control by Chalones

It is evident that in any “typical tissue” the alternative
programs of protein synthesis for mitosis and for tissue
function differ in at least 1 important respect. Any cell
that begins to prepare for mitosis is thereby committed to
complete the process, but any cell that is synthesizing the
proteins needed for normal tissue function is commonly
able to revert to a relatively nonfunctional state from
which it can then proceed to mitosis. Such reversion is
seen in most tissues after wounding, during regeneration,
in tissue culture, and in neoplasia, and it follows that the
state of functional activity typical of most differentiated
tissues may be regarded as unstable. Indeed, the impli-
cation is that not only the functional state of a tissue but
the whole structure of an adult mammal may need at all
times to be actively maintained against the possibility of
collapse into mitotic anarchy. By contrast, the process of
recurrent cell growth and mitosis is evidently entirely
stable, and both in vitro and in neoplasia, cell populations
that are behaving in this way appear to be potentially
immortal.

Thus each of the tissues of an adult mammal seems to
be in a state of equilibrium and all except the most highly
modified types of cells, to be poised between an unstable
functional state and a stable routine of recurrent mitosis.
It is obvious that an understanding of the nature of the
mechanisms that determine and maintain the actual point
of balance within each tissue is of the greatest possible
theoretical and practical importance. One common
theory has been that tissue mitotic activity may be con-
trolled by a tissue mitotic inhibitor in such a way that the
greater the tissue mass within the body space, the lower
will be the rate of cell production (see, for instance, Refs.

The earliest indications that this concept may indeed be
broadly correct came from observations on organ regen-
eration and in particular from studies of the regeneration
of kidney and liver in adult mice and rats. This literature,
which contains much contradictory evidence, has been re-
viewed by, for instance, Swann (467, 468) and Bullough
(73), and it is also discussed below (see p. 1691). The 1st
significant success in the extraction of tissue-specific mito-
tic inhibitors with the necessary characteristics appears
to have been that of Saetren (425, 426), who studied
mitotic control in kidney and liver; the 2nd was that of
Bullough and Laurence (86, 87), who studied epidermis
and suggested that such inhibitors should be called
chalones; and the 3rd was that of Rytömaa and Kiviniemi
(423), who have extracted a granulocytic chalone.

The epidermal chalone is water soluble, and it can
easily be extracted when the epidermis is macerated. It
is not species specific, and it is capable of depressing
mitotic activity in mouse epidermis both in vivo and in
vitro (87). The epidermal chalone will also depress mitotic
activity in cornea, sebaceous glands, and esophageal
lining epithelium, but not in other tissues tested. Con-
versely, aqueous extracts of a variety of other tissues do
not possess the power to depress epidermal mitotic ac-
tivity. More recently it has been shown (78) that the
epidermal chalone is nondialyzable and that it is
destroyed by boiling and precipitated by alcohol. It is
unstable in water solution but stable after lyophilization.
By fractional precipitation most of the chalone present in
the original solution has been recovered in the small pre-
cipitate obtained when the alcohol concentration is raised
from 70% to 80%.

An in vitro analysis of the mode of action of the epi-
dermal chalone has demonstrated 1 particularly important
point, namely that this chalone is unable to exert its full
power as a mitotic inhibitor in the absence of adrenalin
(75, 87), and conversely it can be shown that adrenalin
has no effect on the epidermal mitotic rate unless the
epidermal chalone is present. This raises the whole
question of the relation of the adrenal glands to mitotic
activity, which it is convenient to consider before returning
to the question of the chalones.

B. The Role of the Adrenals

1. The diurnal mitotic cycle.—This discovery of the role
of the adrenal glands in the control of epidermal mitotic
activity has provided an explanation for the old mystery
of the diurnal mitotic rhythm, which is so prominent a
feature in the epidermis (64, 73). It is now evident that
when an animal rests or sleeps the rate of secretion of
adrenalin falls, the power of the chalone is reduced, and
the epidermal mitotic rate rises; conversely, when an
animal wakens and becomes active the rate of adrenalin
secretion rises, the power of the chalone is increased, and
the epidermal mitotic rate falls (83). As would be ex-
pected, adrenalectomy destroys this diurnal rhythm and
causes the epidermal mitotic rate to rise to a constantly
high level (83).

Diurnal variations in the rate of adrenalin secretion
have been described in man (275), rats (161), and mice
(284), and in all cases it is evident that the adrenalin
concentration is greatly reduced during rest and sleep.
The same is true of noradrenalin, but it is now clear that
this substance cannot act as an effective substitute for
adrenaleactomy the marked rise in the epidermal mitotic rate is associated with a reduction to about 0.1 of the normal adrenalin concentration, but there is little if any change in the concentration of noradrenalin (162). The question whether the hormones of the adrenal cortex may change in the concentration of noradrenalin (162). The hormone-dependent tissues (see p. 1703), it is immediately obvious that a diurnal mitotic rhythm is commonly, if not universally, present except when the mitotic rate is either very low or very high.

In adult mammals diurnal mitotic cycles, in which the highest rate of cell division develops during periods of rest or sleep, have been described in the epidermis of mice and rats (70, 73) and in that of men (433), in cornea (6, 197, 501), in lens epithelium (428), in oral epithelium (6, 490), in esophagus (6, 64, 137), in stomach mucosa, in intestinal mucosa (6, 64, 197), in rectal mucosa, in pancreatic exocrine epithelium (136, 331), in epidermis, and possibly in erythrocyte production (223).

In addition, in tissues that are mitotically inert in normal adult mammals, typical diurnal mitotic cycles may develop during regeneration after partial hepatectomy (21, 266, 287, 421); in mammary gland during pregnancy (319); and in liver (60, 136), kidney (6, 136), adrenal cortex (138), outer orbital gland (136), and epiphyseal cartilage (447, 448) during the juvenile growth period.

In tissues like active hair roots, which show an extremely high mitotic rate, no diurnal rhythm is present; in tissues like those of the adult liver and kidney, which show an extremely low mitotic rate, no diurnal rhythm is detectable, but this may be due to the inadequacy of the usual techniques.

Of considerable theoretical importance are the recent demonstrations that the diurnal mitotic rhythm is not confined to the visible stages of mitosis but that it also affects the preceding phase of DNA synthesis. Alov (6) has described rhythmic changes in the numbers of cells undergoing DNA synthesis and also in the rate of RNA synthesis in cornea, tongue, liver, and lymphocytes, and he has indicated that both these rhythms coincide with the mitotic rhythm; he failed to find any similar phenomenon in intestinal mucosa, which is a highly active tissue in which the mitotic rhythm is poorly defined. The existence of a diurnal cycle in DNA synthesis has also been found in epidermis and tongue by Oehlert and Block (375), in cornea by Tchoumak (480), and in epidermis, tongue, esophagus, and forestomach, but not in duodenal mucosa, by Pilgrim et al. (388).

It is interesting, however, to note that no such diurnal rhythms appear to exist in fetal tissues, including epidermis (509).

Although more evidence is needed, it nevertheless begins to appear that a diurnal rhythm in the rate of DNA synthesis may be found in any tissue that, having only a moderate mitotic rate, shows a diurnal rhythm in the numbers of cells engaged in mitosis, and that in tissues such as duodenal mucosa, in which the mitotic rate is high, there may be little if any sign of either rhythm.

Finally, in agreement with the suggestion that the diurnal mitotic cycle is inversely related to the diurnal cycle in the rate of adrenalin secretion, it is well known that any stressful situation results in both increased activity of the adrenal glands (16, 150, 161, 162, 221, 313) and decreased mitotic activity of the tissues. Such decreased mitotic activity has been described in epididymis (66, 77, 83), cornea (361), liver (307), and erythrocyte production (51) during stress by starvation; in epidermis during prolonged exercise (65, 83); in cornea during prolonged swimming (6); in epidermis (68) and cornea (174, 500) during excitement or pain; and in all the tissues of young rats after general disturbance induced by repeated changes of boxes, diet, or companions (459). In juvenile rats stress by starvation has also been shown to depress DNA synthesis in epithelium of tongue, forestomach, and hindstomach, and in liver, pancreas, and adrenal gland (286).

2. Adrenalin and the glucocorticoid hormones.—Turning next to the adrenal hormones themselves, it has been shown, both in vivo and in vitro, that adrenalin is the most potent epidermal mitotic inhibitor known (68, 71, 83, 87, 114), that the glucocorticoid hormones are also capable of exerting an anti-mitotic action if they are given in sufficiently large doses (68, 482), and that the mineralocorticoid hormones are without any apparent effect (68).

Besides epidermis, adrenalin has been shown to depress mitotic activity in cornea (48, 174), in lens epithelium, and in intestinal mucosa (138). In this last tissue with its high mitotic rate the depression caused is relatively small and short lived, and in active hair bulbs, which have perhaps the highest mitotic rate shown by any adult tissue, adrenalin has no effect at all even when it is injected in near-lethal doses. This last observation is of critical importance in that it indicates clearly that, by itself, adrenalin is not a mitotic inhibitor. The fact that its inhibitory action on mitosis is obviously tissue specific may be taken as indicating that before adrenalin can act as an inhibitor it must either combine or otherwise cooperate with some substance present in the tissue. On the basis of the evidence obtained from epidermis, it is suggested that this substance is the tissue-specific chalone.

Regarding the glucocorticoid hormones, it is generally believed that their secretion rate is directly related to the secretion rate of ACTH from the anterior pituitary gland, and it is also known that stressful conditions lead both to a higher rate of ACTH secretion (281, 387, 427) and to an increased blood flow through the adrenal gland (230). The rate of ACTH secretion is increased by the most diverse types of stress—chemical, environmental, and

8 W. S. Bullough and E. B. Laurence, unpublished results.
9 M. J. Coleman, personal communication.

4 M. Towison, personal communication.
psychologic—and the implication is that the various types act on the anterior pituitary gland through a common nervous pathway, which possibly includes the hypothalamus (see Refs. 229 and 427).

In such circumstances it is not surprising to discover that the secretion rate of the glucocorticoid hormones shows a pronounced diurnal rhythm (160, 209, 216, 222, 424, 497, 498); in addition it has been found that the reactivity of the adrenal cortex to ACTH is higher when an animal is awake (182). The details of the diurnal changes are complex and more difficult to interpret than those of the adrenal rhythm. However, the evidence is such as to support the possibility that the glucocorticoid hormones may play some role in mitotic control. If they act similarly to adrenalin then 2 things should follow: 1st, they should inhibit mitosis selectively in the tissues that normally show a diurnal mitotic rhythm; and 2nd, when the stimulus of ACTH is removed by hypophysectomy there should be a rise in the mitotic rate of these same tissues.

The published evidence is unfortunately not extensive, but on the 1st point it is already known that cortisol, or hydrocortisone, does inhibit mitosis in epidermis (68, 115, 258, 482), gastric mucosa (406), and regenerating liver (60); all these tissues show a diurnal mitotic rhythm. Conversely, it is known that cortisol has little or no effect on mitosis in duodenal crypts, intestinal lymph nodes (406, 413), and active hair bulbs (352); these tissues show little if any diurnal mitotic rhythm. Thus it is already obvious that, as with adrenalin, the action of the glucocorticoids is not mitosis specific but tissue specific. Again the implication may be that a tissue-specific cofactor, such as a chalone, may be needed before the anti-mitotic action can develop, but the information available is still insufficient to indicate whether the chalone-adrenalin inhibitory complex also normally contains a glucocorticoid element.

In passing it is interesting to note that interference with the adenalin mechanism in embryos, whether by cortisol injection or by adrenalectomy, may cause serious growth abnormalities (see Refs. 245, 345, 346).

Regarding the effects of hypophysectomy, this operation is so drastic that it is not surprising to find conflicting evidence. However, although the disordered metabolism that follows removal of the pituitary has been found to depress mitosis in regenerating liver (60), there is strong evidence that this operation can result in a dramatic increase in the mitotic rate of epidermis, sebaceous glands (145, 147, 481), and stomach mucosa (30), all 3 of which normally show a diurnal mitotic cycle. Conversely, hypophysectomy has been found to cause no change in the mitotic activity of the duodenal crypts (405), which normally show only slight signs of a diurnal mitotic cycle. It seems probable that in an operation of this magnitude the results obtained may be greatly affected by the technic used.

It is unfortunate that no study of the effect of hypophysectomy on the diurnal mitotic cycle appears to have been made with mammals. However, with amphibians, in which hypophysectomy is an easier operation, Scheving and Chiakulas (434) have found that in the corneal epithelium of salamander tadpoles, not only is the over-all mitotic rate increased, but the diurnal mitotic rhythm is eliminated.

3. Conclusions.—The evidence concerning diurnal mitotic rhythms, the anti-mitotic effects of stress, and the actions of adrenalin and other adrenal hormones has been listed at some length, partly because so much of it has not previously been extracted from the large Russian literature and partly because it illustrates so clearly that close similarities may exist between the mitotic control mechanisms of different tissues. In particular it shows that the type of mitotic control mechanism described for the epidermis by Bullough and Laurence (83, 87) is unlikely to be unique. Since it is now evident that in epidermis the hour-by-hour variations in the mitotic rate are inversely related to the hour-by-hour variations in the concentration of the chalone-adrenalin inhibitor within the cells, it may be presumed that similar chalones, which show similar relations with adrenalin, may exist in other tissues that show a diurnal mitotic rhythm, and perhaps even in all the tissues of the body.

It follows that, after allowance is made for the effects of the varying adrenalin concentrations, the degree of mitotic activity typical of any tissue may be explicable either in terms of the chalone concentration within the cells or of the degree of stability of the chalone-adrenalin complex. Thus, a tissue with a naturally low mitotic rate may have either a high chalone cell content or a chalone with a high affinity for adrenalin; a tissue with a naturally high mitotic rate may show the opposite condition. In both these extreme situations the diurnal changes in the adrenalin concentration would be unlikely to exert much effect.

A possibility exists that the glucocorticoid hormones may also be important factors in the mitotic control mechanism.

C. THE WOUND REACTION

In considering the reaction of tissues to wounding, attention is confined to the changes in the mitotic rate. Other aspects of wound healing, including the important question of cell migration, have been reviewed by, for instance, Gillman and Penn (189), Johnson and McMinn (273), Slome (453), and Abercrombie (2).

The account given above of the recognition and extraction of the epidermal chalone was itself the outcome of a study of the mitotic reactions of the cells closely adjacent to an epidermal wound (81, 84). It is well known that such cells may develop a mitotic rate more than 10 times the normal maximum rate seen during sleep, and for a long time it has been commonly held that this is the consequence of the diffusion outwards from the wound of a mitosis-stimulating "wound hormone." This old theory was critically examined by Bullough and Laurence (81), who showed that the mitotic reaction in the neighborhood of an epidermal wound is in fact due not to the presence of a mitotic stimulant but to the temporary absence of a mitotic inhibitor. By means of differential wounding they also showed that the epidermal mitotic inhibitor is tissue specific and that at least 2 other tissue-specific mitotic inhibitors also exist in mouse skin—1 each in the hypodermis and the hair roots (82).
As regards epidermis, it now appears that the mitotic inhibitor is the epidermal chalone, and it follows that a hypodermal chalone and a hair root chalone (see p. 1686) may also exist. If the high mitotic activity associated with local damage is due to a reduction in the chalone concentration, then as the mitotic rate rises the mitoses should show an increasing independence of the inhibiting action of adrenalin, and when the mitotic activity reaches its maximum they should be unaffected by adrenalin and should show no diurnal rhythm. It has been found that this is in fact the case, although it is possible that in epidermis the mitotic activity never achieves complete independence. The situation may be similar to that in the duodenal mucosa, which may respond to an adrenalin injection by a small and short-lived mitotic depression (158) and may show slight signs of a diurnal mitotic rhythm (64). Epidermal cells adjacent to a wound may therefore always retain a small quantity of chalone with which adrenalin can react.

It may be noted that the mitotic activity adjacent to an epidermal wound may not remain uniformly high from hour to hour as has commonly been believed, but that it may proceed in a series of waves that have nothing in common with a diurnal rhythm (235). This phenomenon has been clearly demonstrated by Harding and Srinivasan (226), who have found waves of DNA synthesis passing outwards from the edge of a corneal wound at an approximate rate of 17μ/hr. Hell and Cruickshank (235) suggest that this phenomenon is the outcome of an induced temporary synchrony in the cell cycle, and since it may be suggested that a normal function of the chalone-adrenalin complex is to inhibit the duplication of DNA, the sudden withdrawal of the chalone would be expected to have this effect.

The failure of adrenalin to limit the high mitotic rate associated with wounds is a point of considerable practical importance since it is obviously essential that any tissue damage should be repaired as quickly as possible, and since it is evident that, in addition to any normal stress, the presence of a wound may itself be a stressful factor. In this connection it is interesting to find that from about 1 to 8 hr after the infliction of a skin wound a high concentration of monoamine oxidase develops around the cut (402, 457), and it is possible that, by destroying adrenalin at an unusually rapid rate, this may help to stimulate the preparations for mitosis during the period before the chalone concentration is significantly reduced.

It must be emphasized that the pattern of the mitotic reaction seen after mechanical injury is essentially similar to that seen after damage by chemicals, radiation, or a variety of parasites (see Refs. 73, 277). After any kind of injury the affected cells, if they are not too heavily damaged, will themselves react by mitosis; if they are too heavily damaged, of if they are killed or removed altogether, then it is the closely adjacent cells that react. In either case the reaction could be due to the failure of the damaged cells to synthesize adequate amounts of their characteristic chalone or to an unusually rapid diffusion of the chalone away from the damaged area [it is known that the water relations of the cells are severely disturbed (see Ref. 492)], or to these 2 factors combined. Even in normal epidermis there is clearly a steady loss of chalone into the dermis and the blood, and it follows that it must be constantly synthesized within the cells (81).

It is important to note that the mitotic response to damage in the epidermis does not begin until after a delay of about 24–36 hr. However, a much earlier reaction, beginning in about 3 hr, leads to a great increase in the RNA content of the damaged cells (492, 494, 495, 523–25), and in about 12–24 hr there is a considerable increase in the DNA content (226, 235). The increase in the RNA content may be related to an increased rate of protein synthesis as the cells begin to alter their pattern of activity, perhaps especially in preparation for mitosis, while the increase in the DNA content is undoubtedly due to chromosome duplication in preparation for mitosis.

It may also be noted that considerable changes occur in the concentration and distribution of various enzymes as shown by histochemical methods (see Refs. 401–4), but the significance of these changes is not yet understood.

Although the details of the wound reactions are best known in the case of the epidermis, it is important to emphasize that both the premitotic and the mitotic reactions follow an essentially similar pattern in all tissues that are capable of mitotic activity and that have been studied from this point of view. This has been confirmed, for instance, in hair follicles (17, 82), tongue (45), esophagus (342), hypodermis (82), gall bladder and urinary bladder (340, 341), gastric mucosa (255), liver (100, 101, 330), uterus (508), and mammary gland (368). In all these tissues the mitotic reaction to local damage is itself strictly local, and as in epidermis (45, 81), it commonly takes the form of a gradient with the highest mitotic activity close to the wound edge. However, it is obvious that in different tissues the time taken for the full development of this reaction may vary widely; in epidermis, for instance, the response is relatively rapid, while in hypodermis it is relatively slow (82). It is possible that such differences may reflect the differing times required for different cell types to change from one program of synthesis to another, and they may also be at least partly dependent on the times taken for the local chalone concentrations to fall to a low enough level.

However, the most important single point emerging from a survey of the mitotic reactions shown by a variety of tissues to damage of any kind is that they are all essentially similar. It may therefore be concluded that the results obtained from an analysis of the reactions of any one tissue, such as wounded epidermis, may apply equally to most, if not all, other tissues that are capable of a mitotic reaction. In particular, since in the case of damaged epidermis the evidence now available favors the theory that the high mitotic activity is due to a reduced concentration of the epidermal chalone, it may follow that essentially similar chalone mechanisms may also be present in a wide variety of other tissues.

D. REGENERATION

1. Local and general mitotic reactions.—It is evident that the effect of a small wound can only be to damage or destroy a minute proportion of the total cell mass of the tissue, and that the consequent local reduction in the chalone concentration is unlikely to have any significant general effect on the chalone concentration in the tissue.
as a whole. This may be why all attempts to show that the presence of 1 wound can affect the process of healing of a 2nd wound made at a distance have always failed (1, 42).

Since it is known that the epidermal chalone normally diffuses into the adjacent tissues, it appears that each chalone may show a pattern of distribution in which the highest concentration is in the tissue of origin, a lower concentration is in the intercellular fluid, and the lowest concentration is at the point or points where the chalone is either degraded or eliminated. This implies a steady balance in which the rates of chalone production, diffusion, and loss are adjusted so that the chalone concentration within the cells is adequate to maintain the mitotic rate typical of that tissue.

It is obvious that such a system could be disturbed either locally, owing to a reduced chalone concentration within a small area, or generally throughout the whole tissue, owing either to a generally reduced rate of chalone production or to a generally increased rate of chalone loss. A local disturbance would, of course, be the result expected of a wound, while a general disturbance would be expected to follow extensive tissue damage or destruction or removal. Thus in the case of the liver a small wound leads only to a local mitotic reaction, as mentioned above, but when about 10% of the liver is removed a general mitotic response is seen throughout the whole organ, and when from 10% to about 70% is removed the mitotic reaction that develops is in direct proportion to the amount of tissue excised (see Refs. 60, 192). Essentially similar results have been obtained by measuring the rate of DNA synthesis; not until some 9–12% of the liver is removed does a general response develop, and beyond this threshold the number of cells that synthesize DNA is directly proportional to the amount of liver removed (62, 325).

The reactions of the liver remnant after partial hepatectomy have been closely studied, especially by histochcmical technics (see Ref. 60). These details are largely irrelevant to the present argument, but in stressing the essential similarity between the general regeneration reaction and the local wound reaction it is important to note that the nature and sequence of the cellular reactions are closely similar in both cases. In particular, a rise in the rate of RNA synthesis becomes obvious within 6 hr after partial hepatectomy (232, 436) and within 3 hr in wounded epidermis (494); an increased synthesis of DNA is seen from about 12 hr both in regenerating liver (for extensive literature see Ref. 60) and in wounded epidermis (226, 235); mitotic activity rises to a high level in 24–30 hr in regenerating liver (59, 109, 110, 228) and in 24–36 hr in wounded epidermis (81); and there are many other similarities. It must be noted that the figures given here for regenerating liver refer to the parenchymal cells; the non-parenchymal cells have a reaction that is slower by about 1 day (212, 228). Similarly in wounded skin the reaction of the hypodermal cells is slower than that of the epidermal cells (82).

Descriptions of the cellular changes during kidney regeneration are less detailed, but these changes are also evidently similar (108, 143, 144, 150, 163, 276); indeed in all cases of wounding or of regeneration, the essential cell reaction is a temporary change from synthesis for tissue function to synthesis for mitosis.

It is now clear that neither in the liver (see Ref. 60) nor in the kidney (204, 449, 514) can this change be due to an increased functional load or to an increased blood supply. Also, since the degree of the mitotic response is in direct relation to the amount of tissue removed, it is clear that it is related to an absence of tissue and not, for instance, to the amount of tissue damaged. In both organs it is generally believed that some humoral agent is the controlling factor, though opinion is sharply divided as to whether this agent is a mitotic stimulant (see especially Ref. 380) or a mitotic inhibitor (see especially Ref. 73) produced by the tissue or organ involved. Indeed it is often considered that both mitotic stimulants and mitotic inhibitors may be involved (see Refs. 60, 366, 510), and it has also been suggested that regenerative growth may be under the control of hormones produced by some endocrine gland and transported to the reacting tissue in the blood (see Ref. 1).

The most important single piece of evidence in favor of the humoral nature of the controlling agent comes from experiments with parabiosis in rats. In such experiments it is found that partial hepatectomy in one partner is followed by a regenerative response in the livers of both partners, and it is therefore most probable that the message passing from the operated to the unoperated rat must be carried in the blood (61, 116, 256, 515). The few reported failures to obtain such responses may be explicable in terms of technic (60). It may be added that essentially similar reactions have recently been obtained with liver autografts in rats, in terms of both mitosis (503) and DNA synthesis (310).

2. Hormones and regeneration.—The suggestion that regenerative growth by mitosis may be under the control of "orthodox" hormones has been considered, for instance, by Abercrombie (1) and Weinbren (510). In particular, attempts have been made to demonstrate some influence on liver regeneration by the hormones of the anterior pituitary gland (106, 173, 243). In brief, it has been found that, although hypophysectomy may result in a reduced liver size (391), the effects of the pituitary hormones on the regenerative process are not clearly defined. Weinbren (510) has concluded that any effects seen are more likely to "reflect the changes in general metabolism in hypophysectomized animals rather than any interference with the basic restorative process," and similarly, in relation to normal replacement growth, Bullough (73) has concluded that "most of the pituitary hormones, as well as such other hormones as thyroxin and insulin, are important to mitotic activity only in so far as they ensure the balanced working of the general metabolic complex."

There is, of course, a special group of tissues and organs that are under the direct control of particular mitogenic hormones (see p. 1703), but with these exceptions, and also with the exception of certain adrenal hormones, there is at present no indication that any of the orthodox hormones plays a direct part in the regenerative response. The growing belief is that those humoral agents that are
evidently involved in the control of the regenerative response will prove to be organ or tissue specific, to be synthesized in the organs or tissues on which they act, and therefore to be internal secretions that cannot be classed with the orthodox hormones. It is the thesis of this review that these agents may be chalones that act as tissue-specific mitotic inhibitors in the manner of a negative feedback mechanism.

3. Chalones and regeneration.—If this theory is correct then it should be possible to explain mitotic homeostasis in organs such as kidney or liver in the following manner. When the mitotic activity of the component tissues is low, this is because of the high chalone concentration existing within the cells. This high concentration is maintained by a rate of chalone production that is in balance with the rate of chalone loss, perhaps mainly into the blood. This rate of loss must be at least partly dependent on the steepness of the diffusion gradient from the tissue to the blood, and therefore the lower the chalone concentration in the blood the greater will be the rate of loss. When a large part of an organ system such as the liver is removed, the blood chalone level must also fall, and this must result in an increased rate of chalone loss from the remaining part of the organ. On the assumption that in this situation the surviving cells cannot significantly increase their rate of chalone production, the chalone concentration within these cells may be reduced to a level at which mitotic activity is no longer inhibited. In such circumstances, the observed relation between the degree of tissue destruction and the degree of the mitotic response, whether local or general, is readily understandable.

Since it is also suggested that each tissue is controlled by its own specific chalone, it is obviously possible that the concentrations of the various chalones within the surviving part of an organ system may be reduced at different rates, or that different degrees of reduction may be needed in different tissues before the mitotic response develops. In either case the differing reaction times observed for instance in the tissues of the liver (3) would also be readily understandable. In this connection, it may be recalled that in regenerating liver, mitotic activity develops first in cells around the branches of the portal vein as though this is the area from which the chalones are first drained (257).

The end of the regenerative response will, of course, come when the organ system is restored to its normal size in relation to the total body space, and when, in consequence, the normal chalone balance is re-established.

4. The experimental evidence.—A considerable proportion of the recorded experimental results relating to both the liver and the kidney can be interpreted to fit a theory of this type, which is closely similar to that put forward by Glinos (192, 193). Most of the experimental work has involved either alterations in the volume of liquid in the body or injections of macerates or aqueous extracts of the organs concerned in attempts to change the concentration of the humoral agents.

Considering first the question of the concentration of a humoral agent in the blood, some of the most important pioneer work was performed by Glinos and Gey (195), who induced mitotic activity in normal liver by diluting the blood, and by Glinos (190, 191), who performed the converse experiment of inhibiting mitotic activity in regenerating liver by increasing the concentration of the blood, which he did by limiting the water intake. However, it must be noted, first, that in carefully controlled experiments involving massive changes in blood concentration Bucher (60) has found "no consistent activation or suppression of regeneration," and second, that increasing the concentration of the blood by limiting the water intake may result in a state of stress similar to that produced by limiting the food intake (83). The belief that stress may inhibit mitotic activity in regenerating liver is supported by Jaffe's (266) report of the existence of a diurnal mitotic rhythm.

Contradictory interpretations of results are a feature of the literature on liver and kidney regeneration. In particular, while many authors have concluded that regeneration is initiated by a reduced concentration of a mitotic inhibitor, an equal number consider that it is controlled by the production of a mitotic stimulant. The literature has been extensively reviewed on many occasions (see especially Refs. 60, 73). Here it may be briefly noted that the serum of normal rats is reported to be capable of limiting mitotic activity in regenerating liver (261, 283, 461, 462, 466); that the serum of rats with regenerating livers may stimulate mitosis in the livers of normal rats (177, 249, 254, 299, 301, 455, 536); that the serum of hepatectomized rats may produce a greater than usual mitotic rate in livers of other hepatectomized rats (4, 445, 455, 461, 462); and that the dilution of serum, for instance with saline, is said to be able to induce mitosis in normal liver (60, 195, 365).

However, in none of these experiments has it been critically established whether the results obtained are explicable in terms of a mitotic inhibitor or of a mitotic stimulant. It may be suggested that part at least of the confusion may be due to the fact that the concentration of any humoral agent in the blood is likely to be relatively low and that, consequently, it might be more promising to investigate the actions of tissue macerates or extracts. There is a considerable literature derived from this type of experiment, but again the results recorded are contradictory, some for instance indicating that normal liver macerates or extracts contain a mitotic inhibitor, some that they are inactive, and some that they contain a mitotic stimulant.

Considering first those extracts that are described as containing a mitotic inhibitor, the earliest positive results appear to be those of Saetren (425, 426), who showed that when a kidney macerate is introduced into the body cavity after unilateral nephrectomy it is capable of inhibiting regenerative mitotic activity in the remaining kidney and, similarly, that when a liver macerate is used it inhibits mitotic activity in a regenerating liver fragment. Each of these actions is described as organ specific, and macerates of organs other than kidney and liver did not produce any similar effects. Saetren's work is almost unique in that he paid particular attention to dosage, and he noted that the most powerful inhibitions were obtained when the amount of macerate introduced was equivalent to
the amount of organ removed. He also noted that the active agents were heat labile and nondialyzable; and in this respect they resemble the epidermal chalone (78).

Support for these conclusions has been provided by Stich and Florian (462) and Molimard (353). It has also been noted by Saetren (423) that damaged liver contains no appreciable amount of the inhibitory agent and by Stich and Florian (462) that the same is true of regenerating liver. However, directly contradictory results have been recorded by Blomqvist (46), Paschkis (380), and Tumanischvili (496), who consider that macerates or extracts of liver, whether normal or regenerating, contain some mitosis-stimulating agent. For a full survey of this literature see Paschkis (380), Weinbren (510), and Bucher (60).

Finally, mention must be made of the suggestion by Malmgren and Mills (329) that a mitotic stimulus may be obtained only from a liver macerate that has been heated. They believe that liver tissue may contain both a mitotic inhibitor and a mitotic stimulant and that in normal circumstances their actions cancel each other out. When, however, the inhibitor is destroyed, the stimulant is unmasked and is able to act.

The only conclusion that can be drawn from all this literature is that, in the particular contexts of the various experiments, each and all of the results obtained may be correct and that therefore the conditions in which the experiments were carried out, and in which the observations were made, may be of critical importance in evaluating the conclusions that have emerged. In particular a survey of the literature shows that in both the serum and the organ extract experiments little attention has usually been paid to dosage, which commonly appears to have been very low; or to the degree of stability or instability of the extracts; or to the age of the animals, which were commonly young rats that had not yet reached their full size so that a varying degree of mitotic activity may naturally have been present in the kidney and the liver; or to the state of the reacting tissue, in which the mitotic rate may vary from hour to hour according to a diurnal rhythm (266) and from day to day during the process of regeneration; or to the proportion of the organ system removed, which determines the magnitude of the mitotic response and for optimal experimental purposes should be only 50% of the possible maximum; or to the time lag allowed either after the operation or after the injection before the mitotic response was measured; or in some cases to the adequacy of the criteria used in measuring the response; or to the manner in which the experimental manipulations may have affected the animals, especially in terms of the degree of stress imposed. These and other difficulties that stand in the way of the proper evaluation of the available evidence have been stressed by Weinbren (510), Bullough (73), Bucher (60), and MacDonald et al. (326), and it is obvious that further experiments involving a more systematic approach and more critical techniques are now needed to resolve all the various contradictions.

5. Mitotic inhibition and stimulus.—One possible explanation for the conflicting results that have been obtained emerges from the suggestion that both an inhibition and a stimulus may normally be produced after a macerate injection and that which of these is recorded depends on the time lag allowed between the injection and the observation. Thus Weinbren (510), using rats with regenerating livers, found a mitotic depression after 1 day and a mitotic stimulus after 2 days, while Wilson and Leduc (527), using young and adult normal mice, found a mitotic depression during the 2nd and 3rd days and a mitotic stimulus during the 5th–8th days. The differences in the timing of these responses may reflect differences between normal and regenerating livers, between the dosages used, and perhaps also between the species.

If it is confirmed that the reaction to the injection of a tissue homogenate is a rapid mitotic depression followed by a later mitotic stimulus, then 3 obvious possibilities exist. First, the homogenate may contain both an inhibitor and a stimulant (329), and these 2 elements may act successively; 2nd, if the homogenate contains a mitosis-inhibiting chalone, the initial depression it induces may be followed by a later compensating stimulus; and 3rd, if the tissue homogenate is injurious to the cells, or if it becomes injurious when it autolyses in the body cavity, the initial mitotic depression may be due to tissue damage, which, through reduced chalone production, ultimately leads to an increased mitotic rate.

Regarding the 1st possibility, little or nothing can be added to the evidence already given. However, regarding the 2nd possibility, it is known in the case of epidermis that the chalone-adrenalin complex, when fully active, imposes a complete inhibition at a point just prior to the onset of mitosis, although it evidently has no similar effect at any point in the long phase of DNA duplication (83, 88). After such an inhibition has been imposed for several hr abnormally large numbers of cells accumulate, all fully prepared to enter mitosis together as soon as the effectiveness of the chalone-adrenalin inhibition is reduced. Thus, if the mitotic counts are made a few hr after the experiment begins, a mitotic depression may be recorded; whereas if the counts are made later, when the inhibition is losing its power, an apparent, but unreal, mitotic stimulus may be found. It must also be added that this double effect may result from the action of endogenous adrenalin if for any reason the experimental treatment results in serious stress, but in this case the mitotic reactions should be equally obvious in all the tissues that normally show a diurnal mitotic cycle.

The 3rd possibility—that the injected macerate is injurious, or that it becomes so—raises again the old theory that the stimulus to regenerative growth by mitosis, which develops after tissue damage of any kind, is due to the production of "necrohormones" (see Refs. 290, 483). It seems reasonable to regard such substances, if indeed they can be proved to exist, as essentially the same as the hypothetical "wound hormones." However, since Bullough and Laurence (81, 82) have provided evidence that "wound hormones" do not exist at all, some doubt as to the existence of "necrohormones" also arises.

6. The effects of metabolic damage.—It does, however, remain possible that tissue extracts may contain substances that might be derived, for instance, from the lysosomes (see Ref. 370), that would not normally be
view comes from Saetren (425), who found that a kidney
easily explicable. Some confirmation for this point of
factors the application of which leads through tissue
damaged for 10 days by ureter obstruction no longer
cause damage to the liver cells, as well as to certain other
cells, and consequently result in a raised mitotic rate.

When such damaging agents are applied to mice clear
signs of cell damage in the liver precede the mitotic re-
action (528), and similarly injections of liver homogenates
have been described as causing “some degree of necrosis”
before the development of the mitotic response (15, 290).
This point was also considered by Wilson and Leduc
(527), who recorded an initial mitotic depression followed by
a mitotic stimulus after injections of macerates of liver,
kidney, and even boiled egg yolk and concluded that although “no injury was evident in most of our
experiments, it would be difficult to rule this possibility
out completely.” With any cytotoxic agent the question
of dose is obviously important. If it is too high the
damage may be too great, and indeed Wilson and Leduc
(527) have indicated that the higher the dose of liver
macerate the lower the ultimate mitotic stimulus. Ac-
ccording to Bullough (73), the correct dose for the
stimulation of mitotic activity can “be defined as that
which is sufficient to cause some cellular damage but not
sufficient to damage the cells so seriously that they can
no longer divide.”

In considering the wide variety of chemical and physical
factors the application of which leads through tissue
damage to increased mitotic activity, Bullough (73) has
emphasized that the “common factor in all these seem-
ingly diverse situations is evidently a particular level of
disturbance of, or damage to, some aspect of cell me-
tabolism” and that in all probability “such damage results
not in the production of a stimulating ‘wound hormone'
but in the failure to produce some factor which had pre-
viously prevented the cells from indulging in their basic
functions of growth and mitosis.” An essentially similar
conclusion has been expressed by Tsanev4 (492, 494, 495),
who has shown that when mouse skin is damaged by
pressure in such a manner that no cells are killed, and
therefore no mitosis-stimulating “necrohormones” are
produced, there is a sharp rise in epidermal mitotic ac-
tivity after about 24 hr. This agrees with the observa-
tion of Bullough and Laurence (81) that massage is all
that is needed to disturb the metabolism of the cells and
to raise the mitotic rate in ear epidermis.

Other situations in which cell damage may lead to an
increased mitotic rate are those in which ligatures have
been applied or tension has been induced. Thus it is well
known that a raised mitotic rate occurs in the kidney
after theligature of either the ureter (31, 239, 240) or
the renal artery (144). If it is assumed that such treat-
ment results in a reduced rate of chalone production in
the damaged kidney, then the rise in the mitotic rate
seen both in that kidney and in its opposite partner is
easily explicable. Some confirmation for this point of
view comes from Saetren (425), who found that a kidney

4 R. Tsanev, personal communication.
sequence of their "direct or indirect interference in DNA synthesis." The question of the points of action of chalones, adrenalins, and glucocorticoid hormones is discussed below on p. 1710.

8. Other evidence on regeneration.—In conclusion 2 other points of interest may be mentioned. First, it is evident that the mitotic reaction to partial hepatectomy, including DNA synthesis, develops much more rapidly in young animals than it does in old animals (see Ref. 60). The livers of these young animals still normally show many mitoses and may therefore contain a relatively low chalone concentration. A similarly low chalone concentration may also exist in livers damaged, for instance, by cirrhosis, and it is therefore interesting to note that the mitotic reaction to partial hepatectomy develops most rapidly in the livers that have suffered the greatest amount of such damage (400). Again this is in accord with Saetren's observation (425) that damaged liver contains less than the normal chalone concentration.

The 2nd point of interest is that after unilateral nephrectomy in a pregnant rat, the regenerative mitotic response is seen only in the remaining maternal kidney; there is no reaction in the fetal kidneys (203). It thus appears that maternal chalones are unable to pass the placenta, and this would appear to be a necessary precaution that prevents the maternal mitotic control mechanisms from interfering with fetal growth. There are also no diurnal rhythms in DNA synthesis in fetal tissues, including epidermis (509).

9. Conclusions.—In view of the conflicting evidence and the conflicting opinions it is particularly difficult to reach any general conclusions regarding the mechanisms underlying regenerative growth. It is clear that much of the confusion arises from inadequately planned experiments and especially from a general failure to study the mitotic reactions from hour to hour during a sufficiently long period of time. There is also the possibility that tissue extracts may contain not only the typical chalone but also certain toxic substances which, by damaging cells, may induce complex pathologic changes in the mitotic rate.

However, in spite of these difficulties, the following points may be emphasized. First, it is evident that with increasing tissue damage the local mitotic reactions seen alongside wounds grade imperceptibly into the general mitotic reactions of regeneration; there is such a close and detailed similarity between these local and general reactions that it is reasonable to conclude that they must be essentially similar. Since the mitotic reaction alongside a wound is most readily explicable in terms of the chalone theory, and since the evidence concerning the mitotic reaction in regeneration is not inconsistent with this theory, it is also reasonable to believe that regenerative growth may be basically explicable in terms of a negative feedback chalone mechanism. The 2nd point, which lends support to this conclusion, is the evident involvement of adrenalins and the glucocorticoid hormones in the control of regenerative growth.

E. THE MITOTIC CYCLE

1. The phases of the cycle.—It is well known that any cell, in passing from one mitosis to the next, traverses a series of phases of activity, and it is of considerable importance that these should be adequately defined and that the point or points of action of the chalone-adrenalin complex should be determined. There is a large literature devoted to attempts to disentangle and define these various phases (for early evidence see Ref. 252; for later evidence see Refs. 334–37) and also to determine the times spent by different types of cells both in the whole mitotic cycle and in each of the phases within the cycle (see Refs. 37, 132, 306). Techniques for determining the duration of the mitotic cycle and of its various parts are described by, for instance, Fry et al. (178), Smith and Dendy (454), Puck and Steffan (396), Quastler (399), and Hof and Ying (247).

The 4 phases commonly recognized as constituting the mitotic cycle are: mitosis itself (also called "M"); the "1st gap" (or "G1"); the phase of DNA synthesis (or "S"); and the "2nd gap" (or "G2"), from which the cell passes into mitosis once more. However, with increased knowledge this simple analysis of the mitotic cycle is no longer adequate, and on present evidence it is possible to recognize at least 6 phases, which were recently defined by Bullough (74). Still more recently, it has become possible to suggest slight modifications to these definitions to indicate more precisely what may be happening in each of the phases, and to relate them to that other important cell activity, which is specialization for tissue function. The 6 phases, arranged in sequence, are: apophase (a new term defined below); dichophase (from which a cell passes either to specialization for function or for mitosis); prophase, which is itself divisible into 3 phases (early prophase, phase of DNA synthesis, and antephas); and mitosis itself (see Chart 1).

Apophase: This is a term proposed here for the first time (and derived from apo, which implies "moving away from") to describe the period (commonly included in G1) between the end of mitosis and the beginning of the dichophase. Although this is a phase about which little is known, it is reasonable to suggest that the main preoccupations of the cell at this time may be recovery from the previous mitosis and growth in bulk so that the cell recovers the size typical of its kind. Although it is well known that cell growth does not necessarily follow mitosis, it seems probable that it may commonly do so in the cells.
of adult mammalian somatic tissues. This phase may therefore be regarded as the final part of the mitotic cycle, in which the cell returns to its normal state.

Dichophase: This is the period (commonly included in $G_2$) during which the cell is committed either to specialize for tissue function or to specialize for mitosis (the term is derived from δφοσ, which expresses movement towards). It appears that the dichophase may sometimes be short but that commonly it may be protracted. Its significance is discussed on p. 1701.

Prophase: This is the period during which the cell completes all the necessary syntheses to enable mitosis to begin (the term is derived from προφθαλμος, which expresses movement towards). At the moment it can be subdivided into the early prophase (commonly included in $G_1$), the phase of DNA synthesis (or $S$), and the antephase (or $G_2$). The most dramatic of these phases, and consequently the one that has been most intensively studied, is that of DNA synthesis. It is commonly forgotten that this cannot be the actual beginning of the preparation for mitosis but that at least 1 earlier phase must exist.

2. The initiation of the prophase.—The prophase may be defined as beginning with the synthesis of the 1st of the sequence of enzymes that underlie the mitotic process, and it is already known that accelerated RNA and protein syntheses do occur before the onset of the phase of DNA synthesis (250, 251, 257, 316, 322). It has been suggested that 2 enzymes in particular are likely to be of critical importance at this early stage. The 1st is that enzyme, or group of enzymes, which promotes histone synthesis; it is evident, for instance during liver regeneration, that the histone content of the nucleus begins to increase before the onset of DNA synthesis (217, 257). The 2nd is the DNA polymerase, without which the phase of DNA synthesis could not begin. Mazia (337) has questioned whether enzymes of these kinds may be synthesized directly on the chromosomes or whether, like other proteins, they are produced in the cytoplasm. He has also questioned whether the DNA polymerase alone is capable of inducing DNA synthesis and has shown that another enzyme may also be needed to prime the DNA before it can respond. Busch et al. (99) have suggested that DNA may be inactive when combined with histone and that the DNA strands are "released for replication by the removal of specific histones protecting against their replication," and it is possible that this removal may be the essence of the priming process. However, emphasis must be placed on the concluding words of Busch et al. (99) that "it is painfully apparent that we are just at the earliest stages of comprehension of the role of proteins in regulation of synthetic activities of the cell and in mitosis."

3. The phase of DNA synthesis.—It is significant that once the phase of DNA synthesis has begun it evidently always proceeds to completion. This is, of course, also true of the whole process—DNA synthesis-antephase-mitosis—but it is not yet certain whether the actual point of no return is at the beginning of the phase of DNA synthesis or at some previous point in early prophase. It follows that although active RNA and protein synthesis are necessary to initiate DNA replication, they may not be necessary to sustain it (225, 322). Indeed there is some evidence that during this phase the rate of RNA synthesis is normally depressed; that it is not stopped entirely may be due to the fact that the DNA molecules do not appear to replicate simultaneously and that consequently at any given time a certain number are always in a state in which they can initiate RNA synthesis (372, 394, 451). In microorganisms it has been suggested that the process of replication may pass in a linear manner along the entire genome (321), and in HeLa cells there is evidence that the average rate of DNA synthesis may reach its maximum at approximately the midpoint of the phase (485). It has also been postulated that, since the start of the phase of DNA synthesis may be dependent on the separation of the DNA from the histones, so also the end of the phase may involve their final recombination (217).

4. Antephase and mitosis.—The final period of preparation for mitosis is the antephase, which extends from the end of DNA synthesis to the beginning of mitosis. In different tissues and in different circumstances it has a duration of from about 1 to many hr, and with the chromosomal syntheses already completed, it appears to be the time when the final cytoplasmic syntheses are completed. Taylor (479) has shown that a partial inhibition of protein synthesis results in a prolonged antephase.

Unfortunately relatively little is known of these final syntheses, but it has been suggested that they probably include the production of those molecules that, at the appropriate moment, become reversibly aggregated to form the spindle apparatus (see Ref. 516). The possible binding mechanism used in the assembly of these macromolecular units has been discussed by Mazia (333, 334).

Another suggestion has been that the antephase is the time when an adequate store of energy-rich molecules is established to support the cell throughout mitosis. This suggestion has been widely accepted (70, 355, 467)—and indeed it has been shown that the spindle apparatus carries an ATPase (336)—but recently some important contrary evidence has been published (see Ref. 337). The theory originally arose from a study of mouse epididymis (see Ref. 79) that showed that mitotic activity is dependent on glucose and oxygen, but that any cell entering mitosis becomes independent of these substances. It also became apparent that the actual point of inhibition caused by glucose or oxygen lack is towards the end of antephase.

Later, similar results were obtained with cleaving sea urchin eggs (see Ref. 467), but recently these results have been criticized by Epel (153), who has shown that these eggs at all times possess an energy store sufficient to support full activity for about 20 min, by which time the ATP level falls to about 50 % of the normal. If at this moment the cells are part way through the process of mitosis, then they fail to complete the division. Doubt as to the establishment of an energy store has also been expressed by Amoore (8–13), who has studied pea root tips. In these he has shown that the effect of oxygen lack appears to be exercised through some interference with a "mitotic ferrous complex."

However, although these conclusions may be accepted, they do not at the moment seem to apply to such adult mammalian tissues as epidermis, or even to embryonic tis-
sues (128). In adult epidermis the blockage imposed by oxygen lack develops immediately in any cells in late antephase, but it does not develop in any cells actually in mitosis, even though this process may last for several hr (88). Thus, in the case of epidermis, no evidence exists to contradict the conclusion that the antephase may be a time when the energy debts of the dividing cell are paid in advance. However, this is certainly a matter that needs further investigation; the results of the experiments of Gelfant (184), which may appear to be relevant, have already been criticized (85) and need not be discussed here.

It remains to consider the manner in which a cell passes from antephase into mitosis. It is possible that this happens simply as the natural consequence of the completion of all the necessary preparations, but it is also possible, as suggested, for instance, by Mazia (337) and Taylor (479), that the transition to mitosis may be triggered by the synthesis of some particular enzyme. These suggestions are hypothetical, and the question remains unanswered.

The activities of a cell in mitosis are dramatic and include especially the assembly of the spindle apparatus, the separation and movement apart of the chromosomes, and the production of new cell walls. It is remarkable how little general agreement exists as to the manner in which any of these tasks is accomplished (see especially Refs. 335–37). However, there does appear to be general agreement that during mitosis both RNA and protein synthesis are depressed and even arrested (285, 393), and it is believed that this may be the consequence of the condensation of the chromosomes, the breakdown of the nuclear membrane, the disappearance of the nucleolus, and the disruption of the endoplasmic reticulum. It is evidently because of this dismantling of the cell machinery that all preparations for mitosis must be completed in advance and that, once a mitosis has begun, it is so immune to outside interference as to give the impression of being an all-or-none reaction (see Refs. 70, 73).

5. RNA and protein synthesis.—From this survey the suggestion emerges that the essential RNA and protein syntheses of the mitotic cycle may take place in a sequence of periods of high and low activity. Setting aside the phase of general protein synthesis that may commonly characterize the apophase and that may be regarded merely as a final phase of recovery from the previous division, the following periods can be recognized: a phase of protein synthesis, which initiates preparations for the next mitosis (early prophase); a phase during which the chromosomes, being concerned with DNA synthesis, are not in a state to induce active protein synthesis (phase of DNA synthesis); a 2nd phase of protein synthesis in preparation for mitosis (anaphase); and finally the phase of mitosis itself, during which protein synthesis is again depressed. It seems probable that if the early prophase and the anaphase are the times when protein synthesis is most active, then they may also be the times when metabolic inhibitors are able to exert their maximum effects and in particular when any natural mitotic homeostatic mechanism, acting through an inhibition of the syntheses that precede mitosis, may be expected to operate most powerfully.

In agreement with these conclusions it is already well known that the phases of both DNA synthesis and mitosis show a high degree of independence once they have begun and that the 2 points in the mitotic cycle that are most sensitive to interference are located before the onset of DNA synthesis and before the onset of mitosis. Thus Mazia (337) has concluded that no case is known in which DNA synthesis fails to be completed once it has begun, and Bullough (70) has reached a similar conclusion regarding mitosis itself. By contrast it is well known that cells may be arrested for days or months or years before they enter the phase of DNA synthesis, and Bullough and Laurence (83), by strengthening the chalone-adrenalin inhibitor, have shown that cells in antephase may be prevented from entering mitosis for periods of at least 2 days.

6. The action of the chalone complex.—This raises the question of the point or points in the mitotic cycle at which the chalone-adrenalin complex exerts its inhibitory action. It is at once clear that any interference in apophase is most improbable. If apophase can be partially defined as a period of general cell growth, and if the chalone complex suppresses the special syntheses needed for mitosis, then there is little reason to suspect any action at this time.

The first possible points of action of the chalone complex may be the dichophase, the transition from dichophase to early prophase, and the early prophase. Although no adequate investigation has yet been made, certain evidence is already available. Thus Bullough and Rytömaa (90) have shown that the granulocytic chalone, produced by mature granulocytes, reduces the numbers of bone marrow myelocytes that enter the phase of DNA duplication, while Brown et al. (57) have shown that in a stressful situation, which may be presumed to involve the production of excessive amounts of adrenalin, there is also a reduction in the numbers of intestinal cells entering the phase of DNA duplication. It has been shown above that stress and adrenalin provide the explanation for the diurnal mitotic cycle, and it is therefore significant that diurnal variations in the numbers of cells commencing DNA synthesis have been described in a wide variety of mammalian tissues (388). It is also interesting that these variations are found only in tissues that also show a diurnal mitotic cycle; in tissues in which the mitotic rate is so high that no clear cycle is evident, there is also no diurnal variation in the numbers of cells entering the phase of DNA synthesis.

If these results are interpreted to mean that the chalone-adrenalin complex controls entry into the phase of DNA synthesis in the same way as it controls entry into mitosis, then another parallel may be expected. It is known that in a stressful situation, such as starvation, cells may be arrested in antephase in such a way that large numbers accumulate and are ready to enter mitosis together as soon as the adrenalin concentration falls (83); it is now known that starvation reduces the numbers of cells entering the phase of DNA synthesis and that there also appears to be an accumulation of cells, in dichophase or early prophase, that enter the phase of DNA synthesis in abnormally large numbers when food is again provided (104, 286).

To this may be added the obvious evidence that after wounding or during regeneration, when it is suggested that the chalone concentration falls, unusually large numbers of cells enter the phase of DNA synthesis. The implica-
tion is that these cells had previously been prevented from taking this step by the inhibitory action of the chalone, and it follows that all the available evidence combines to support the suggestion that the chalone-adrenalin complex acts to inhibit the entry of a cell into the prophase.

Considering next the phase of DNA synthesis, the evidence of Pilgrim and Maurer (389) suggests that there is no diurnal rhythm in the duration of this phase. This suggestion in turn may be taken to indicate that adrenalin, and therefore the chalone-adrenalin complex, has little if any effect on a cell once it has begun to synthesize DNA. This conclusion is strengthened by the observation of Bullough and Laurence (83) that, both in vivo and in vitro, a high concentration of adrenalin results in the accumulation of abnormally large numbers of cells in antephase. This can only mean that adrenalin does not prevent the cells that are synthesizing DNA from progressing into the antephase. However, while not contradicting this conclusion, it has been noted by Rytömaa that not only does the granulocytic chalone reduce the number of cells entering the phase of DNA synthesis, but that it also slows the passage of cells through this phase. This is a matter for further investigation but already it is clear that cells in the phase of DNA duplication are not readily susceptible to inhibition.

In contrast, the evidence reviewed above overwhelmingly to the conclusion that the antephase is highly susceptible to the inhibitory action of the chalone-adrenalin complex in a wide variety of tissues. This is shown by the existence of diurnal rhythms; by the anti-mitotic effects of stress in vivo, and of adrenalin and chalone in vivo and in vitro; and by the raised mitotic rate after adrenalectomy and after chalone reduction by wounding. It is also interesting, and important to emphasize, that the main point of inhibition appears to be towards the end of antephase, perhaps only a matter of minutes before the cells enter the earliest recognizable stage of mitosis. Thus, in experiments in which epidermis containing no mitoses was taken from stressed mice and placed in a saline medium, high mitotic activity developed immediately (83). This may lend some support to the idea that mitosis is initiated by some trigger synthesis (335, 479) that is particularly sensitive to inhibition.

As regards mitosis itself, it has been found in epidermis that the chalone complex can act to slow the process but not to stop it and, conversely, that in the close neighborhood of wounds, where the chalone concentration is evidently reduced, the cells pass through mitosis more quickly than usual (88). However, when the situation is studied more closely it is found that the chalone complex has little or no action on a cell once it has entered mitosis; the slower passage through mitosis is the result of the action of the complex in antephase. It is interesting to note that the slower passage through mitosis that results from a lack of oxygen or glucose is also partly or wholly due to an inhibition in antephase, and it may be recalled that colchicine blocks mitosis in metaphase only as a result of its previous action on the cell in antephase. Although the full significance of these observations is unknown, it does appear that the progress of a cell through mitosis is usually influenced only by earlier events.

F. Conclusions

The evidence reviewed above suggests that mitotic homeostasis in the tissues in an adult mammal may commonly depend on the anti-mitotic actions of tissue-specific chalones and that these actions are strengthened by adrenalin and perhaps also by the glucocorticoid hormones. It appears that the chalone may be synthesized at a constant rate within the cells on which it selectively acts, while the concentration of the adrenal hormones varies according to the degree of stress felt by the animal. It may be noted that, since the chalone mechanism evolved to suit conditions in wild animals, which may commonly be subject to considerable stress, mitotic homeostasis may operate at less than optimum efficiency in well-kept domesticated animals.

The diurnal mitotic cycle appears to depend on the instability of a chalone-adrenalin complex, which evidently breaks down when the adrenalin concentration falls during rest or sleep. The details of this cycle are now seen to be more complex than was originally thought. When the adrenalin concentration rises after a period of sleep, cells are inhibited from entering the prophase, but those cells that are already in the prophase continue their preparations until they are arrested in the antephase; the few that manage to enter mitosis pass through it relatively slowly. When the adrenalin concentration falls during rest or sleep, cells enter freely into both the prophase and mitosis. The cells that enter mitosis are a mixed group, being partly those that are then completing the prophase and partly those that have accumulated in antephase during the previous waking hours. The cells that complete prophase during sleep pass through mitosis relatively quickly, but those that had previously been held in antephase pass through mitosis even more quickly. The result is that the beginning of a sleep period is characterized by a particularly high rate of mitosis; later in the sleep period the mitotic rate falls to a lower level that is determined by the rate at which cells are then completing the prophase (see Chart 2).

In considering the different types of diurnal mitotic cycles seen in the various tissues it is evident that they can be arranged to show every gradation between 2 extremes. These extremes are illustrated, on the one hand, by tissues such as those of the kidney and liver, in which

![Chart 2](attachment:image.png)

**Chart 2.**—Diagram illustrating a typical diurnal mitotic cycle. The highest mitotic activity is at the beginning of the sleep period (from Coleman).
the mitotic rate is so low that the counting techniques now in use are inadequate to determine whether or not a diurnal cycle exists, and on the other hand, by tissues such as that of active hair root, in which the mitotic rate remains constantly at the maximum. In an idealized form the range of types of diurnal rhythms lying between these extremes is illustrated in Chart 3, and it appears logical to suggest that the mitotic rate, which determines the type of cycle seen, may be in inverse proportion to the chalone content of the tissue. The evidence also suggests that when, after tissue damage, the chalone concentration is reduced and the mitotic rate rises, the type of diurnal cycle also changes and that in extreme cases, when the mitotic rate rises to a maximum, the diurnal cycle disappears.

It has been stressed that when the chalone concentration falls after tissue damage there is a collapse of functional specialization and a reversion to specialization for mitosis, and the hypothesis has been advanced that the choice between specialization for function and specialization for mitosis is normally made in terms of the chalone concentration within the cells. If this is true, then a chalone may be regarded as acting on the genome in the manner in which an effector is believed to act in a microorganism.

This hypothesis differs fundamentally from that of Weiss (514), which explains tissue growth in terms of tissue-specific "templates" and "anti-templates." The "templates" are regarded as catalysts in the production of that specialized "diversity of compounds" that is typical of the particular cell type, while the "anti-templates," which diffuse throughout the body and have a certain superficial resemblance to the chalones, act by inhibiting the "templates." This hypothesis implies that when, within the cells of a tissue, there is active synthesis of the specialized tissue proteins, then mitosis leading to tissue growth naturally follows, whereas in fact it is evident that when a cell indulges in synthesis for tissue function, this automatically involves an inhibition of synthesis for mitosis.

It is also difficult to perceive any relationships between the chalones, which are regarded as tissue-specific inhibitors, and such substances as "retine," which has been described by Szent-Györgyi and his colleagues as a nonspecific inhibitor (233, 470-72).

IV. MITOTIC AND FUNCTIONAL HOMEOSTASIS

A. THE BASIC MECHANISM

1. Mitosis and cell function.—If the chalone-adrenalin complex acts in the manner of an effector to determine which region of the facultative genome shall be active at any given moment, then when the efficiency of the chalone complex is high cells may tend to prepare for tissue function, whereas in fact the efficiency of the chalone complex is low they may tend to prepare for mitosis. Thus the primary function of the chalone complex may be the promotion and maintenance of tissue function, and the inhibition of mitosis, by which the existence of chalones has been recognized, may be merely the secondary outcome of this. There may even be a hidden diurnal rhythm in the number of cells preparing for tissue function, and if so it would of course be the reverse of the diurnal mitotic rhythm.

It has often been suggested that mitotic activity and functional activity are mutually exclusive. Thus, considering normal epidermis, it could be suggested that mitotic activity is the exclusive function of the unspecialized cells of the basal layer, and that once a cell has moved into the more superficial layers and has commenced keratin synthesis, mitosis becomes impossible. However, when this example is examined in greater detail, it is found that the basal cells of the germinative layer already contain bundles of fine fibrils, which are regarded as the precursors of keratin (54, 347, 374, 442). In fact the cells are clearly recognizable as epidermal cells, and indeed mitotic activity may commonly occur, as in human epidermis, in the basal layer of the stratum spinosum in which the fibrils are even more conspicuous.

Evidence that cells showing clear signs of their special nature and function may also indulge in mitotic activity has often been given; examples of this are seen during endochondral osteogenesis (535), erythrocyte production (373), and granulocyte production (381). However, in all cases of this kind it is clear that beyond a certain stage of cell specialization mitosis no longer occurs. Mäkelä and Nossal (328), studying the transformation of lymphocyte to plasma cell, noted an inverse relation between the antibody-forming and the DNA-synthesizing capacity of the cell, and Wessels (517) has made the important point that "the fact that mitotic figures are occasionally found in cells in the early stages of specific synthesis may be less significant . . . than the fact that no mitotic figures are seen in the more fully differentiated cells." It seems that this comment, made especially in relation to pancreas acinar cells, is generally true of all cell types, and as Weiss (513) has said, "the general impairment of proliferative capacity in cells undergoing terminal specialization can be ascribed to the fact that most of their substance, including mitotic prerequisites, is diverted to the building of differentiation products."

It thus appears that mitotic activity and specific cell function are indeed mutually exclusive but that mitosis only ceases beyond a certain point in the range of increasing preparation for tissue function. It is obvious that a graded effect of this type could be related to the effects of a steadily increasing concentration of the chalone complex within the cell, and certainly the evidence does not run
counter to the suggestion that mitotic homeostasis and functional homeostasis may be achieved by one and the same control mechanism. The maintenance of any tissue in a functional state automatically involves the control of its mitotic activity.

In this connection it is interesting to note that when certain tissues are incubated in vitro the cells tend to lose their specialization for function and to prepare for mitosis. This collapse of organization is readily explicable if it is postulated that a small fragment of tissue immersed in a relatively large volume of nutrient medium suffers such a marked reduction in the intracellular chalone concentration that the cells revert to mitotic activity. It is also relevant to note that when, as a result of this activity, a large enough cell mass is built up, the cells limit their preparations for mitosis and recommence their specialized syntheses. This has been described in vitro in mouse fibroblasts, which, when their numbers approach a critical level, begin to produce collagen (210, 211), and in pancreas acinar cells, which in similar circumstances begin to produce zymogen (517, 518). This latter case is especially interesting since it is the innermost cells that first cease mitotic activity and commence zymogen production; the peripheral cells, which might be expected to possess a lower chalone content, continue to undergo mitosis (see also Ref. 364).

One problem in such in vitro reactions is the source, if any, of the adrenalin cofactor. No information on this point is available, but there is evidence that a chalone may itself possess considerable activity which, in vivo, is merely augmented by the adrenalin.

When small groups of already specialized, or partly specialized, nondividing cells are placed in vitro, it seems most probable that the process by which they reacquire a mitotic ability must be the same process by which similar cells in vivo reacquire a mitotic ability after wounding or during regeneration. It is this kind of evidence that serves to stress the normal instability of the specialized tissue functions and also to emphasize that functional homeostasis is merely the obverse of mitotic homeostasis.

However, it is also obvious that in many, if not all, functional tissues there are cells that have entirely lost any capacity for mitosis. It seems improbable that the epidermal cells of the stratum granulosum, or those granulocytes that have been released from the bone marrow, can ever divide again. It is obvious that this must be true of the nonnucleated erythrocytes, and it is also obvious that this phase must be the final one in the life of a cell and that it can end only in death. It is evident that the proportion of the cell population in this final phase varies from tissue to tissue: it is relatively high in the granulocyte system and relatively low in the epidermis. In the nervous system and in striped muscle, all cells might be considered to be in this phase.

It appears that in any typical tissue there exist at least 4 main categories of cells (see Chart 4): the progenitor cells (P) that are involved in mitotic cycles; the immature cells (I) that have ceased, or are ceasing, to divide, and are preparing for tissue function; the mature cells (M) that may or may not be functional and that, if circumstances dictate, are capable of reversion to mitosis; and the mature cells (D) that are perhaps always functional but cannot revert to mitosis and are approaching death. It must be emphasized that the boundaries between the 4 cell categories are probably not as sharply defined as they appear in Chart 4 and that in fact each category may merge imperceptibly into the next. Epidermis is an unusual tissue in which these cell categories are stratified and in which a 5th category of cells—dead ones—are retained as the stratum corneum. In most other tissues the various cell types seem to be mixed indiscriminately.

2. Life expectancy of cells.—It is also important to note that the life expectancies of the 4 categories of cells are quite different. Cells of type P form a potentially immortal population, as is shown by their behavior in vitro and in neoplasia. Cells entering I and reaching M thereby acquire a certain limited life expectancy since M normally leads only to D and to death, and this life expectancy is evidently typical of the tissue to which they belong. However, in unusual circumstances involving a fall in the chalone concentration or, in certain tissues, a rise in the concentration of some specific mitogenic hormone, the cells in I and M can return to P and thus apparently become rejuvenated. Cells in D can only go forward to death, and their life expectancy may perhaps prove to be relatively constant in any one tissue.

In considering the balance within a tissue between the processes of cell production, cell function, and cell death it is obviously inadequate to consider only mitosis and function. The life expectancy of the cells entering I and M and the consequent rate of death from D are also critically important factors. It must be concluded that in any adult tissue not only is the average rate of cell gain by mitosis in exact balance with the average rate of cell loss by death, but the proportion of the cell population that is preparing to be or actually is functional (I, M, and D) is dependent on the life expectancy of the cells entering I. Since the chalone complex appears to play an important role in the maintenance of tissue homeostasis it is interesting to consider what influence, if any, it may have on the life expectancy of the cells in I, M, and D and thus on the rate of cell death. From many examples of tissues with widely differing mitotic rates it is possible to suggest that, as in duodenal mucosa, a low chalone concentration may lead to a high mitotic rate combined with a short life expectancy in I, M, and D, while a high chalone concentration, like that in the liver, may lead to a low mitotic rate combined with a long life expectancy, especially perhaps in M.

It thus becomes important to consider whether the chalone mechanism may act not only to promote the func-
tional maturity of the cells but also to prolong their life expectancy. In this connection it may be recalled that when, by long-continued stress induced by partial starvation, Bullough and Ebling (76) maintained the epidermal mitotic rate of adult male mice at a quarter of its normal level for 4 weeks, no change was recorded in either epidermal thickness or sebaceous gland size. It was obvious that in these 2 tissues the rate of cell loss must have been reduced to a quarter of its normal level. The converse situation has been described by van Scott and Ekel (439), who have shown that with increased epidermal mitosis in psoriasis the life expectancy of the cells was reduced from the normal 27 days to only 4 days, and by Skjaeggestad (452), who, from a study of the epidermis of hairless mice, has concluded that all hyperplasia-producing agents also induce an increased rate of cell loss. Similarly in the rat liver MacDonald (323) has described how normal cells divide at intervals of from 191 to 453 days, while with a raised mitotic rate in cirrhosis the cells have a life span of only about 26 days. The conclusions must be that when the efficiency of the chalone complex is decreased, the raised mitotic rate is matched by a shorter life expectancy and therefore a higher rate of cell death, and that when the efficiency of the complex is increased, the reduced mitotic rate is matched by a longer life expectancy and therefore a lower rate of cell death.

It must, however, be emphasized that only within limits is a change in the mitotic rate exactly matched by an opposite change in the life expectancy of the functional cells. In epidermis Bullough and Laurence have noted that when, in middle age, the number of mitoses increases to about 3 times the normal figure the thickness of the epidermis remains unchanged, while van Scott and Ekel (439) have noted that when, in psoriasis, the number of mitoses increases to almost 30 times the normal figure the epidermis becomes thickened. In this latter case it is evident that the decrease in the cell life-span was inadequate to offset completely the increase in the mitotic rate, and the tissue increased in size until it became stabilized at a new point of balance.

Thus, the evidence suggests that the homeostatic mechanism of which the chalone complex forms an important part controls the mitotic rate, the degree of synthesis for tissue function, and the length of life of the cells in I, M, and D, and the question arises how one mechanism could exert such a variety of actions. The simplest suggestion is that they all may be the outcome of the one basic chalone action, which is to promote the active synthesis of those proteins on which the specialized functions of the cell depend—an action that not only involves blocking any alternative types of synthesis, but also maintains the functional efficiency of the cells at a higher level for a longer period. This hypothesis is illustrated in Chart 5. The examples shown are taken from mouse data and include the duodenal mucosa, in which the mitotic rate is high and the life expectancy of the cells in M and D is about 2 days (311); the ear epidermis, in which the mitotic rate is moderate and the life expectancy of the cells in M and D is about 25 days (439, 443); and the liver, in which the mitotic rate is low and the life expectancy of the cells in M and D is about 3000 days (323). These are all, of course, normal and stable situations; but if, by tissue damage leading to a lowered chalone concentration, the mitotic rate rises in either the epidermis or the liver, then it may be expected that temporarily these tissues will move closer to the condition shown by the duodenal mucosa.

If this argument is taken to its theoretical limits then at one extreme, with no chalone present at all, M and D would disappear and all cells would remain in P; some such situation may perhaps be found in cases of advanced malignancy. At the other extreme, with an excess of the chalone complex, all cells would enter and remain in M, and their life span would be infinite; in effect this may be the case in the mouse liver, in which the estimated cell life-span of 3000 days greatly exceeds the possible life-span of the animal. It is, of course, obviously important that the degree of efficiency of the chalone mechanism. If this were not so then any tissue in which mitotic activity declined and finally ceased would be in danger of total disappearance as its cells passed steadily through M to D and death.

3. The nature of the dichophase.—It has been suggested above that the decision whether to specialize for mitosis or for tissue function is taken by a cell in dichophase in terms of the intracellular chalone concentration. However, it is evident that while in some tissues this decision may be taken promptly, in others the cells may remain in a state of indecision for a long time.

Tissues in which the decision is taken promptly are those with a high mitotic rate. An example is an actively growing hair root, the cells of which appear to lack any effective chalone control and therefore to pass almost directly from apophase into prosphase. The speed at which this occurs, together with the shorter duration of

![Chart 5](chart.png)
prophase and mitosis (88), results in recurrent mitotic cycles, each of which is completed in the shortest time needed for the essential syntheses. In an adult mammalian tissue it does not seem possible to reduce the duration of the mitotic cycle to less than about 12 hr (80, 440).

As the effectiveness of the chalone control increases in a tissue the duration of the whole mitotic cycle also increases, and it has been shown by Bullough and Laurence (88) that this is at least partly due to the increased time spent by the cells in prophase and mitosis. However, it is well known that the duration of the phase of DNA synthesis is not likely to exceed about 10 or 12 hr (see Refs. 103, 291, 312), the anaphase about 16 hr [which is about the maximum time that an animal may normally remain awake (see Ref. 83)], and mitosis about 6 hr [which is about the longest time taken by an epidermal mitosis with excess adrenalin (see Ref. 88)]; and although little is known of the length of the apophase, it is possible that this too may only last for a matter of hours. Thus even in extreme conditions the total duration of the whole cycle, prophase-mitosis-apophase, seems unlikely to exceed some 2 or 3 days.

However, it is well known that in tissues with moderate or low mitotic rates a mitotic cycle may take very much longer than this (see Refs. 37, 306); examples are the cells of the basal epidermal layer, which undergo mitosis about once in 25 days (439, 443) and the cells of rat liver, which may undergo mitosis only once in about 200–400 days (323). It therefore appears probable that in such tissues the cells must spend a considerable time resting indecisively in the dichophase.

It seems probable that any cell entering the dichophase is exposed to conflicting urges towards mitosis and towards functional maturity; this is expressed diagrammatically in Chart 6. With a low chalone concentration the decision to enter mitosis again would be quickly taken, the dichophase would be very short, and few if any cells would enter I, M, and D; with a high chalone concentration the dichophase would be equally short, the cells would rapidly enter M and become functional, and P would be depleted and even eliminated; but with an intermediate chalone concentration the dichophase might be very long and might consequently contain a large number of cells. The actual length of time that any particular cell remains in dichophase appears to be a matter of chance and may vary widely between cells, but in any particular tissue the average length of time a cell remains in dichophase may be precisely definable. The variable effects of chance on individual cells are shown when a cell population is artificially synchronized so that the cells leave mitosis in unison. It is found that progressive desynchronization occurs in the part of the cell cycle that may now be recognized as the dichophase (485).

When the chalone concentration falls after any type of cell damage, not only do the cells in dichophase tend to move towards the prophase, but the functional cells in M also tend to move back into the prophase. Epidermal cells already in dichophase may react to wounding by commencing DNA synthesis in as little as 4 hr (235); functional liver acinar cells, after hepatectomy, pass back through dichophase to commence DNA synthesis in about 20 hr (see Ref. 60).

Thus on the present hypothesis the dichophase emerges as perhaps the most critical of all the phases through which a cell passes and also as a phase in which, if the chalone concentration is at some indeterminate level, a cell may remain indecisively poised between the 2 possibilities that are open to it.

4. Conclusions.—In any stable adult tissue the balance between cell gain and cell loss appears to be determined, 1st, by the characteristic chalone concentration, and 2nd, by the characteristic life expectancy of the maturing cells. The primary function of a chalone may be to promote maturity, and since it is commonly believed that chalones are produced mainly by mature cells (73, 260, 348), chalone production may be self-promoting.

The characteristic life expectancy of the maturing cells varies, perhaps widely, among different tissues, but within any one tissue it changes inversely with the mitotic rate. It is important to note that when such a change takes place it appears to apply equally to the new cells then being produced and to the already mature cells.
It is also probable that the mitotic rate and the life expectancy of the mature cells have an inverse log-log relationship (see Chart 7). The result is that beyond a certain point the increasing mitotic rate outpaces the increasing cell death rate so that the tissue increases in mass until it reaches a new point of balance. However, in order to obtain a small increase in tissue mass it is necessary that there be a great increase in the mitotic rate.

It follows that the proportion of tissue mass to total body mass tends to remain remarkably constant, and indeed it seems possible that tissue mass itself might be determined by the combined actions of the rate of chalone production and the life expectancy of the mature cells. Thus it might be suggested that a tissue of large mass and low mitotic rate may be characterized by a relatively low rate of chalone production and a long cell life; a tissue of small mass and high mitotic rate, by a relatively high rate of chalone production and a short cell life; a tissue of large mass and high mitotic rate, by a relatively low rate of chalone production and a short cell life; and a tissue of small mass and high mitotic rate, by a relatively high rate of chalone production and a short cell life.

B. THE VERSATILITY OF THE MECHANISM

1. Methods of alteration of tissue mass.—It is evident that the point of balance between cell gain and cell loss, which may also determine the tissue mass, is normally remarkably stable. Only after wounding or during regeneration, when the mitotic rate is greatly increased, is this normal balance disturbed. However, the disturbance is temporary and self-cancelling, and as the chalone concentration returns to normal the original point of balance between cell gain and cell loss and the original tissue mass are both regained. Only if cell damage is constantly inflicted, for instance by any form of tissue irritant, can a new point of balance be established in a tissue of increased size.

However, certain tissues and organs must be able readily to increase their mass in response to particular needs. Thus the tissues of the accessory sexual organs become enlarged during the breeding season in response to the action of certain mitogenic hormones, and the mass of the erythrocytic tissue is increased during oxygen lack in response to erythropoietin. From such examples the impression is gained that the mitogenic hormones and the poietins may constitute a 2nd order of mitotic and functional control imposed on the basic chalone control mechanisms of the target tissues.

2. The mitogenic hormones.—It is well known that a number of hormones can exert a profound effect on the mitotic activity of certain so-called target tissues. It must be emphasized that the present argument is concerned only with those hormones that, in physiologic concentrations, exert a direct effect on the mitotic rate. Other hormones, not considered here, may be important to mitosis but only in so far as they ensure the balanced working of the general metabolic complex; in a normal adult mammal these are always present in adequate concentrations.

The best known examples of mitogenic hormones are the androgens and estrogens, although considerable information already exists regarding thyrotropin (1, 429). It is, however, surprising how little of the vast literature that has been devoted to these substances is concerned with the mitotic response of the target tissues, and this is especially true in the case of the androgens, which are normally vaguely described as stimulating the "growth" of the male accessory sexual organs (see, for instance, Refs. 376, 395). It is, however, evident that such growth does involve a raised mitotic rate and also that the mitogenic stimulus of the androgens is steadily maintained throughout the whole period of reproductive activity. This conclusion has been confirmed by studies of the steady mitotic stimulus exerted by testosterone on such nonsexual tissues as the epidermis (89, 359) and the sebaceous glands (146, 360).

It is also evident that the mitotic stimulus exerted by the estrogens, which is felt especially in the female accessory sexual tissues but also in other nonsexual tissues, must be steadily maintained. However, when the stimulus is first applied it results not in a constantly elevated mitotic rate but in a series of sharply defined waves of mitotic activity (see Refs. 63, 154, 157). The reaction is complex, and it also differs in its timing in different tissues. Thus the lining epithelia of vagina and uterus show their earliest reaction in about 12 hr (39, 385) and reach maximum mitotic activity in from 24 to 36 hr, while the lining epithelium of the Fallopian tube and the smooth muscle cells of the uterus may take 2-3 days to reach maximum mitotic activity (63). Similarly, in a normal estrous cycle the vaginal and uterine epithelia reach their 1st mitotic peak in late diestrus, while the other 2 tissues do not do so until estrus (38, 63, 154, 155). It is also obvious that, at the peak of the mitotic reaction, those tissues that react the fastest contain the highest proportion of cells in mitosis.

This situation is reminiscent of the different reaction times of the tissues of the skin after wounding (81, 82), and indeed there are obvious similarities between tissue reactions to a reduced chalone concentration and tissue reactions to an increased mitogenic hormone concentration.

After the 1st mitotic reaction to the estrogenic stimulus there is usually a period of at least a day during which the mitotic rate falls to a low level (63, 154, 155). Then if the estrogen concentration remains high, this is followed by a 2nd wave of mitotic activity, which is commonly smaller than the 1st wave. After this the waves are damped down and the mitotic rate settles at a moderate level, which however is considerably higher than that of the ovariectomized controls (383).

A full explanation of this phenomenon is not yet available, but Epifanova (156) has argued that the high initial mitotic stimulus may be reduced because of an estrogen-induced rise in the adrenaline and glucocorticoid hormone content of the blood. It is well known that estrus is a time of stress when the adrenal glands increase considerably in size (69) and when unusually large amounts of adrenaline are absorbed by the uterus (530, 531). However, it has been shown that, in mice stressed for long periods by starvation, "the uterus responded normally to physiological amounts of estradiol" (49), and this sug-
gests that the stimulus of an estrogen may be more powerful than the inhibition of the adrenal hormones.

As an alternative explanation it may be suggested that the mitotic waves are closely similar in form to those which occur in the epidermis alongside wounds (226, 235) and which have been plausibly explained in terms of an imposed mitotic synchrony. It seems that any type of sudden stimulus to mitotic activity may result in an unusually large number of cells entering prophase and mitosis approximately in unison, and if the stimulus continues the cells may also enter a 2nd mitotic cycle while still preserving some of their synchrony.

The evident similarities between wound reactions and estrogen-induced reactions help to suggest a possible relationship between the chalones and the mitogenic hormones. Since chalones are regarded as tissue specific and since mitogenic hormones tend to be target-tissue specific, the simplest explanation would be to suggest that each mitogenic hormone selectively neutralizes the chalones of its target tissues. In this connection Jensen (271) has already remarked that “it is curious that so little attention has been given to the possibility that estrogen may inhibit some growth-restraining factor, for many aspects of its action are more in keeping with an inhibitory rather than a stimulating role” and that the key property of any estrogen “might well be an ability to associate with some cellular factor and inhibit its function.” The result of such an action would be a reduction in the effective chalone concentrations in the target tissues, and if this interpretation is correct then certain consequences should follow.

In the 1st place, while the mitotic rate of the vaginal and uterine epithelia in the ovariectomized animal may be too low to show any diurnal rhythm, as the effective chalone concentration is reduced by the rise in concentration of the estrogenic hormone, a diurnal rhythm may appear [compare the situation in regenerating liver (266, 421)]. That this is indeed so is indicated by Bertalanffy and Lau (38), who have shown that diurnal mitotic cycles are evident in most phases of the estrous cycle in the vaginal and uterine epithelia. In the vagina the diurnal cycle disappears when mitotic activity is at its maximum, as it is also known to do in epidermis during the period of maximum reaction to a wound, and in both cases this could indicate a severe reduction in the effective chalone concentration.

In the 2nd place, if a hormone-induced mitotic reaction is basically similar to a wound reaction, then in threshold or subthreshold situations the 2 should be additive. In this connection it has recently been shown by Prop that, when mammary gland tissue is maintained in vitro, mitotic activity may indeed be stimulated when a subthreshold hormone stimulus is combined with a subthreshold wound stimulus. This is an important point on which further evidence is needed. With a normal wound in a hormone-dependent tissue the mitotic reaction is so great that the hormone is unable to induce the development of any more mitoses (for example, see Refs. 63, 368), and indeed in a full wound reaction there should be little or no chalone left for the hormone to neutralize.

In the 3rd place, if a mitogenic hormone normally acts by neutralizing a chalone, then the increased mitotic rate should result in a shorter dichophase and in a shorter expectation of life in the cells in the functional phase. Regarding the 1st point, Epifanova (156) has noted that estrogenic treatment may cause a 7-fold increase in the numbers of mitoses seen and a 7-fold decrease in the length of the interphase, and Peekham and Kieckhofer (383) have shown that the whole mitotic cycle may be completed in about 13 hr. Regarding the 2nd point, in an ovariectomized animal the rate of mitotic activity in the vaginal lining is very low and the life expectancy of the mature cells is very long; after treatment with estrogen the life-span of the nondondividing cells is reduced to between 30 and 45 hr (383). Even more remarkable figures demonstrating an inverse log-log relationship between hormone-dependent mitotic activity and the life expectancy of the nondondividing cells are given by Ebling (146, 147) in relation to the sebaceous glands of normal, castrated, hypophysectomized and testosterone-treated adult rats (see Chart 7).

These results are particularly important since they emphasize that although a target tissue, and the organ of which it forms a part, may become larger in size when stimulated by a mitogenic hormone, they do not become as large as would be expected from a consideration of the mitotic rate alone.

It is, of course, obvious that mitogenic hormones induce a variety of cell reactions besides the increased mitotic rate; in particular, the new cells that are produced begin to function in a new way (see Refs. 151, 271). For example, under sex hormone influence, considerable cytoplasmic changes occur in the uterine cells (180), the vaginal epithelium produces keratin and thus becomes cornified (97), the cells of the male accessory glands secrete the seminal fluid (395), and in the thyroid the pituitary thyrotropic hormone also stimulates the synthesis and release of the thyroid hormone (198). Such reactions involve the synthesis of unusual quantities of messenger RNA (107, 200, 282), which leads to the active synthesis of new proteins both in accessory sexual tissues (502) and in nonsexual tissues (433, 441).

It appears that when a target tissue is unstimulated and the mitotic rate is negligible the majority of the cells, which have an indefinitely long life-span, are mature but nonfunctional. They may be regarded as belonging to type M, shown in Chart 8. The hormonal stimulus causes them to revert to type P and thus to begin mitosis. Many of the newly formed cells then pass to maturity again, but now because of a severely reduced life expectancy they pass from type M, which is nonfunctional, to type D, which is functional. Thus they are able to function actively in the manner typical of the tissue in the hours or days or weeks that remain before they die. The sequence of this reaction is shown in Chart 8.

When the hormone is withdrawn and the chalone concentration is again high the mitotically active type P cells are transformed to type M and the functional type D cells must go forward to death, with the result that the tissue shrinks in mass.

3. The poietins.—Most of the dispersed cells of the
body fall into 3 groups—the granulocytes, erythrocytes, and lymphocytes—and the manner in which these are produced is of particular interest. The granulocytes are the end product of the process of granulopoiesis, which is generally believed to be stimulated and controlled by a substance called granulopoeitin, while the erythrocytes are the product of erythropoiesis, which is stimulated by erythropoietin. The more complex problem of the lymphocytes is dealt with in the next section.

The mechanisms by which control is exercised over the processes of cell production and cell loss in the granulocytic and erythrocytic systems are evidently complex, and they are not yet fully understood. Regarding the granulocytic system, Patt and Maloney (381) have stated that “little is known about its beginnings and endings, its dimensions and functions, and even less about its controls.” In this system most of the available information refers to the neutrophils, which are polymorphonuclear leukocytes. These are considered to originate from a population of stem cells, which give rise in the bone marrow to a sequence of cell types—the myeloblasts, promyelocytes, and myelocytes—that show increasing degrees of maturation but also undergo mitosis (90). Finally, they give rise to the metamyelocytes, which show no mitosis and mature into granulocytes. These are stored in the bone marrow until they are released for a limited life in the blood and the tissues (see Ref. 122).

The situation in the erythrocytic system is essentially similar (see Ref. 292), and the cells are usually considered to originate from the same stem cells as those that give rise to the granulocytes. In the bone marrow the maturation of the erythrocytic progenitor cells proceeds through a sequence of cell types—the pronormoblasts, basophilic normoblasts, and polychromatinc normoblasts—that show increasing degrees of maturation and that all undergo mitosis. Finally, the oldest cells become transformed into reticulocytes, which are incapable of mitosis and mature into erythrocytes. These are released into the blood immediately after they are formed, and they then have only a limited life-span.

Described in this way, the granulocytic and erythrocytic systems each constitute a typical tissue system, like that for instance of the epidermis, in that they contain cells that are mitotically active, cells that show increasing maturation accompanied by decreasing mitotic activity, and mature functional cells with a limited life expectancy. However, they differ markedly from a normal tissue in that they evidently share the same group of mitotically active stem cells, which must therefore be considered to be pluripotential.

Although the emphasis in the literature has been mainly on the possible manner of control of these 2 systems by their respective points, in the present context it is necessary to consider whether each may contain a typical chalone mechanism. Since in both systems the mature cells are dispersed it would, of course, be necessary to suppose that any chalone produced by them would have to pass via the blood stream to reach and influence the dividing and maturing cells in the bone marrow. It would also be expected, since in both systems the rate of cell production is very high, that the chalone concentration in the blood may be low. In such circumstances a reduction in the already low chalone content of the blood might have relatively slight stimulatory effect, but an increase either in the chalone concentration or in the activity of the adrenals might have a marked inhibitory effect.

As regards the granulocyte tissue, the evidence in favor of the existence of a negative feedback control of the chalone type has been assembled by Osgood (377, 378). In particular it has been shown that a decrease in leukocyte numbers leads to increased granulocyte production (see Ref. 122) and that an increase in leukocyte numbers inhibits granulocyte production, that fresh blood contains some factor that inhibits granulocyte production while old blood does not (520). However, in addition to the basic negative feedback mechanism, Osgood (377, 378) has proposed a theory of mitotic control in the granulocyte system and in tissues in general that is fundamentally similar to the chalone theory outlined above. However, in addition to the basic negative feedback mechanism, Osgood lists a long series of secondary mitotic controls, which includes vitamins, electrolytes, nutrients, temperature, CO2 tension, pH, and blood and lymph flow. Such factors, if they influence mitosis at all, are most likely to do so in a nonspecific manner and are unlikely to form any significant part of a tissue-specific mechanism.

Some attempts have been made to extract a granulocytic chalone from mature granulocytes (122, 123, 423), and recently Rytömaa and Kiviniemi have obtained a substance having the required characteristics when tested on the mitotic rate of bone marrow cells in vitro. This substance is water soluble, unstable, nondialyzable, and relatively low in molecular weight, all of which characteristics it shares with the epidermal chalone.

\[^8\] T. Rytömaa and K. Kiviniemi, personal communication.
As regards the erythrocytic tissue, relatively little attention has been paid to the possible existence of an erythrocytic chalone. However, it is well known that erythrocyte production is depressed after the transfusion of large numbers of red blood cells, and it seems probable that the erythrocytic tissue will prove to be similar to the granulocytic tissue in possessing a chalone mechanism (see Refs. 218, 295, 464). If this is so then the chalone may be produced in the circulating erythrocytes and it may influence only the mitotic activity of the erythrocyte progenitor cells.

If such a mechanism is present in both the granulocytic and erythrocytic systems, it follows that these systems may show a reduced mitotic rate during stress, and possibly even a diurnal mitotic rhythm. Similarly, they may show changes in the mitotic rate after adrenalectomy or after injections of adrenalin or glucocorticoid hormones. It is unfortunate that the literature on these various points, while voluminous, mainly records variations in the numbers of granulocytes and erythrocytes in the blood, and these may commonly depend on processes other than the mitotic rate (see, for instance, Refs. 27, 56, 199).

The granulocytic and erythrocytic tissues differ from most other tissues so far considered in that they must be able to adjust their rates of cell production not only to changes in tissue mass but also to changes in functional demand. In the case of the granulocytes, it is evident that mature circulating cells are eliminated not according to their age but according to the work they have to do in combating bacterial invasion, since in these cells function leads to death (122, 422, 484). In the case of the erythrocytes the mature circulating cells are eliminated, as in a normal tissue, according to their age, but the cell numbers must be able to increase if, for any reason, the oxygen supply to the tissues becomes inadequate (see Refs. 263, 464). In both these tissues it is evident that these adjustments are made through the actions of the 2 poietins. The sites of synthesis of these 2 substances are not yet certain, but granulopoietin may be produced in functioning or postfunctioning granulocytes, while erythropoietin may be produced in the kidney or other tissues (264, 351, 384, 411).

A second and more surprising way in which the granulocytic and erythrocytic tissues appear to differ from ordinary tissues is that throughout life both may continue to be formed from a common stem cell population, which may perhaps be located in the bone marrow. It is even possible that the stem cells may form part of the lymphocytic system (see Ref. 533). Although in the adult mammal the stem cells may prove to comprise 2 or more separate groups, each leading to only 1 mature cell type, the present evidence is generally held to indicate that they are pluripotent cells (29, 293). If this is accepted then, as Lajtha (292) has pointed out, there are 2 ways in which an increased number of mature cells may be obtained. First, an increased number of stem cells may differentiate to enter either the granulocytic or the erythrocytic tissue systems, and second, there may be an increase in the number of mitoses through which each maturing cell passes. Lajtha concludes that "it is quite possible and indeed likely that the two mechanisms require different controlling mechanisms," and it is worth considering what these might be.

If the stem cells are indeed pluripotent, this may imply that, unlike most tissue cells, they contain more than 1 set of genes that are potentially capable of directing the maturation of more than 1 type of cell. It appears in fact that in these cells the final step in differentiation has not been taken and that when it is taken, whether in the direction of the granulocyte or of the erythrocyte, the process must be akin to tissue induction in the embryo. In their final form the cells may be expected to contain only 1 functional set of genes, which will lead to only 1 type of maturation.

From what is now known of the chalones it seems most improbable that they could control this type of induction, and indeed specific chalone synthesis may itself be the consequence of a specific type of induction. It is the poietins that evidently act as inducers. Erythropoietin has no significant effect on either the mitotic activity or the rate of maturation of the erythrocytic progenitor cells (7, 294); its primary effect is merely to promote the conversion of the stem cells into erythrocytic progenitor cells (7, 294). In a similar way granulopoietin appears to promote the conversion of stem cells into granulocytic progenitor cells. The probable relations of the poietins and chalones of the erythrocytic and granulocytic systems are illustrated in Chart 9.

Thus the actions of the poietins are fundamentally different from those of the mitogenic hormones, although both groups of substances are similar, first, in that they are produced in response to a trigger mechanism operated by circumstances external to the animal, and second, in that they both induce the increased mass of their particular target tissues.

This theory leaves unanswered the problem of mitotic control in the stem cells themselves, but this is a problem that can hardly be attacked until these cells have been recognized and localized. Possibly they may synthesize

![Chart 9](chart9.png)
their own chalone or possibly they may respond to one or all of the chalones of their daughter tissues. In this connection it may be important that the mitotic activity of lymphocytes, which are evidently stem cells, can be suppressed by granulocytes (123, 124), possibly through the action of the granulocytic chalone.

4. Mitotic control in lymphoid tissue.—From a consideration of a wide range of evidence, Yoffey (533) has suggested that although the lymphoid tissue may appear to be entirely separate from the granulocytic and erythrocytic tissues, in fact all 3 may together constitute 1 large tissue system—the lymphomyeloid complex. He has concluded "that the different parts of the complex are in close functional relationship, and that through the blood stream there is in all probability a constant interchange of cells between them." At the moment this remains only an interesting possibility.

The lymphoid system is obviously highly complex, but it evidently produces only 2 types of "mature" cell, the small lymphocyte and the plasma cell (see the review by Yoffey (534)). The production pathway of the small lymphocyte is often believed to begin with the reticular cell, which remains static in the lymphopoietic center and which, by a series of successive mitoses, gives rise to the large lymphocyte, the medium lymphocyte, and finally the small lymphocyte. If this is the basic mitotic system of the lymphoid tissue, and if the small lymphocyte is considered to be a "mature" cell, then the system displays the usual pattern, whereby increasing maturity is matched by decreasing mitotic activity.

However, it is by no means certain what role the small lymphocyte plays in the body. Its rate of production is high. In the thymus Leblond and Sainte-Marie (305) consider that the division of 1 reticular cell usually results in the production of 128 small lymphocytes, and in the mesenteric lymph node Grundmann (214) estimates that 1 reticular cell may give rise to 64 small lymphocytes. This rate of production argues either a short life-span or a rapid transformation into some other type of cell, or both. All that is known is that large numbers of small lymphocytes migrate via the lymph and the blood to the tissues, and it is believed that large numbers may settle in the bone marrow (206, 207).

This system is essentially similar to that seen in the erythrocytic and granulocytic tissues, and this suggests the possible existence of a chalone control mechanism. There is no direct evidence to support this suggestion, but considerable indirect evidence has been reviewed by Dougherty (139), who shows that the glucocorticoid hormones, administered in excess, result in an involution of the lymph nodes, thymus, and spleen that is characterized by a sharply reduced mitotic rate and a lower lymphocyte output. The same effect is seen after any kind of stress, and there is also a marked diurnal rhythm in the lymphocyte content of the blood. These stress effects are eliminated by adrenalectomy, which increases the mitotic rate and raises the number of lymphocytes in the blood while simultaneously reducing their life-span. Furthermore, during recovery after several hr of imposed stress, the blood lymphocyte content not only quickly returns to normal but for a few hr exceeds it, an effect which could be the result of a stress-induced accumulation of cells in anaphase. It is evident that all these effects are closely similar to those described for epidermis and other tissues in which they are dependent on the existence of a chalone mechanism.

The 2nd and apparently separate mitotic system of the lymphoid tissue is that by which the plasma cell is produced. Leblond and Sainte-Marie (305) regard this as an alternative form of maturation to that which gives rise to the small lymphocyte, and they describe the sequence of mitoses and of cytoplasmic maturation which they believe to occur between the large lymphocyte and the plasma cell (see also Ref. 369). In sharp contrast is the point of view expressed by Yoffey (533, 534), who regards the plasma cell as being derived from the small lymphocyte (see also Refs. 205, 208). He describes the way in which this cell grows in size and becomes transformed into a pyroninophilic cell, which then undergoes successive mitoses to form a group of daughter plasma cells. Certainly it appears that the small lymphocyte can be stimulated to grow in size and undergo mitosis both in vivo (205, 390) and with phytohemagglutinin in vitro (338, 371).

It is now generally agreed that the function of the plasma cell is the synthesis of antibody. Whether the small lymphocyte or the reticular cell is the parent cell, it is the sudden stimulus of the invading antigen that results in the production of the pyroninophilic cell and in the burst of mitotic activity that ceases only when the plasma cell matures and fills with antibody (22). It is well known that each invading antigen calls forth the production of an appropriate antibody, and therefore it must be concluded that each plasma cell is narrowly committed to only 1 program of protein synthesis. Yoffey (534) has described the plasma cell as "conditioned" and has commented that "whatever theory of antibody production may ultimately prove to be correct, it is clear that in the primary response there is some conditioning of the lymphoid cells, whether genetic or otherwise." Medawar (343), expressing a similar point of view, has emphasized that "the antigen, like the inducer, is an agent that commits a cell to a certain pathway of differentiation—to one pathway among several that it might have taken, and that lie within the genetic capability of the cell." This point of view is closely similar to that expressed above when the actions of the poietins were likened to those of embryonic inducers.

If it is tentatively accepted that the small lymphocyte may be the immunologically competent cell then a speculative theory can be developed as follows. The supply of small lymphocytes is maintained by the mitotic activity of a population of stem cells, which are the larger lymphocytes and the reticular cells. To maintain itself in balance this population must be controlled by some feedback mechanism that limits the mitotic rate and promotes the maturation of the small lymphocytes. The role played by the adrenals indicates that this may prove to be a typical chalone mechanism, and if so then the system will be self-balancing since the loss of small lymphocytes, whether by death or differentiation, will lead to a lowered chalone concentration in the blood and lymph.
On this view the small lymphocyte is regarded as a cell in which the final step of differentiation has not been taken and which therefore still possesses a number, perhaps a very large number, of genetic possibilities. The antigen, acting as an inducer, limits these possibilities to only 1—the synthesis of the appropriate antibody—and at the same time evidently frees the cell from the mitotic control that had previously held it inert. If final differentiation always includes an acquired ability to synthesize a specific chalone, then a newly differentiated cell may always lack sufficient chalone and therefore always undergo mitosis until, in the plasma cell, the intracellular chalone concentration rises to an effective level. Once again, although there is no direct evidence available to support the theory that a chalone mechanism is present, there is some indirect evidence derived from a study of the actions of glucocorticoid hormones. White (522) has recently reviewed evidence that these hormones reduce the production rate of the plasma cells, but not the rate of antibody production in any cells already formed.

A plasma cell is immunologically active and, being fully differentiated, it is unable ever again to revert to a state of immunologic competence. Indeed it is generally believed that plasma cells may be short lived (121, 369, 437). The basic response of the lymphoid system to antigen stimulation is in fact the creation of a new type of tissue that was not present in the body before, and although the plasma cells may all belong to type D (see Chart 4) and therefore die, some cells, perhaps of type M, may survive for long periods. These long-lived cells may superficially resemble the short-lived unconditioned lymphocytes (see Refs. 168, 207), and they may be able to take part in a secondary response. Such a response would then involve a double reaction: the increased activity, perhaps including mitosis as well as antibody production, in already existing tissue cells; and the induction of new cells into the tissue. This would imply that an antigen may normally act, directly or indirectly, on both activity, perhaps including mitosis as well as antibody production, in already existing tissue cells; and the induction of new cells into the tissue. This would imply that an antigen may normally act, directly or indirectly, on both the demands of function, which are mediated through the inducer-like action of an antigen. With the great variety of possible antigens, such a system must be unusually complex, and the homeostatic mechanisms controlling mitosis and cell maturation are also likely to be more complex than in any other tissue system of the body.

5. The growth of hair.—It has been suggested above that the relationship of tissue mass to body mass is a function of the rate of chalone production in the mature cells. The question now arises how the mitotic rate may be controlled in such a tissue as hair, in which the mature cells die so rapidly that no significant mass of chalone-producing cells can be built up. The high mitotic activity of the hair bulb results in the production of a shaft of dead cells, which are obviously incapable of chalone production, but when the hair reaches its correct length, or mass, the mitotic activity ceases. It is obvious that some mechanism must control the phases of activity and inactivity of the cells, and since it seems unreasonable to expect that this mechanism may be unique, it is worth considering how a typical chalone mechanism might be modified to produce this effect.

The complex pattern of hair growth has often been described (see Refs. 80, 112, 357). Reduced to its simplest terms it involves 4 successive phases of inactivity and activity. In the 1st phase the cells of the hair bulbs are in a resting condition (called telogen) in which no mitosis is ever seen; in the 2nd phase (early anagen) the cells show increasing mitotic activity leading to the lengthening of the follicle; in the 3rd phase (anagen) the cells show continuous high mitotic activity, and it is the duration of this phase that determines the length of the hair; in the 4th phase (catagen) the mitotic activity decreases and the follicle shortens to enter the resting condition once more. In any 1 area of skin all the follicles may follow this rhythm in unison, as is the case in mice and rats (141, 142); alternatively, each follicle may follow its own individual rhythm, as is the case in guinea pigs and men (112). In either case it seems that the control mechanism must be intrinsic in the individual follicle and not systemic (141, 142).

Considering first the resting follicle, it is evident that the cells are similar to those of any ordinary inert tissue in that they are able to react by high mitotic activity to any form of wounding, and thus it appears that they must normally be controlled by a high chalone concentration. In contrast, the cells of the actively growing follicle show an extremely high rate of mitosis, a mitotic cycle length of only about 12 hr (80, 111), and a complete lack of response to stress or adrenalin. Indeed the cells react as though little or no chalone exist within them.

One obvious possible explanation for the rhythm of hair growth lies in the suggestion that it depends inversely on a rhythm of chalone production, and this agrees with the suggestion of Chase (112) and Chase and Eaton (113) that the onset of hair growth may be due to “a more or less rapid loss or leaching of an inhibitor.” This theory is expressed diagramatically in Chart 10, and the evidence both for and against it has recently been summarized by Ebling and Johnson (149).

If this theory is basically correct then it should follow...
that the adrenal hormones should be without any effect in either the resting or the actively growing phases, but should exert a pronounced action during the transition periods. Thus, for instance, when the chalone concentration falls towards that critical level at which mitotic activity begins, the enhancement of the efficiency of the chalone complex by stress or by adrenalin injection should delay the onset of the phase of hair growth, while the weakening of the efficiency of the chalone complex by adrenalectomy should accelerate it. As regards stress, Blumenthal (47) has shown that when mice are maintained on a reduced diet the initiation of new waves of hair growth is delayed or even prevented (see also Chase, 112).

As regards the adrenal hormones, Mohn (352) and Ebling and Johnson (149) have shown that the initiation of hair growth is accelerated by either hypophysectomy or adrenalectomy and that it is delayed by injections of ACTH, cortisone, or adrenalin. Once again it appears that a glucocorticoid hormone may be involved as a third partner in the control mechanism.

Thus the available evidence tends to support the theory that hair growth may be controlled by the normal type of mechanism, but, whereas in other tissues the rate of chalone production may be relatively constant, in the hair bulb it appears to be rhythmic. This rhythm may be inherent in the genes directing chalone synthesis or it may be imposed or modified by factors in the follicular environment that affect the activity of these genes. The available evidence strongly suggests that the ultimate control lies in a gene or group of genes that oscillate between activity and inactivity. For any particular type of hair the durations of the various phases of the rhythm are evidently constant, but between different types of hair they vary widely. Oscillating genetic control of this type may be analogous to the oscillating genetic control of estrous cycles (see Ref. 215).

It is, however, evident from the latest results of Ebling and Johnson (148, 149) that the follicular environment may exercise a moderating influence on the basic hair growth rhythm. In a similar manner the follicular environment may modify gene expression at the agouti locus within the melanocytes associated with the hair root (43, 446); perhaps the most spectacular example of this is in such northern species as the varying hare and the arctic fox. In these the color of the growing hair is dependent on the expression of the melanocyte genes, which is determined according to the season by the physiologic condition of the animal.

6. Conclusions.—From this survey it appears that the mechanism of mitotic and functional homeostasis in certain adult mammalian tissues is subject to a 2nd order of control by mitogenic hormones and that in a few tissues the situation is also modified by the actions of poietins or antigens that augment the tissue mass by inducing the final differentiation of stem cells.

Regarding the mitogenic hormones, it is clear that because of the effective inverse balance between cell gain and cell loss a great increase in the mitotic rate is needed before any significant change in tissue mass can occur. It seems that the effects of these hormones are dramatic mainly because the target tissues in their unstimulated condition commonly show no mitosis at all. Their manner of action supports the suggestion that they may neutralize the chalones of the target tissues, and their action on the genome is therefore indirect. It is interesting that in insects Kroeger (288) has also concluded "that hormones . . . cannot act directly on the genetic loci, and that there must be an intermediate system which relates the hormonal stimulus to the loci."

It seems possible that the hormone-dependent tissues are merely those that are characterized by an exaggerated dependence on hormones, which also operate to some degree in all normal tissues (63). The evidence suggests that target-tissue specificity is expressed in a quantitative rather than a qualitative manner, and regarding the sex hormones Bullough (63) has emphasized that most tissues may possess some sensitivity, which gives them the potentiality for enlargement in relation to the sexual processes should the need ever arise. Examples of accessory sexual tissues that seem to have evolved in this way are the posterior region of the stickleback kidney, the thumb pads of the frog, the comb and wattles of the cock, the sexual skin of the monkey, and the mammary gland. In all such cases it is evidently the tissues that become modified, through changes in their mitotic and functional homeostatic mechanisms, to enable them to react more strongly with the hormones. The hormones themselves have evidently remained unchanged. It must also be emphasized that the mitogenic hormones probably exert a wide variety of actions inside cells, and that only one or some of these are directly related to mitosis.

It is interesting that all the known mitogenic hormones, such as androgens, estrogens, prolactin, and thyrotropin, are under the control of the anterior pituitary gland, which is itself influenced by the nervous system and thus by information entering through the sense organs. Although in most laboratory mammals the rate of secretion of the mitogenic hormones may be wholly controlled by inherent genetic mechanisms, as in the case of the estrous cycle (see Ref. 215), in most wild mammals the rate of secretion of these hormones is modified by environmental changes that act as trigger mechanisms. This is particularly obvious in relation to seasonal breeding cycles (see Ref. 72).

It is interesting to note that in laboratory animals the mechanism controlling hair growth is also evidently genetically controlled, while in most wild mammals the growth of a new coat is a seasonal phenomenon. Here again gene action is evidently modified by seasonal changes. However, although the time of onset of hair growth...
growth may be determined in this way, the end of anagen appears to be determined solely by gene action.

It has been stressed that increased tissue mass cannot easily be obtained through increased mitotic activity but that in the granulocytic and erythrocytic systems increased tissue mass is simply achieved by the incorporation of new cells that were not previously part of these tissues. This is essentially an exploitation of an embryonic mechanism whereby, under the direction of either granulopoietin or erythropoietin, relatively undifferentiated stem cells are induced to undergo their final differentiation. The poietins, like the antigens that act in a similar manner on the relatively undifferentiated, immunologically competent lymphocytes, are also part of a mechanism whereby the animal can respond to environmental change. Although the manner of control of erythropoietin production is not yet clear (see Refs. 20, 379), granulopoietin production seems to depend on the rate of granulocyte destruction, which is a function of the work the granulocytes have to do. Similarly in the case of the lymphocytes, antigen induction is dependent on unpredictable circumstance.

It is evident that mammals possess only limited stem cell populations, and in this they differ markedly from many amphibians in which the regeneration, for instance, of entire new limbs indicates the existence of stem cells with great potentialities. However, the recurrent growth of antlers in deer has not yet been analyzed from this point of view (see Refs. 201, 202).

C. THE BREAKDOWN OF THE MECHANISM

1. The mechanism itself.—The essential features of the theory of mitotic and functional homeostasis outlined above are summarized diagrammatically in Chart 11; for simplicity, regulator genes are not included. The process of induction, by which the tissue originated, involved the suppression of all genes that are relevant to other types of tissue (the "blocked genes"). The remaining genes that are, or may become, functional form the facultative genome (170), and they include genes that dictate the synthesis of general metabolic enzymes (the "essential genes"), genes that dictate the synthesis of those special enzymes on which the tissue organization depends (the "tissue genes"), and genes that dictate the synthesis of those enzymes that underlie the mitotic cycle (the "mitosis genes"). In the chart the syntheses controlled by the tissue genes and the mitosis genes are regarded as occurring in a series of steps, each of which is under separate gene control. In each case only one synthetic pathway is indicated although, of course, there must be many. One group of tissue genes (the "chalone genes") are regarded as specifying both the structural details and the rate of synthesis of the tissue-specific chalone, which then promotes the activity of the remaining tissue genes. If the chalone concentration falls (as in wounds) not only does this promotion cease but many of the already functional cells lose some or all of their special characteristics. It is not clear whether in promoting tissue specialization the chalone acts alone or whether adrenalin (and perhaps a glucocorticoid hormone) is also involved.

It is, however, clear that the power of a chalone to inhibit the activity of the mitosis genes is strengthened by adrenalin. Since it seems reasonable to believe that the mitosis genes may be the same in the cells of all tissues, it is difficult to understand how within any one tissue they could develop the ability to respond only to the chalone of that tissue. One possibility is that a tissue-specific, chalone-sensitive trigger mechanism may operate at the beginning of the phase of DNA synthesis and another at the beginning of the phase of mitosis. Both these phases, once begun, are self-supporting.

Finally, it should be emphasized that although a chalone-adrenalin complex appears to be a central feature of the homeostatic mechanism, the effects of withdrawing chalone and those of withdrawing adrenalin are very different. When most of the adrenalin is withdrawn (as after adrenalectomy) the epidermal mitotic rate rises 2-
or 3-fold (83); when most of the chalone is withdrawn (as after wounding) the epidermal mitotic rate rises perhaps 10- to 50-fold (81). Also, the adrenaline effect is immediate, evidently because this hormone is so rapidly gained or lost, whereas the chalone effect takes many hr, perhaps because it is more stable and less easily lost, but perhaps also because it may act most powerfully in early prophase.

Although in Chart 11 the situation shown is obviously oversimplified and inadequate, it is at least useful in indicating some of the main points at which the homeostatic mechanism may be broken, either temporarily or permanently.

2. Carcinogenesis.—The effects of a temporary breakdown of the homeostatic mechanism have already been considered at length; the question of a permanent breakdown leads to a consideration of the relation of the chalone theory to the cancer problem. The current theories of carcinogenesis have been reviewed and criticized by Hieger (241, 242), but even more valuable for present purposes are the recent statements by Foulds (170-72).

It is clear that one of the most important theories concerning carcinogenesis derives from the work of Bernblum (34) and of Rous and Kidd (416), who distinguished between a process of initiation, whereby a number of dormant and unrecognizable tumor cells are formed within the tissue, and a process of promotion, whereby these dormant cells are caused to multiply to produce a visible tumor. Initiation, which is irreversible, may perhaps occur spontaneously or may be the result of any kind of carcinogenic action; promotion, which at least in its early stages is reversible, may also be spontaneous or may be hastened by a promoting agent. The inadequacy of such a simple analysis has been stressed by Foulds (171, 172), who has pointed out, first, that the action of a carcinogen is quantitative. With smaller doses fewer tumor cells are produced, and these, after promotion, develop only into papillomas, nearly all of which regress. With larger doses more tumor cells are produced, and these, after promotion, give rise either to papillomas with the power to progress into carcinomas or occasionally to carcinomas ab initio. Second, Foulds emphasizes that, whether spontaneously or after treatment with carcinogens, tumors may arise at one or many sites within a tissue, appearing at random both in space and time, and that occasionally they may even involve a whole organ. Third, Foulds concludes that a benign tumor is subject to 4 possible fates—"namely regression, indolent persistence, growth without qualitative change, and progression to malignant neoplasia"—but that the common tendency is for any tumor to become progressively more malignant.

To explain the cellular changes that underlie these phenomena one widely held theory is that initiation involves 1 or more somatic mutations, and it is evident that these mutations might involve damage to the genes that previously controlled mitotic and functional homeostasis (55). In Haddow's (220) words, it is possible to "regard the problem as one of the impact, upon the genonal integrity, of any of a host of reagents", and it is now evident that similar damage can be inflicted on cells in vitro (25, 26). It is even known that a specific chromosome abnormality is usually associated with chronic myeloid leukemia (38). If the gene damage leads to the production of "foreign" proteins, then presumably the immune mechanism will be activated, and as Burnet (93) has suggested, this may be one of the main defences against cancer. If, however, the mutation leads to the weakening and ultimate cessation of gene action then the immune mechanism will presumably not operate, and there is indeed considerable evidence that tumor cells are deficient in specific proteins and show a loss of immunologic specificity (see Ref. 93).

In a carefully reasoned criticism of the theory of somatic mutation, Rous (415) has emphasized the relative lack of evidence that such mutation does commonly occur and has also stressed the important point that all carcinogens are not mutagens and all mutagens are not carcinogens. This latter point is also emphasized by Trainin et al. (489), who however conclude that it is "probable that only a small proportion of mutagenic agents would share the common site or sites of action which one would postulate to be involved in the initiation of carcinogenesis."

Hieger (241, 242), too, has objected that gene damage must be a comparatively rare event and that if, for cancer to develop, several distinct mutations are needed, as indeed seems to be the case, then the chances of initiation decrease "to practically zero." However, Burnet (94, 95), while agreeing that "at any given genetic locus an error in replication occurs with a frequency in the range 10^-9 to 10^-7 per replication," has pointed out that in an animal as large as a man some 10^4 cells may be constantly reproducing themselves. However, all such arguments omit a number of important considerations. First, they do not take into account the effects of carcinogenic agents in increasing the mutation rate (see Ref. 296), and it must be emphasized that many so-called spontaneous tumors may have been induced in this way by as yet undetected carcinogens. Second, they do not consider the possibility that gene damage, whether spontaneous or induced, may occur in nondividing cells, and indeed Curtis (127) has presented evidence that nondividing mammalian cells accumulate deleterious mutations at such a rate that by late middle age "virtually all cells carry many gene mutations." Third, they omit consideration of the possibility that gene damage or failure may be at least partly dependent on inherited gene weaknesses, and that in such animals as man it may even be "that the majority of malignancies are of this kind" (see Refs. 91-93).

If for any of these reasons gene function is impaired then the reaction of the damaged cells would be expected to vary according to the type of gene or genes involved. For the present argument the simplest situation would be one involving some failure in the genes that determine the production rate or the structure of the tissue chalone, and even a relatively slight failure would tend to give the cells some proliferative advantage. According to the Darwinian point of view advocated by Burnet (96), this might seem sufficient to ensure the appearance of a tumor, but in fact it is now clear that certain counterchecks would be expected to come into operation to limit and even to prevent any extra mitotic activity. First, if only single cells, or small groups of cells, are damaged, the chalone...
concentration within them should remain normal because of diffusion inwards from the surrounding undamaged cells. An increased mitotic rate should not be seen until the group has grown to such a size that its central cells are beyond the effective range of chalone diffusion, and judging from the length of the chalone diffusion path adjacent to a wound (81), this critical cell mass might have a diameter of a little less than 1 mm.

Beyond this point, so long as the rise in the mitotic rate is only moderate, a 2nd countercheck might begin to operate. This would depend on the expectation that any rise in the mitotic rate would be offset by a reduction in the life expectancy of the mature cells. Thus the latent period described by Berenblum (34) and Rous and Kidd (416) may include the whole of the period from initiation to the end of the period of effectiveness of this 2nd countercheck. This question is considered further in the next section.

So far only the expected consequences of damage to the chalone genes have been considered. It is obviously possible that gene damage might also involve, or even only involve, those other tissue genes that dictate the synthesis of the typical tissue proteins. The result of this would be expected to vary widely according to which genes were damaged and in what degree. In the 1st place the damage might involve the gene operators through which the chalone mechanism ultimately acts, and this could have similar consequences to chalone lack. Second, the damage might upset the balance between cell gain and cell loss either by reducing the rate at which the tissue genes synthesize tissue protein (183, 414) or by increasing the life expectancy of the mature cells. It seems probable that delayed or inadequately realized maturity may increase the proportion of the cell population that remains capable of division, and in this connection Berenblum (36) has emphasized that promoting agents commonly appear to operate "by producing a delay in maturation of the dormant tumour cells". Regarding the life expectancy of the mature cells, it is known that this is enhanced by the chalone complex, but it is possible that this action may operate through a mechanism that might be separately subject to damage.

In the 3rd place, the variety of possible damage to the tissue genes could account for Fould's (170) conclusion that "tumours derived from the same tissue by the same carcinogenic procedure may be extremely varied and intergraded" so that "it is probable indeed that no two tumours are exactly alike in every respect." Foulds (170) also emphasizes that tumor characters are not static but tend to change progressively, that such progression occurs independently in different characters in the same tumor, that it occurs in tumors that are no longer growing, and that it may be continuous in large numbers of small steps or discontinuous in small numbers of large steps. Commonly such progressive changes evidently also involve further gradual breakdown in the mitotic and functional homeostatic mechanism, which may lead to greater malignancy. Although the evidence is still inadequate, the view of Klein and Klein (279, 280) that this is due to progressive gene damage may be tentatively accepted.

One effect of such tissue gene damage might be seen at the cell surface. It is commonly believed that the cell surface structure is tissue specific, with the result that the cells of each tissue tend to hold together. With progressive malfunction of the genes controlling surface structure, a point may be reached when that structure becomes so modified that the cells separate and metastasis results.

One final possibility may be mentioned—namely, that the gene damage might involve the mitosis genes. In this case it is probable that the cells would be unable to divide or that they would die during attempted division, and it is indeed one of the aims of radiotherapy to inhibit tumor growth in this way.

From this very brief review of the evidence it appears that both initiation and progression may involve sudden and irreversible changes in the genome; that with increasing age such gene damage seems to occur at random in most if not all tissues; that this process may be augmented by carcinogens and supported by inherited gene weaknesses; and that if, by any route, such damage leads to malfunction of the mitotic and functional homeostatic mechanism, so that the balance between cell gain and cell loss is disturbed, a tumor may result.

3. The chalone complex and the latent period.—Between the process of initiation and the appearance of a tumor there is the latent period, which can be shortened by the application of a promoting agent. This process of promotion is usually neither sudden nor irreversible, and it is important to question whether the speed at which it proceeds may be related to the state of the mitotic homeostatic mechanism, not only in the damaged cells but also in the surrounding normal tissue. It has already been noted that the latent period may be shortened either by hyperplasia (220, 242) or by a delay in the maturation of the dormant tumor cells (36), and it has also been emphasized that in normal tissues any weakness of the chalone complex leads both to increased mitotic activity and to delayed maturity.

The length of the latent period is evidently determined by chance, and in most epithelia there is the added possibility that the damaged cells may be shed. Since, if the animal lives long enough, all those latent tumor cells that are not shed may produce tumors, the difference between an increased chance and a decreased chance should lie mainly in an earlier or a later average time of appearance of the tumors. The question to be answered, therefore, is whether with an inefficient chalone complex, as after tissue damage or adrenalectomy, the latent period will be shortened, while with an efficient chalone complex, as during stress, the latent period will be lengthened.

4. Promoting agents.—To take first the question of tissue damage, there is an old hypothesis that, given sufficient time, irritation or ulceration or scarring automatically leads through hyperplasia to cancer. Although this theory of the origin of cancer is inadequate today (242), it does remain possible that an increased mitotic rate may increase the chance of a genetic mistake and tend through chalone lack to favor the multiplication of any tumor cells that may be so formed. There is certainly strong evidence that epidermal wounding or irritation will promote the earlier appearance of epidermal tumors in carcinogen-
treated skin (175, 176, 327, 397, 398, 416, 417, 450). Similarly it has been reported that regeneration following partial hepatectomy promotes the earlier appearance of tumors in carcinogen-treated liver (194, 303, 304) and that primary hepatic carcinoma occurs with greater frequency in cirrhotic livers. However, both wound repair and regeneration are normally short-lived episodes, and it is therefore not surprising that a stronger promoting action can be obtained by repeated tissue damage (397, 398).

A 2nd way in which mitotic homeostatic mechanisms may be damaged is by adrenalectomy, and because of the increased mitotic rate this may also be expected to act in the manner of a promoting agent. Unfortunately most investigations have failed to distinguish between the processes of initiation and promotion, or have related solely to the growth of established tumors, which may be adversely affected by the general metabolic disturbance (32, 33, 409, 469). Recently, however, Trainin (488) has made a careful analysis of the effects of adrenalectomy and has shown that, although it has no effect on initiation, it strongly enhances promotion. This is in agreement with Law (302), who found that adrenalectomy increases the incidence of spontaneous lymphoid leukemia, and with Metcalf (349), who found that high leukemia AKR mice are characterized by low production of glucocorticoid hormone.

The 3rd type of promoting agent includes irritant chemicals, such as croton oil, which are often considered to act through cell damage and consequent hyperplasia. However, the theory that hyperplasia is the essence of promotion has been critically discussed by Berenblum (35, 36), who has suggested that promotion must depend on a dislocation of the normal balance between cell gain and cell loss and that a promoting agent may exert its action more by preventing cell maturation than by promoting mitosis. On present evidence it is difficult to distinguish the relative importance of these 2 processes, and it is possible that in different circumstances either or both may have the same consequence, which is the build-up of a group of damaged cells sufficiently large for the central area to be freed from chalone control.

After successful promotion the tumor cells are commonly able to continue their multiplication without further assistance. It follows that the act of promotion must either have altered the nature of the tumor cells in the direction of autonomy or have enabled them to realize characteristics they already possessed. As they emerge from their dormancy the tumor cells may give the impression either that they are lacking sufficient chalone or that they are no longer able to respond to whatever chalone is present. If the 2nd alternative is correct then the cells should be unable to respond to induced changes in either the chalone content or the adrenalin content of the tissue. In fact, however, it is evident that newly formed tumors are commonly able to respond to such changes. Thus Trotter (491) has shown that in the liver partial hepatectomy results in a greatly increased mitotic rate in small adenomatous nodules (see also Refs. 185, 320), and further she has pointed out that as malignancy increases this power of reaction is lost. In a similar manner certain types of tumors continue to show a diurnal mitotic rhythm (317–19, 505), while others that are perhaps more malignant show no diurnal rhythm at all (135, 197).

It may therefore be concluded that both the dormant tumor cells and the cells of benign tumors may normally be capable of responding to the chalone mechanism. Indeed, even after a tumor has appeared, it may still possess such a relationship between cell gain and cell loss that it may reach a new point of balance, and, at least for a time, it may remain in the state of “indolent persistence” described by Foulds (171). Commonly, however, a progressive loss of functional maturity may lead to a progressive reduction in the chalone concentration and so to an increased mitotic rate, which is dependent both on a progressive shortening of the cell cycle length (410) and a progressive increase in the proportion of cells involved in mitosis. In addition, those cells which do manage to mature should die more quickly, and at the theoretical extreme, growth would be explosive. An approximation to this extreme may be seen in many ascites tumors, in which the cell cycle may be reduced to as little as 11.5 hr (132) and in which no mature cells are present. Unfortunately, relatively little precise information is available on the mitotic rates or on the durations of the cell cycles of tumor cells as compared with those of their tissues of origin (see Refs. 132, 344).

It seems probable that at some point during progression the cells may lose their ability to react to any remaining chalone and that from then onwards they are fully autonomous. It has been found, for instance, that malignant leukemic granulocytes may continue to produce a chalone-like substance capable of inhibiting mitosis in normal granulocytic progenitor cells (429) but that they themselves are unable to respond to it (123, 124). With respect to those few tumors that are already malignant when they first appear, it may be assumed that their loss of ability to respond to the normal homeostatic mechanism occurred in 1 large step during either initiation or dormancy. In either case promotion would be unnecessary and growth would be rapid.

5. Retarding agents.—If a weakened mitotic and functional homeostatic mechanism is an essential feature of promotion then a strengthened mechanism may prolong the period of dormancy of the tumor cells. It is apparent that in those tissues, such as liver and kidney, which have a naturally low mitotic rate, and presumably therefore a naturally powerful mitotic control mechanism, the mutation rate may be high (127) but the chances of promotion are so low that the cancer incidence is also low; in those tissues, such as epidermis, which have a weaker mitotic control mechanism, the chances of promotion are much greater; while in those tissues, such as the basal cells of the duodenal crypts, which apparently possess a very weak mitotic control mechanism, the high chances of promotion may be powerfully offset by the high rate of cell loss. Curtis (127) has noted that, while a mitotically inert tissue spontaneous mutations build up to a high level, in a mitotically active tissue they remain rare.

The simplest way to strengthen the homeostatic mitotic mechanisms of the tissues is by means of stress, which leads to a higher rate of secretion of adrenalin and glucocorticoid hormones. If the chalone mechanism in the
damaged cells is even partly active, this should retard the multiplication of the latent tumor cells and delay the growth of benign tumors. Direct studies of the effects of stress are few, but it has already been shown that a longer latent period is found in mice stressed by high temperatures (179, 506, 507), low temperatures (513), or excessive noise (324), and recently Anderson (14) has concluded that “apprehension [gives] some protection against carcinogenesis.” Stressful conditions have also been shown to delay the growth of existing tumors, which may therefore have been relatively benign (289, 332, 407, 419).

However, the most extensive and important work on the effects of stress on carcinogenesis is that of Tannenbaum and Silverstone (473–78) and of Rusch et al. (418, 420), although none of this work was originally considered in terms of stress. Tannenbaum was the first to show that with a wide variety of latent tumor cells, both spontaneous and induced, promotion is delayed and even prevented by a restricted diet, but determined attempts to explain this effect in terms of the diet itself have failed. It has been noted, however, that such treatment results in an increase in the size of the adrenal gland (49, 432, 478) and in an increase in the secretion rate of both adrenalin (196) and the glucocorticoid hormones (182).

A survey of the large literature on the effects of restricted diets and carcinogenesis has been made by White (521). From this it is clear that not only may promotion be prevented but the growth of what are probably relatively benign tumors may be delayed (see also Ref. 248). One important observation, made by Pearson (382), shows that when underfeeding retards the growth of an implanted tumor, the cells may show signs of reacquiring some degree of functional maturity, and this is, of course, exactly what would be expected with a strengthened chalone complex if the process of progression towards malignancy had not gone too far. It is also clear that when progression has gone too far a restricted diet has no effect (521).

If this retardation is indeed due to a strengthened chalone complex then a similar action should be exercised by adrenalin or the glucocorticoid hormones. The effect of adrenalin does not seem to have been tested, but the effect of the glucocorticoid hormones is well known. In particular, the careful work of Trainin (488) has clearly shown that, while hydrocortisone has no effect on initiation, it strikingly inhibits promotion. The cortisone inhibition of spontaneous leukemia (267, 499, 529), of induced skin tumors (28, 152, 187), and of induced skin and lung tumors (188) is probably due in each case to a similar inhibition of the process of promotion. Contrary evidence that cortisone acts to increase the yield of induced skin tumors (436) may be due to changed hair follicle activity (444).

The effect of glucocorticoid hormones on the growth of existing tumors differs in different cases, but a reduced growth rate has been described in a lymphosarcoma (234, 463), in lymphoid leukemia (181, 244, 274), and in various other tumors (465). However, such glucocorticoid-sensitive tumors commonly progress to a state of glucocorticoid resistance (18, 297, 298). This change has the stable character of a mutation, and it presumably denotes progression towards greater malignancy. There are several known cortisone-resistant types of tumor, but it is interesting that their cells may still bind glucocorticoid hormones in a normal manner (98). This may possibly suggest that the chalone mechanism remains and that the cells have lost their power to react to it.

Such evidence tends to confirm the conclusion that both latent tumor cells and relatively benign tumors may normally be subject in some degree to the mitotic homeostatic mechanism of their tissue of origin and consequently that anything that strengthens this mechanism may delay the appearance and retard the early growth of a tumor. The only known retarding agents are glucocorticoid hormones, and it is obviously important to discover whether adrenalin and the various chalones are similarly active. Regarding chalones it has been noted that when excess blood, presumably containing the granulocytic chalone, is introduced by transfusion, chronic myeloid leukemia may be suppressed (520) while acute leukemia is unaffected (123). Such unresponsive malignant cells may fail either to respond to a normal chalone concentration or to produce significant amounts of chalone: the 1st alternative may be illustrated by malignant leukemic granulocytes that continue to exert a chalone-like inhibition of mitosis in granulocyte progenitor cells, and the 2nd by other malignant leukemic granulocytes, which are no longer able to inhibit lymphocyte mitosis (see Refs. 122–24).

6. Conclusions.—The theory that the processes of initiation and progression may commonly be due to gene damage, acquired or inherited, leads to the pessimistic view of the cancer problem described, for instance, by Rous (415) and Burch (93). In Burnet's words (94), “Everything suggests that, somatic cells being what they are, the impact of the environment must inexorably lead to an accumulation of mutant cells, some of which will have malignant descendants if life persists long enough.” However, it is already clear that the average time of tumor appearance may be considerably delayed by retarding agents. The agents so far known are those adrenal hormones that are produced in response to stress and that act to strengthen the mitotic homeostatic mechanism. Their potential power has been strikingly illustrated in the experiments of Tannenbaum (473, 474), in which the latent tumor cells have remained suppressed throughout the entire lifetime of the animal. These experiments, which hold out the hope of large-scale cancer prevention, may yet prove to be some of the most important in the history of cancer research. It is also evident that retarding agents that act by strengthening the homeostatic mechanism may delay the growth of visible tumors, at least as long as these remain in the earlier stages of progression.

In the converse situation, the promoting agents, which advance the time of tumor appearance, may all act by weakening the homeostatic mechanism with effects that are evident in terms of mitotic rate, cell maturation, or cell life expectancy. One natural factor that may act in this way is increasing age. It has been noted in a number of species, including man, that from middle age onwards there is commonly a progressive degeneration of the adrenal cortex (130, 140, 268–70), and it has also been
reported in both mice and men that middle age may be a
time of increased mitotic activity (67, 487). However,
the effect of middle age on adrenal function is not yet
clear, and the situation is further confused by the differing
reactions of individuals and of strains within 1 species
(486). Little is known of changes in the rate of adrenalin
secretion, although in men Kärki (275) has concluded that
"the adrenal medulla maintains its activity up to a high
age," while in mice Bullough and Laurence3 have indirect
evidence indicating that the adrenalin output may be
much reduced. In the case of the glucocorticoid hor-
mones the evidence is also inconclusive. On the one
hand, it is generally agreed that in man the rate of ex-
cretion of the hormone derivatives declines progressively
during middle age (224, 278, 519), but on the other, it
appears clear that this does not reflect any significant
change in the plasma hormone level (246, 519). It
appears that a reduced adrenal secretion rate may be offset
by an impaired capacity to metabolize and excrete the
glucocorticoid hormones and possibly also to utilize them.
Although this is a matter that needs further investigation,
remains possible that in middle age adrenal deficiency,
perhaps combined with a quieter and more settled life,
may provide a more favorable environment for the multi-
plication of latent tumor cells.

Other factors that may affect the situation in particular
tissues are the mitogenic hormones and the poietins. If
the theory that one function of a mitogenic hormone is to
neutralize the chalone of its target tissue is correct then
the resulting mitotic stimulus may be more powerfully felt
in any latent tumor cells that are producing less than the
normal amount of chalone or reacting less adequately to
whatever chalone is present. The problem of hormonede-
dependent tumors and of the progressive changes that
occur in them has recently been discussed by Foulds (170,
171). While a tumor remains responsive to a hormone, it
may "grow so long as an appropriate (hormone) stimulus
is maintained, regress when it is withdrawn and recur, at
the same place and with the same characters, when it is
restored (170)." This is, of course, exactly what would be
expected of any group of cells which are only moderately
damaged and which maintain just sufficient control to
limit their own growth. The hormone would then act
rather in the manner of a promoting agent, increasing
mitotic activity and reducing maturation, but when the
hormone is withdrawn these processes would be reversed
and the tumor might even regress completely. When,
however, progressive changes towards greater malignancy
occur, some of the cells might become so free from the
normal mitotic control that they no longer would need the
hormone stimulus and ultimately might even lose all their
ability to react to the hormone.

By contrast, it seems improbable that the poietins,
which may not affect the mitotic rate, play any significant
part in the cancer problem. Theoretically a breakdown
in mitotic and functional homeostasis may occur in the
progenitor cells of the erythrocytic, granulocytic, and
even the plasma cell systems, but these cells are not af-
fected by the poietins. Alternatively the breakdown
could occur in the relatively undifferentiated stem cells,
and in this case, too, the presence or absence of the poietins
would be unlikely to exert any significant effect.

V. GENERAL SUMMARY AND
CONCLUSIONS

The problem of mitotic homeostasis, with which this
review began, is now seen to constitute only a part of
the far larger problem of tissue homeostasis, which may be
analyzed most profitably in terms of the control of gene
expression. The programs of syntheses that lead to the
creation and maintenance of a tissue are evidently de-
determined by a sequence of gene actions that are in turn
determined by a sequence of humoral controls. There
are at least 3 main levels of these controls: the basic in-
duction mechanisms by which the tissues are formed, the
chalone mechanisms by which they are maintained in a
functional state, and in some cases the hormone mech-
nisms by which tissue function may be modified.

A. The Role of Inducing Substances

There is a general belief that tissue induction is achieved
through the interaction of an inducing substance with
cells that are competent to react, and that the process
involves the activation of one region of the genome and
the inactivation of most of the other regions. In mam-
als this inactivation may be permanent, and in the case
of most tissues it is completed in the embryo. However,
it is also evident that in the adult mammal, with which
this review is primarily concerned, certain groups of stem
cells, which retain at least part of their embryologic
competence, are always present.

These stem cells appear to belong to 2 related groups,
the 1st perhaps retaining only the dual potentiality of
induction by poietins to form either granulocytes or
erthrocytes, and the 2nd perhaps retaining the multiple
potentiality of induction by antigens to form a variety of
plasma cells. It is interesting that neither the granulo-
cytic nor the erythrocytic cell populations are regarded
as self-maintaining in the manner of normal tissues since
their mitotic potentialities are insufficient to meet their
high rates of cell death. Thus in both cases maintenance
of tissue mass depends on continued induction of the stem
cells. In these unusual circumstances it is possible that
the tissue chalone mechanisms may be relatively poorly
developed, but it is nevertheless clear that some consider-
able degree of chalone control does exist and that it may
also influence the stem cell population.

The organization of the lymphocyte-plasma cell system
is less well known. It is probable that it may contain the
most complex system of interrelated tissues in the body,
and that the details of its mitotic and functional homeo-
static mechanisms may be equally complex. However,
it would be surprising if these mechanisms proved to in-
volve anything more than variations on the 3 main types
of homeostatic control.

It has been noted that the granulocytic, erythrocytic,
and plasma cell systems are all tissues that must be able to
meet sudden and urgent demands for large numbers of
extra cells. It has been emphasized that such sudden
tissue augmentation is not readily achieved through in-
creased mitotic activity and that recruitment by induc-
tion from the large stem cell populations is a more efficient method.

B. The Role of the Chalones

It is evident that the creation of a tissue does not guarantee its continued functional efficiency and that at all times most mammalian tissues must be supported against the tendency of their cells to revert to a stable program of recurrent mitosis. It appears that, with induction complete, the tissue cells normally retain at most only 3 groups of active or potentially active genes: the 1st concerned with the synthesis of general metabolic enzymes, the 2nd with tissue-specific function, and the 3rd with synthesis for mitosis. Together these groups of genes constitute the facultative genome, although it is possible that the 1st group may sometimes be nonfunctional and that the synthesis of the general metabolic enzymes may be directed at the ribosome level. The 2nd and 3rd groups are never fully functional simultaneously, and it now appears that the choice between tissue function and mitosis may be decided in terms of the intracellular concentration of the tissue-specific chalones. Thus the main task of the chalone systems of the body may be to maintain the whole functional organization of the animal against collapse into cellular anarchy.

It is also evident that mitotic and functional homeostasis is not a static affair; it responds to changing circumstances and is subject to various checks and counterbalances. The homeostatic mechanism continually maintains the tissue mass in correct relation to the body mass and reacts appropriately in conditions of tissue damage, whether local or general. Once the tissue mass is correctly related to the body mass, the situation is stabilized by the counterbalance provided by the inverse ratio between the mitotic rate and the life expectancy of the functional cells. Because of this, the point of balance between cell gain and cell loss is not easily disturbed and tissue mass tends to remain constant. When the mitotic rate is greatly increased, for instance by chalone lack through persistent cell damage, the tissue mass increases only moderately; when the mitotic rate is reduced towards zero, the increased length of life of the functional cells is such as to prevent any change in the tissue mass. It is not yet clear how the length of life of the functional cells may be influenced in this way. However, since nothing seems to affect the life expectancy of the nonnucleated erythrocytes, it is possible that the time of cell death may be controlled at the gene level, possibly through the intermediary of the lysosomes.

The homeostatic mechanism is also responsive to the effects of stress. Adrenalin (but not noradrenalin) and the glucocorticoid hormones strengthen the action of the chalones, and this, by reducing the rate of cell gain and cell loss, may have some survival value, especially perhaps during starvation.

C. The Role of the Hormones

From time to time, in certain mammalian tissues, mitotic and functional activity must be modified to meet particular needs, and it is evident that this must involve some interference in the chalone mechanism. In the case of the hair growth rhythm such interference may be dependent on genes that oscillate spontaneously between activity and inactivity, but usually it seems to depend on the actions of diffusible substances that are synthesized in other tissues and may perhaps all be classified as hormones. Of these the best known are the various mitogenic hormones, such as the estrogens, which act to promote both mitosis and tissue function, perhaps through the neutralization of the chalones of the target tissues. When such tissues are unstimulated, it is evident that the mature cells are long-lived and nonfunctional; when they are stimulated by chalone neutralization, the raised mitotic rate is accompanied by a reduced life expectancy of the mature cells, which become functional as they pass towards death.

It is important to recall that such mitogenic hormones as the estrogens are widespread throughout the animal and plant kingdoms in situations in which their functions must be radically different from those they exert in an adult mammal. It seems indeed that certain types of molecules, which possess a pronounced ability to interfere in biochemical reactions, may have been incorporated in an opportunistic manner in the control mechanisms of a wide variety of cellular activities. In these cases it is clearly the mechanisms that have evolved while the active substances have changed little.

Although at the moment only relatively few hormones are known to be active in mammals as part of this 3rd order of tissue control, it seems probable that a wide variety of other active substances may also be involved. These may perhaps include, for instance, promine and retine (470), the nerve growth factor (314), and the skin factor (119, 120), all of which have been omitted from the present discussion because they form such a heterogeneous group. Indeed, it appears that this 3rd order of tissue control may prove to be mediated through the activities of such a varied assortment of substances that there may be little common pattern except in their ultimate disturbance of the chalone mechanism.

It may be emphasized that many adult hormone mechanisms are so organized as to regulate tissue functions in terms of seasonal or other environmental changes.

D. The Problem of Gene Damage

If all levels of mammalian tissue organization are maintained through the activities of particular substances which, directly or indirectly, control the expression of the genome, then some at least of these substances may be regarded as acting in the traditional manner of the effectors of the microorganisms. In these organisms versatility in the face of a changing environment is obtained at the gene level by the variety of the possible alternative synthetic programs that may be activated by a wide range of effectors. In the metazoans such versatility is evidently obtained by the variety of the restricted genomes represented by the various tissues, and in this situation the part played by effectors is less obvious. In this connection it is important to attempt to determine how restricted the genome of a typical tissue may be. Of the possible syntheses a cell may undertake, it has already been mentioned that those relating to the general meta-
bolic enzymes may perhaps sometimes be directed by long-lived messenger RNA, but those relating to tissue function and to mitosis, being effector-controlled alternatives, must be directed at the gene level.

It is evident that during life damage may accumulate at both the DNA and long-lived RNA levels and that by middle age such damage may be widespread. It follows that the fewer the number of active or potentially active DNA and RNA molecules that remain in a cell, the less is the chance that this accumulated damage will have serious consequences. Indeed the greatest tissue stability may be achieved through a final blockage of the entire genome, which possibly occurs both in striped muscle (532) and in neurones. In this situation cell replacement is impossible and molecular replacement is directed at the ribosome level; the accumulation of molecular damage at this level may cause an increasing burden of malfunction, nonfunction, and even cell death, but it cannot lead to the rapid death of the animal itself through unregulated growth by mitosis.

In most tissues, for a variety of obvious reasons, it is necessary to replace cells rather than molecules, and the possibility of gene damage leading to cancer may be the price that such tissues must pay for the retention of a facultative genome capable of directing synthesis for mitosis. It is an indication of the remarkable efficiency of the homeostatic mechanism that this price is so rarely paid in the tissues of wild animals; that it is more often paid in the tissues of domestic animals and of man may be directly related to their relatively longer and less stressful lives.

ACKNOWLEDGMENTS

The ideas here put forward have emerged during a long study of mitotic control in adult mouse tissues, a study that would never have been possible but for the continuous and generous financial support of Organon Laboratories Limited. These ideas have also been tested and often radically modified in the course of innumerable discussions, especially with Dr. E. B. Laurence, Dr. W. J. Tindall, Dr. C. L. Hewett, and Dr. L. Foulds in London, with Dr. J. I. D. Homan, Mr. H. de Jager, and Dr. W. Hondius in Holland, and with Professor H. Teir and Dr. T. Boldingh in Holland, and with Dr. E. B. Laurence, Dr. W. J. Tindall, Dr. C. L. Hewett, and Dr. L. Foulds in London, with Dr. J. D. H. Homan, Mr. H. de Jager, and Dr. W. Hondius Boldingh in Holland, and with Professor H. Teir and Dr. T. Boldingh in Holland. Later, supported by a grant from the Damon Runyon Memorial Fund for Cancer Research, New York, growth by mitosis have also been tested and often radically modified in the course of rapid death of the animal itself through unregulated growth by mitosis.

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