Regulation of Growth in Neoplasia

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Preface

This book is a sequel to the volume entitled 'Neoplasia and Cell Differentiation' published in 1974. The great interest in the field of Neoplasia and Cell Differentiation has prompted us to bring out this volume, which presents three extensive and authoritative reviews of growth processes and their regulation, especially in neoplasms in vitro. I have little doubt that these will stimulate much thought and research into the mechanisms involved in the control of growth processes in the two closely allied fields of neoplasia and cell differentiation.

I would like to thank the contributors for the assiduity and enthusiasm with which they devoted themselves to the writing of the reviews. I would also like to thank the Publishers for a cool and competent handling of this project which has resulted in a well-produced book.

Newcastle upon Tyne, June 1981 *G.V. Sherbet*

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Regulation of Growth and Cell Division in the Whole Organism

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I. Introduction

Current methods of treating cancer involve drastic measures. Surgery is disfiguring while radiotherapy and chemotherapy often make the patient feel extremely weak and debilitated. None of these treatments is particularly effective since, with some notable exceptions, the best that can be hoped for is a few years remission of the illness. Advances that have been made during recent years have not entailed the introduction of new methods so much as the refinement of existing methods, in particular by combining different treatments and by empirical determination of the optimum dosages and means of administration. Over the past decade or more, there has been a growing interest in the immune surveillance theory of cancer, which proposes that the body is capable of recognising and rejecting potentially malignant cells and that cancer develops because of a defective surveillance mechanism. This theory has come under criticism for a number of reasons: in many spontaneous cancers there is no evidence for tumour-associated antigens, in experimental systems the rate of production of T lymphocytes does not correlate well with the incidence of tumours nor is there good evidence that natural killer (NK) cells protect against tumours [110]. Nevertheless, clinical trials involving manipulation of the immune response frequently improve the chances of survival in patients with some forms of leukaemia and carcinoma [201].

A common feature of all the forms of treatment so far mentioned, namely surgery, radiotherapy, immunotherapy and the use of cytotoxic drugs, is that their object is to eradicate the tumour tissue by destroying the cells of which it is composed. The need for such a strategy is based upon

the widely held view that in changing from the normal to the neoplastic state, cells have undergone some form of irreversible change. While the main body of clinical observation is consistent with this idea, long-term or even complete remissions, sometimes in the absence of conventional treatment, are by no means uncommon and even when those remissions which seem explicable on the basis of immunological rejection are excluded, there remains a considerable body of evidence, both clinical and experimental, which suggests that the neoplastic state of some tissues can be reversed. Turning to the biological evidence, there is much less support for the view that neoplasia is irreversible. One firmly held belief about neoplasia is that the cells involved have undergone some form of true genetic change or 'somatic mutation'. If this were true, then neoplasia would indeed be irreversible, since the chances of any particular chromosome aberration or gene mutation reverting to normal genotype are very small. While it is true to say that some forms of neoplasia are associated, possibly causally, with a true change in genotype, such evidence is lacking in the majority of cases and in many forms of cancer the biological and clinical data may actually be inconsistent with the somatic mutation hypothesis [209]. There is much evidence to support the idea that cancer is a form of atypical cell differentiation due to changes in the mode of gene expression rather than alteration of genotype and that, in a large proportion of tumours, genetic abnormalities are observed because aberrant cells, which occur even in normal tissues, have a better chance of survival in the neoplastic tissue.

The idea of neoplasia as a state of abnormal differentiation is therefore tenable and this has important implications. While differentiation tends to be a very stable state, it is far from irreversible. Modification of the differentiated state is observed during the modulation of cultured tissues [109], in states of pathological metaplasia or in regenerating tissues and changes in the way in which genes are selectively expressed may be observed following experimental procedures such as nuclear transplantation and cell fusion [209]. Cancer research programmes actively support investigations into normal cell physiology and, in particular, into the processes regulating normal cell growth and differentiation. Implicit in this policy is the idea that it may eventually be possible to control neoplasia by manipulation of the natural physiological controls. This is an exciting, if at present distant, prospect.

It is difficult to find any simple feature which distinguishes tumours from normal tissues. The state of neoplasia rather involves a spectrum of

related changes. These include alterations in the biochemistry and morphology of individual cells, invasiveness leading to infiltration of surrounding normal tissues, a loss of cellular adhesion resulting in metastasis and the formation of secondary growths and an imbalance in cell proliferation such that the rate of cell production exceeds the natural loss so that the tumour gradually increases in size. It is this uncontrolled growth which is the most serious clinical feature of the disease. If the physiological control of cancer growth is to become a clinical reality, it must be based upon manipulation of the mechanisms which regulate growth in normal tissues. Growth of the whole organism and of its component organs and tissues is, with few exceptions, due to an increase in the number of component cells and an understanding of how cell division and proliferation is controlled is therefore essential. It is reasonable to ask whether there is a single mechanism which can account for all growth phenomena or whether the means of control differs according to the type of growth process. As an introduction to this enquiry, it is relevant to list the many types of growth in which we might assume some type of cell division control to exist. A detailed review of cell division kinetics and of the methods and terminology employed is given elsewhere [2].

II. Types of Growth Phenomenon

A. Embryonic Growth

The first few 'cleavage' divisions of the single-celled zygote are characterised by a short cell-cycle time in which, following mitosis (M), the cell proceeds directly into DNA synthesis (S) with no G_1 or G_2 stage [101]. During this initial period cell division is synchronous, continuing for at least 6 or 7 consecutive cleavage divisions, but eventually synchrony breaks down with the appearance of the G_1 stage and a general lengthening of the cell cycle [190]. This transition is accompanied by the appearance of specific morphogenetic functions which denote the onset of cell differentiation and which probably involve the transcription of new mRNA. In mammals, in which the embryonic cells have a small cytoplasmic mass, the information needed for new morphogenetic functions may be acquired during early cleavage stages [227] while in larger-celled embryos, such as those of Amphibia, the onset of new DNA-RNA transcription appears to occur some time during gastrulation [68]. Some time during the gastrula stages, regional differences appear in the rate of incorporation of specific

amino acids into proteins [74] and by the end of gastrulation in Amphibia specific antigenic differences can be demonstrated between neural and epidermal ectoderm, mesoderm and endoderm [60]. The early stages of histotypical differentiation in the adenohypophysis [18] and the kidney tubules [78] are characterised by the appearance of specific antigens and this may be a common feature in all forms of tissue differentiation. Nuclear division, occurring during mitosis, appears to be essential for the transcription of new RNA [100] and the expression of new developmental information [227].

B. Developmental Growth

This term is used to denote the period of growth extending from the early stages of cell differentiation up to the time when the organism attains its upper size limit. It should be noted that in some species, such as many teleost fish [63], developmental growth is continuous and, given adequate nutrition, no upper size limit is reached. In many animal species developmental growth is at first exponential [35] and in mammals this phase lasts through foetal life into the early post-natal period when the maximum rates of growth may be observerd [220]. Overall exponential growth of an organ may conceal wide differences in the growth rate of its component tissues. Within the intestine, which as a whole presents an exponential growth pattern until the time of birth, the rate of proliferation of epithelium is considerably higher than that of the mesenchyme [202].

Quite early in development, three stages of the cell cycle – S, G_2 and M – achieve a fairly constant duration, and thereafter the gradual reduction in cell division rate is due mainly to a lengthening of G_1 and a progressive reduction of the growth fraction or proportion of cells actively dividing [35]. This even progression may be interrupted by events such as metamorphosis in insects [250] or puberty in mammals where the sexual [176] and secondary sexual [122] organs may show massive, though temporary, increases in the rates of growth and cell multiplication.

Termination of the period of developmental growth and the achievement of the upper size limit does not mean that growth processes then cease, as will be shown in the following examples.

C. Cell Turnover

Cessation of overall growth occurs when a state of dynamic equilibrium is reached between production of new cells and the loss of old cells. In many tissues this process of cell turnover is very rapid, for example in

parts of the gastrointestinal tract it may be equivalent to total replacement of the epithelial cell population every 2 days [257].

In some tissues, notably the epidermis and intestinal epithelium, cell production is due to the proliferation of 'stem cells' which retain the ability to divide while the maturing and functional cells of the tissue lose the ability to divide and are lost by desquamation [99]. In contrast, the mature liver, kidney [38] and lung [26] do not appear to contain a stem cell population and growth occurs as the result of the proliferation of fully matured functional cells.

It appears that the curtailment and eventual cessation of developmental growth is, in the final stages, due more to an increase in the rate of cell loss than to a reduction in the rate of cell proliferation.

D. Diurnal Variation in the Rate of Cell Division

In tissues which exhibit some measurable rate of cell turnover, the rate of cell production usually shows a marked and regular pattern of variation throughout the day [77]. During the 24-hour period the difference between the maximum and minimum rates of cell division may, in some organs, be as much as four-fold and the time of maximum and minimum rates appears to differ from organ to organ [212]. When animals or human subjects are kept in conditions where environmental cues, such as variation in light intensity, are absent, the strict 24-hour or diurnal cycle breaks down and the subject enters a state of 'free cycling' where the rate of cell division develops a 'circadian' pattern which has a period sometimes shorter and sometimes longer than 24 h [42]. This suggests that animals have an inbuilt timekeeper, possibly mediated by the hypothalamus, which is programmed to an accurate diurnal cycle by environmental changes.

E. Wound Healing, Compensatory Growth and Regeneration

Most animals have some means of repairing damage due to physical trauma and tissue loss. In some cases, repair is limited to the restoration of structural integrity in which damaged functional tissue is replaced by connective tissue. This process is termed wound healing by 'second intention' [244]. In other cases full function may be restored by growth due to the proliferation of cells in the remaining mature functional tissue, a phenomenon known as compensatory growth. In some tissues, for example skin and lachrymal gland [98], the response to damage is purely local: increased cell division is restricted to tissues adjacent to the wound.

In other cases, a response may be found in parts of the organ situated at some distance from the site of injury, as in the liver, or in the undamaged contralateral organ, as is the case in the salivary gland, kidney, lung, testis and ovary [99]. In the case of organs such as the liver [147], kidney [10] or lung [214] which exhibit a contralateral response, a local response may also be found where the extent of damage is small.

In many cases of local damage the essential architecture of the tissue must be preserved if perfect restoration is to occur, otherwise the lost functional tissue is replaced by connective tissue. In zonal necrosis of the liver, perfect healing depends on the reticulin framework of the lobule remaining undamaged [244], in skeletal muscle maintenance of intact sarcolemmal tubes [151] and basement membrane architecture is essential [50], while in nerves regrowth occurs only if the connective tissue sheath remains intact [99].

Regeneration of complete body parts such as limbs may sometimes occur. This is frequently regarded as a feature of 'primitive' species but in fact wide differences in regenerative ability may be found between phylogenetically related groups, such as crustaceans and insects (Arthropoda), which suggests that regeneration is a faculty which evolves in cases where it has some adaptive survival value [98]. In many cases, such as the lizard tail [219] or the Amphibian limb [236], regneration depends on the dedifferentiation, proliferation and redifferentiation into new cell types of tissue elements adjacent to the wound. This process may be termed 'physiological metaplasia'.

F. Hypertrophy and Hyperplasia

Many organs or tissues can become enlarged above their normal size. This may involve enlargement of the individual functional or parenchymal cells (hypertrophy), an increase in the number of such cells (hyperplasia) or both. In the human body, the most extreme form of hypertrophy occurs in the myometrium of the uterus where, during pregnancy, individual smooth muscle fibres increase to ten times their normal resting size [244]. At the time of birth, the average weight of the heart of human infants is 30 g and after this no further cardiac muscle fibres are produced. By the time that adult life is reached, these muscle fibres have increased seven-fold and yet further enlargement can occur in response to chronic overload such as is found in lung diseases or systemic hypertension [244]. Hypertrophy of cardiac muscle cells during postnatal development may involve nuclear division in which cells become binucleate while later in life the

nuclei fuse to produce polyploid cells [99]. Increasing polyploidy is also a feature of liver tissue and may be related to the hypertrophy of individual cells [6].

Many types of hyperplasia increase the functional capacity of the organ and have a beneficial value. Lymphoid tissue undergoes hyperplasia in chronic infection and following antigenic stimulation, one example of this being the enlargement of the spleen in malaria [244]. Oxygen deficiency due to prolonged exposure to high altitude is compensated for by increase of up to 50% in the red cell count [45]. In addition to these conditions of 'physiological hyperplasia' there are many states of 'pathological hyperplasia' in which the increase in cell number has no apparent adaptive advantage. One such condition is psoriasis, which is a skin disorder involving a massive increase in the rate of epidermal cell division [242] and an expansion of the germinative zone to three layers of proliferating basal cells [241].

It should be noted that hyperplastic growth is self-limiting in that the balance between cell production and loss is still maintained though at a different rate of turnover.

G. Oedema

Mechanical or chemical injury is often followed by a very rapid increase in volume of the surrounding tissue. Immediately after injury, a protein-containing exudate accumulates due to the escape of fluid from damaged capillaries. These proteins, and their breakdown products, exert an increased osmotic pressure which further encourages fluid retention. This process is termed 'oedema' and does not appear to involve any significant change in cell size or number [85].

H. Neoplasia

In neoplastic conditions (cancers or tumours) the stabilisation typical of normal cell turnover or of hyperplasia breaks down and the result is a progressively growing mass of tissue. There is some evidence that neoplasia in some tissues may originate in areas which have already undergone some form of hyperplastic change [226]. Neoplastic cells almost invariably show some loss or change in normal physiological function in addition to disordered growth. One example of this is the 'oat cell' tumour of the lung in which the loss of differentiated function is so great that it is uncertain whether it derives from connective tissue or from the epithelium [244].

Although the cells of many tumours proliferate more rapidly than the cells of the normal tissue from which the tumour originated, the duration of the cell cycle in many normal tissues can be shorter than that of the most rapidly growing tumours. It appears that the size of the growth fraction and the rate of cell loss are more important than the rate of cell division in determining the growth of normal and neoplastic tissues [142]. Cultures of neoplastic cells can yield apparently normal cells which are incapable of forming tumours. This suggests that the normal cell genome contains all the information required for the expression of the neoplastic phenotype and that neoplasia is due to a faulty developmental process [172], possibly involving some increase in the stability of mRNA whose products regulate the rate of cell turnover [173].

A distinction is often made between benign tumours which are slow-growing and non-fatal and malignant tumours which have a rapid rate of growth and which usually are fatal unless treated [7]. While this distinction is helpful from a clinical point of view, it is uncertain whether there is any true biological difference between the two categories or whether they simply represent different extremes of the same phenomenon of disordered growth.

I. Cell Loss

In addition to the cell loss observed in renewing tissues and referred to above, tissue degeneration due to massive cell loss is a common phenomenon during embryogenesis and developmental growth [65] and examples may be found in most organ systems of the body [92]. It occurs during the pupal stage of development in insects where larval tissues degenerate and are replaced by the proliferation of cells in the imaginal buds [250] and it is also observed in the development of Amphibia where the larval kidney or pronephros completely disappears, to be replaced by the mesonephros or adult functional kidney [166]. The cytological appearance of degenerating cells in tissue renewal and in developmental cell loss is very similar and it has been proposed that a common mechanism, termed 'programmed cell death' or 'apoptosis', is involved [123].

III. The Relative Importance of Cell Programming and of Regulation

The question arises as to whether the various growth phenomena listed above can be explained on the basis of a fixed intracellular

programme or clock which determines the rate at which cells divide and the number of divisions a cell passes through before death or loss or whether these parameters can be modified by regulatory systems involving interaction between cells, between tissues or organs or between the organism and its environment. Observations designed to test the relative importance of programming and regulation were carried out on the intestinal epithelium. In this tissue there is a high rate of proliferation in the stem cells of the crypts but the cell population remains constant due to migration of cells and loss at the tips of the villi [257]. One way of explaining this equilibrium [169] is that it might be due to a cellular programme which determined that, after mitotic division of the stem cell, one daughter remained a stem cell while the other became a 'maturing' cell which was eventually lost. However, it can be shown that in addition to this 'unequal' division, some pairs of daughter cells both remain stem cells while others both progress to maturing cells. This evidence strongly indicates the existence of regulatory processes [47].

The concept of programming cannot, however, be dismissed. Cells from rapidly growing foetal tissues typically retain a high rate of division when maintained in tissue culture as compared with homologous cells from mature tissues [109]. For example, the rate of mitosis in alveolar cells of mouse lung maintained in organ culture for 3 weeks remained higher than in equivalent cells from adult lung kept under identical conditions [216]. This evidence strongly supports the idea of cellular programmes.

Another aspect of intracellular information which, in many situations, seems to be an important determinant in cell division is the cytoplasmic mass [260] or the cytoplasm/DNA ratio. The decrease in cell division rate after the first few cleavage divisions during embryonic development appears to be programmed and it has been suggested [190] that the change is caused by the cytoplasmic/nuclear ratio falling below a critical level. Growth of many insect tissues is due to an increase in cell size rather than to cell division, an observation which seems inconsistent with the concept of cell division being triggered by growth to a critical size. However, in such insect cells there is a high degree of polyploidisation which may correspond to over 10 duplicative steps and the effect of this is to keep the cytoplasm/DNA ratio relatively constant [61]. In some cell systems, some critical amount of G_1 growth is necessary for the induction of the S phase and theoretical studies suggest that the timing of mitosis may be dependent on cytoplasmic mass [81]. Work on yeast cells suggests that the attainment

of a critical cell size is, in itself, unlikely to be the direct stimulus to division but that the important factor is some cellular property which changes with size such as the accumulation of some specific cell component [51]. A similar process has been proposed to account for the lengthening of the cell cycle in early embryonic tissues, namely that preformed cytoplasmic deoxyribonucleotides, which act as a reserve for rapid DNA synthesis, constitute a critical component whose exhaustion determines a reduction in the rate of cell division [133].

It thus appears that cell division rates are, at least in part, determined by intracellular information or programmes which are modified by the process of cellular ageing. The concept of cell ageing has been developed mainly on the basis of studies on cultured diploid cells and there is evidence to suggest that some mammalian cells have a finite life span and that, having passed through a specific number of consecutive mitotic divisions, they are programmed to die [225]. Whether this is generally true for other species and cell types is uncertain, since other types of cultured diploid cell, notably those of Amphibian origin [178], appear to have a much higher limit. However, while the idea of a finite number of mitotic divisions may be open to question, there can be no doubt that cells do age or senesce and that this is due to the progressive effects of cell division. In the development of Amphibian embryos each stage of differentiation is determined by the cells achieving a specific number of divisions [35] and a similar phenomenon has been described in the development of appendages in the chick [256]. It has been suggested [114] that cells count the number of divisions by successive methylation of adenine in DNA and that the process may be reversible, either by the action of a demethylating enzyme or by division in the absence of the methylating enzyme. Other workers have proposed that cell ageing may be due to the accumulation of peroxidized lipids in the cell membranes [205] and cell fusion experiments also favour the idea that at least part of the cell ageing mechanism may reside in the cytoplasm [162].

The control of apoptosis or programmed cell death is believed to involve a different mechanism from that which regulates the cellular ageing phenomena referred to above [144]. Cells from the posterior necrotic zone of the chick wing bud, isolated as early as their location can be determined, will undergo apoptosis even when cultivated in vitro, which suggests that the programming must occur very early in development [80] though, in its earliest stages, the effects may be reversible [144]. The signal which initiates programming may induce the synthesis of