"MATHEMATICAL MODELS OF GRANULOPOIESIS".

Thesis Presented to the University of Glasgow for the Degree of Doctor of Philosophy.

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CONTENTS.

PREFACE.

This thesis describes work carried out while the author was a staff member of the Regional Department of Clinical Physics and Bio-Engineering of the Western Regional Hospital Board, Scotland.

The author was fortunate in having as his immediate colleagues Mr. W. M. Gray, Dr. J. Kirk and Dr. J. S. Orr. The collaborative nature of this association is borne out by the authorships of the twelve papers so far published or in press. Acknowledgement is especially due to Dr. Kirk for his skill in obtaining numerical solutions of some unusual species of equations.

However, those chapters claimed as original (4,6,7,8,9,10) describe work for which the principal responsibility lies with the author. The remaining chapters (1,2,3,5) are of a review or introductory nature. The thesis itself is solely the responsibility of the author who places no restrictions on its reproduction or use for purposes of study or research.

Computer facilities were provided or financed by the Western Regional Hospital Board. The support of the directing staff of the Regional Department of Clinical Physics and Bio-Engineering (Dr. J. M. A. Denihan, Mr. J. McKie) is appreciated.

For some of the computational work, technical assistance was ably provided by Mrs. Helen M. Finlay and Mr. Tony Griffin, both of the Control Section, Yarrow-Admiralty Research Department, Glasgow.

In the course of the work, the author benefited by discussion

or correspondence with a large number of scientific and medical workers, both in this country and abroad. In particular, thanks are due to each of the following for criticisms or suggestions:

Professor J. Anderson, Department of Pathology, University of Glasgow;

Dr. N. M. Blackett, Department of Biophysics, Institute for Cancer Research, Sutton, Surrey;

Mr. David Conkie, Beatson Institute for Cancer Research, Glasgow;

Dr. John Paul, Beatson Institute for Cancer Research, Glasgow;

Dr. Patrick Riley, Department of Biochemical Pathology, University College, University of London;

Dr. Roy Tarbutt, School of Biological Sciences, University of Sussex.

Thanks are also due to Dr. Michael Willoughby, Queen Mother's Hospital for Sick Children, Glasgow and to Dr. J. Whitelaw, Southern General Hospital, Glasgow, for access to certain clinical records.

These acknowledgements do not, of course, imply any endorsement by those acknowledged of the contents of the thesis.

The author's fiancée, Miss Elizabeth G. McClafferty, typed the manuscript and, in effect, edited it as well. Without her help, the thesis would have contained many more errors, inconsistencies and irrelevances than it now does. Without her encouragement, it may never have appeared at all.

SUMMARY.

Haemopoiesis (blood cell production) is a process subject to active physiological regulation. It constitutes one example of a biological process controlled through a hierarchy of feedback loops acting at a range of levels from the molecular to the macroscopic.

The thesis describes mathematical studies of the more macroscopic (physiological) levels of the control of haemopoiesis, with special emphasis on granulopoiesis.

Following review of pertinent background material in cybernetics, physiology and pathology, attention is focussed on the mathematical representation of cellular proliferation and maturation, and a representation formulated in terms of experimental observables is proposed. This leads to the study of a non-linear transcendental equation of the form

$$N(t) = e^{\alpha t} \int_{t-\tau}^{t} e^{-\alpha\psi} \xi(\psi)\, d\psi$$

with τ the unknown quantity. An iterative method of solution is proposed for this equation, which permits kinetic analysis of a certain class of non-steady-state processes. The method is used for the analysis of maturation kinetics of embryonic erythroid cells.

Attention is then turned to the causal basis of the control of haemopoiesis. It is pointed out that certain features of haemopoietic regulation lead to the expectation of oscillatory phenomena and that observation of such phenomena can be revealing.

With this in mind, a simple model is advanced for the control of granulocyte production. The model comprises two feedback loops, one regulating 'de novo' granulopoiesis in accordance with marrow granulocyte numbers and one regulating release from the marrow in accordance with blood granulocyte numbers.

The model is described by the system of delay-differential equations

$$\frac{dG_m}{dt} = \frac{\alpha}{1+\beta\left[G_m(t-\tau)\right]^x} - \frac{\lambda G_m}{1+\mu\,(G_\beta)^\gamma}$$

$$\frac{dG_\beta}{dt} = \frac{\lambda G_m}{1+\mu\,(G_\beta)^\gamma} - \omega G_\beta$$

where G_m , G_β are (respectively) the marrow granulocyte number and blood granulocyte number, both at time t , and α , β , x , τ , λ , μ , γ and ω are parameters, chosen to give maximum physiological realism.

Since 'de novo' granulopoiesis is believed to constitute a drain on primitive 'stem cells', regulation of stem cell number appears necessary. A model for stem cell mitotic autoregulation based on a diffusible inhibitor concept is proposed. This theory leads to the study of a pair of differential equations which, utilizing probable order-of-magnitude differences in relaxation times, may be reduced to the single equation

$$\frac{1}{N}\frac{dN}{dt} = \frac{\phi + \psi N}{k + \lambda N}$$

which admits of a closed analytic solution. This equation exhibits both self-limiting and non-self-limiting modes of behaviour, the biological implications of which are discussed.

With parameters selected for stability, the stem cell mitotic autoregulation loop may be adjoined to the granulopoiesis control system, previously considered, to obtain a composite model. However, the unstable and capricious behaviour of this multi-loop combination renders it physiologically unacceptable. Modifications which restore stability seem to imply heterogeneity of the stem cell population, representation of which lies outside the scope of the study described.

In the following chapter, consideration is given to the physical basis of regulation of 'de novo' granulopoiesis with emphasis on 'in vitro' evidence relating to 'colony stimulating factor' and the possible role of positive feedback in regulating granulocyte numbers in infection. By mathematical formulation of a recently proposed positive-feedback model it is shown that positive feedback systems can be stable in the absence of overt negative feedback loops provided passive damping elements (e.g. cell death) exist and satisfy certain criteria. It is shown, however, that the criteria concerned are incompatible with known features of the regulation of granulopoiesis in infection and the existence of additional, negatively-acting, loops is deduced. Some possibilities in this direction are proposed.

The model studies may illuminate the pathogenesis of some disorders of the control of granulopoiesis, notably cyclical neutropenia and myeloid leukaemia. In the former case, reduced stem cell input, or intramedullary or vascular granulocyte destruction appear the factors most likely to be responsible for cyclical

neutropenia. An additional factor - delayed maturation - a theoretically possible cause of granulocyte oscillations, does not appear to contribute to the pathogenesis of cyclical neutropenia in practice.

The pathogenesis of myeloid leukaemia remains a major problem. Model studies, however, suggest that two features may be of critical importance ; i.e.

(a) slowed maturation of granulocytic precursors

(b) increased input to the granulocytic pathway.

If the indicated association of these two factors is causal, rather than fortuitous, the simplest interpretation implies that cellular differentiation includes an early, labile phase, stability being a property acquired in the course of maturation.

Now that there exist techniques for growing human granulocytes in culture, the computational method described in chapter 4 should permit analysis of development of both normal and leukaemic cells. The proposed theory of cellular maturation in leukaemia might then be open to experimental test.

1. MATHEMATICAL MODELS IN BIOLOGY AND MEDICINE.

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Introduction.

Historically, mathematics has played a less important role in the study of biology than of almost any other natural science. In recent times, it has become standard to describe the results of experimental investigations in terms of their statistical properties and, to some extent, this represents a departure from tradition.

Nevertheless, mathematical theory - and in particular deterministic theory - is utilized in biology to a slight extent only. The work described in this thesis is untypical in that it consists of the application of deterministic mathematical modelling to a specialist area of biology and medicine. Moreover, the mathematical models considered are of a deterministic rather than a stochastic nature.

Purely deterministic models represent, of course, a very idealized conception of what is occurring, in that it is never possible to describe in finite terms the totality of causal influences responsible for a given process or phenomenon. Those influences excluded in any deterministic representation are allowed for in a stochastic model by replacing deterministic certainty by statistical probability.

Nevertheless, deterministic models can be useful in identifying particularly strong causal influences, such as would be exerted by mechanisms responsible for the active regulation of biological processes or system behaviour.

In the present instance, mathematical models are employed in the analysis of the modes of interaction of various elements of particular systems. It is not widely appreciated that mathematical models can be helpful in providing qualitative insights into system behaviour or organization. Not every theoretical precept is capable of mathematical formulation nor is this necessarily useful where it is possible. For a large (and growing) class of systems, however, it is recognized that mathematical modelling is not only useful, but may be indispensible for the conceptual understanding of how the elements interact to generate the properties of the composite system.

Cybernetics and the Theory of Control.

Confronted with a large set of elementary components, the exploration of all possible modes of organization arising from random coupling of the elements would hardly be a feasible undertaking. However, the majority of possible modes of coupling may be eliminated in advance by the application of some relatively simple precepts.

The outstanding characteristic of living creatures, recognized from ancient times, is the 'goal-directedness' of their bodily and mental processes. This observation gave rise to the Aristotlean doctrine of 'teleology', an animistic concept based on the temporal inversion of cause and effect.

Nowadays, it is universally recognized that goal-directedness can arise in two main ways, neither of which violates the principle of causality. In the first place, a selection mechanism rejecting all systems not conforming to the prescribed standard would result in the numerical domination of the appropriate type - provided this type already existed in the initial population. Random mutation, combined with Darwinian selection, is the mechanism which confers goal-directedness on biological evolution.

The second type of mechanism is more elegant conceptually, and avoids the wastefulness of Darwinian selection and can operate over extremely short time scales. This requires a system (which may be a product of the slower evolutionary process) which establishes a causal coupling between the

measured 'distance' (in a generalized sense) and a mechanism capable of increasing or decreasing this 'distance'. Such systems are referred to as 'feedback control systems' both in engineering and biology.

If the system acts to minimize the 'distance', it is a 'negative feedback system' while if it maximizes the 'distance' it is a 'positive feedback system'. The utility of feedback control systems has been appreciated in engineering only within the last century, but feedback control is ubiquitious in biology. The kinship of biological and engineering control systems was emphasized by Norbert Wiener who proposed the name 'cybernetics' for 'the study of control and communication in the animal and the machine' (1).

Certain very general cybernetic principles are useful in devising plausible models of biological control systems. Goal-seeking responses usually involve negative feedback. Goal-avoiding responses, or processes involving 'amplification' may depend on positive feedback. Models of biological control systems are usually built up as a hierarchy of negative and positive feedback loops rather than by randomly coupling the elements together. Mathematical formulation of the causal relationships represented by the system of loops then provides a deterministic mathematical model.

In the past two decades, considerable progress has been made in the analysis of mathematical models of various kinds of control system. This analysis has given rise to a body of knowledge known as 'control theory'.

Unfortunately, the mathematical models which result from consideration of biological control systems are usually very complicated and can seldom be treated by the existing methods of control theory.

Most biological control systems are not 'analyzed' (by application of analytic theorems) but 'simulated' (by numerical solution of the control equations using computers). Pending further developments in the mathematical theory of control, simulation is the best available method for the examination of the properties of mathematical models in biology.

The Control of Haemopoiesis.

Blood cell production - haemopoiesis - is only one of many biological processes of interest from the viewpoint of cybernetics. However, it possesses certain advantages over most other such processes.

Firstly, the spatial distribution of (mature) blood cell populations renders haemopoiesis a more tractable subject of study than other cellular production processes whose end-products are distributed in highly complex patterns and arrangements.

Secondly, haemopoiesis is a relatively accessible example of the process of cytodifferentiation, one of the most fundamental biological problems under current consideration. Moreover, haemopoiesis has for some time attracted the attention of experimental and clinical investigators and a considerable body of information has been accumulated.

Finally, the control of haemopoiesis is not a subject of only academic interest. Under normal circumstances, the haemopoietic control system exhibits the familiar biological properties of homeostasis or adaptive regulatory response to changed conditions. In pathological situations, these features may be absent or deranged with consequences of varying severity. Hopefully, improved understanding of haemopoietic regulation may assist in the alleviation of a group of diseases whose present therapy is necessarily empirical and often inadequate.

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2. PHYSIOLOGICAL ASPECTS OF THE REGULATION OF HAEMOPOIESIS.

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Introduction: Blood and Blood Cells.

Blood is not a true liquid but a dispersed cellular tissue borne by a liquid - the plasma. The coexistence of these different physical phases imparts to blood a rather complicated set of properties in relation to fluid flow and viscosity.

The cellular components of blood are extremely varied. Among the more important cells are the erythrocytes (red blood cells), platelets and leukocytes (white blood cells) - the last named being a very heterogeneous grouping of different types.

Erythrocytes are distinctive cells in more than one respect. Topologically, they are biconcave discs, a shape which may reflect an evolutionary comprise between that optimal for stream-lined flow and that for efficient chemical exchange with the plasma and tissues.

The distinctively red colour of blood (in higher animals) is due to the haemoglobin content of the erythrocytes. Haemoglobin is a chromo-protein molecule with a molecular weight of about 4,000 which can exist in both an oxygenated and a reduced form. Erythrocytes containing oxygenated haemoglobin convey oxygen from the lungs to the tissues while erythrocytes containing reduced haemoglobin convey carbon dioxide in the opposite direction.

An important feature of haemoglobin is that iron is one of its molecular constituents. Red cell precursors which synthesize haemoglobin may therefore be

labelled using radioactive isotopes of iron (Fe^{55} or Fe^{59}). The availability of a selective label has facilitated the investigation of red cell production (erythropoiesis) and more is known about this production process than any other.

Platelets are small disc-like pieces of actively-metabolizing cytoplasm. They result from the disruption of the cytoplasm of giant multi-nucleated precursor cells (megakaryocytes) in the bone marrow. The main physiological function of platelets consists in their aggregation at the site of any bleeding wound and participation in the complex sequence of biochemical events leading to blood clot formation. However, it may be that other functions exist also (1).

As already mentioned, 'leukocyte' is the name applied to any white blood cell. There are three major classes of leukocyte: granulocytes, lymphocytes and monocytes. They differ in morphology and function. The lymphocytes, which are important components of the immunological system and the monocytes, which are large phagocytic cells of the reticulo-endothelial system, will not be further considered (However, see chapter 9).

Granulocytes form a main topic of interest in the chapters which follow. As the name implies, they are leukocytes exhibiting granulation of the cytoplasm. They may be sub-divided into neutrophils, basophils and eosinophils according to their avidity for biological staining reagents and other criteria.

The physiological functions of the granulocytes are not firmly established. However, the neutrophils and (to a

lesser degree) the eosinophils, display phagocytosis - engulfment of foreign bodies - and it is apparent that they participate in defence against bacterial infection.

The relative numbers and cell sizes of the more important blood cells are specified in table 1. As may be seen, neutrophils comprise the great majority of granulocytes and therefore tend to dominate the kinetics of granulopoiesis unless granulocytes are classified differentially.

Cytodifferentiation of Prokaryotic Cells : The Jacob-Monod Model.

In bacterial cells, the process of cellular specialization and adaptive regulation of protein synthesis has been very intensively studied. In 1961, Jacob and Monod (2) proposed a specific theory of the control of protein synthesis in prokaryotic cells. This theory has since been favoured by a considerable body of experimental evidence - notably that accumulating from studies of the metabolism of the sugar lactose by the bacterium Escherichia Coli.

Essentially, Jacob and Monod suggested that bacterial genes are linked together in functionally related and spatially compact groups called 'operons'. A particular gene - the 'operator' - was supposed receptive to a 'repressor' molecule synthesized elsewhere in the genome. On this model, transcription of the operon proceeded spontaneously but was inhibited by the binding of the repressor to the operator. The repressor could however be inactivated by another molecular species - the 'inducer' - so enabling transcription of the entire operon to proceed.

Jacob and Monod argued that if the inducer were a molecule which could be usefully metabolized by the cell, and the proteins coded by the operon were those implicated in the metabolic process, then a teleologically appropiate loop was established, the cell synthesizing enzymes only as they were required (fig. 1).

In a subsequent paper, Monod and Jacob (3) described hypothetical control loops of the above type

which might underlie cellular specialization in non-bacterial cells. However, specialization in eukaryotic cells is evidently a good deal more complicated than in prokaryotic cells and, despite intensive study, it remains very poorly understood.

Cytodifferentiation in Eukaryotic Cells.

The most characteristic difference between cellular specialization in prokaryocytes and eukaryocytes is the high degree of stability of specialized type in the case of the latter.

In Jacob and Monod's scheme, the induced enzyme synthesis is terminated by the departure of the inducing molecule. Even if the inducer is internally synthesized (3), a transient blocking action should be enough to destabilize the induced pattern of protein synthesis.

This lability is not typical of terminally differentiated eukaryocytes. To take the example of higher animals, all somatic cells in the adult derive from a single fertilized ovum, yet they comprise a large variety of distinctive specialized types none of which exhibit any propensity for 'de-differentiation' back into a fertilized ovum.

Where 'de-differentiation' is referred to (e.g. by tumour pathologists) it usually means a loss, transient or otherwise, of the distinctive phenotype characteristic of the cell type concerned. This phenomenon is logically distinct from true 'de-differentiation' involving the reacquisition of the properties of an ancestral cell type.

Apparently, cytodifferentiation in eukaryocytes involves one or more irreversible steps before its completion - which is not to say that such steps are not preceded by a reversible phase (see chapter 10). One kind of irreversible process would be the loss from the cell of all

genes not required for the maintenance of the specialized phenotype. An extreme example of this is the extrusion of the entire nucleus, as happens in the development of the mammalian erythrocyte. However, such mechanisms appear very exceptional. Moreover, the well-known 'totipotency' of plant cells, as well as recent studies on reactivation of quiescent genes by cell fusion (4) provides evidence that the genome of terminally differentiated eukaryocytic cells is usually intact.

The alternative to gene loss in differentiation is the imposition of some restriction on the expression of the genome. In theory this restriction could be upon either the 'transcription' of the genetic DNA to messenger RNA or the 'translation' of this RNA to protein. Although Harris (5) has strongly argued for translational control, most workers consider that some regulation of transcription must occur, with or without translational blocks.

A scheme favoured by some molecular biologists involves a relatively non-specific blocking of transcription by histone molecules, with a specific de-blocking mechanism mediated by acidic proteins (6,7) or special kinds of RNA (8,9). Tsanev and Sendov (10) have further proposed that the small number of histone types could be the 'building blocks' of an epigenetic code, just as the four bases in various nucleotide sequences constitute the genetic code.

At any rate, the positive regulation of gene switching in the course of cytodifferentiation seems highly probable. The study of the control of presumed switching by physiological signals is a very active research field, and

developing blood cells are favourite subjects for these studies.

The Development of Mammalian Blood Cells.

Phenotypically at least, cytodifferentiation in the cells of higher organisms is a temporally protracted process. This may be interpreted on the following hypothesis: The onset of cytodifferentiation results from the arrival of an inductive stimulus at one or more receptor loci of an ancestral cell type. This stimulus removes blocks at either or both of the transcription and translation level and permits protein synthesis to proceed. The newly-synthesized RNA and protein molecules can, in their turn, remove or impose further blocks on synthesis, so promoting a cascade process whose outcome is the establishment of stable pattern of protein synthesis appropriate to the mode of differentiation induced.

This process is time-extended, and the phenotype of the cell gradually alters as different proteins and nucleic acids make their appearance. Usually, development is accompanied by cell divisions and it is possible that division is obligatory for certain developmental steps to take place (11).

In the case of many cell types - amongst them the main types of mammalian blood cell - experimental techniques have been developed which permit the classification of a particular developing cell as being at one of several defined stages.of the differentiation process. The progress of the cell, or its daughters, from one recognizable stage to another provides a means of 'tracking' the process. As might be expected, classification is least difficult for

late, well-characterized cells and is most difficult for cells in which the process of specialization has only recently begun.

When the developmental ancestry of erythrocytes, granulocytes and platelets is traced backwards in time, the ancestral lines are seen to be convergent, but classification tehcniques cease to be reliable just before the lines coalesce. The straightforward interpretation is that these three cell types derive from a common precursor cell of uncertain morphology. Direct evidence, based on chromosome examination of developing blood cells in vitro, supports this interpretation (12,13).

The common ancestral cell, generally called a 'stem cell' (14) is not of course 'undifferentiated' being, like all somatic cells a specialized derivative of the fertilized ovum, but its differentiation is evidently non-terminal inasmuch as stem cells can develop into (at least) three different kinds of terminally differentiated cell types according to the inductive stimuli which impinge upon them.

The development of erythrocytes, granulocytes and platelets from a common stem cell is schematically depicted in fig. 2.1. The terminology is that employed in the recent comprehensive review by Metcalf and Moore (15).

Evidently, blood cell development provides a rare opportunity to study, in adult organisms, differentiation processes which, in most tissues, occur only in the embryonic phase.

In adult mammals, haemopoiesis is largely confined to the active (red) bone marrow of the skeleton and - to a lesser

extent - the spleen. In the embryo however, the anatomy of haemopoiesis is rather more complicated and embryonic 'extra-medullary' sites of haemopoiesis, normally inactive in the adult, may be reactivated in adult life in disease states or other abnormal conditions.

Haemopoiesis in the Mammalian Embryo.

Embryonic haemopoiesis is a multi-phasic process in which the anatomical site of blood cell production changes with gestational age. Haemopoiesis is initiated in the so-called 'blood islands' of yolk-sac mesoderm proximate to endoderm.

The region of mesoderm which is close to endoderm apparently provides a 'permissive' environment for haemopoiesis, as a diminished haemopoiesis can occur in mesoderm even if endoderm be totally removed (16,17).

Thereafter, haemopoiesis becomes established in the liver before moving, finally, to marrow and spleen - the sites usually active in the adult. The mechanism of transition of haemopoiesis from one anatomical region to another has attracted a good deal of attention. Current views are that a blood-borne migration of stem cells, originally derived from yolk-sac, is responsible (18,19).

A simple mechanism might be that haemopoietic stem cells are rather unadhesive to most tissues and accumulate preferrentially in tissues and organs whose adhesiveness, or suitability as a site of stem cell proliferation, alters with embryonic development. However, in the context of immunology, de Sousa (20) has proposed an active cellular homing process ('ecotaxis') guided by appropriate signals, and this could be important in the present context also. Each of these suggestions is speculative and direct evidence is lacking.

In most species, the shift of haemopoiesis from one site to another is accompanied by a change in the composition of the

haemoglobin synthesized by erythroid precursor cells. As a rule, the haemoglobin type is not organ-specific, but an important exception is the mouse embryo, in which 'embryonic haemoglobin' is synthesized by cells resident in yolk-sac only (15).

Baglioni (21) has proposed that the number of cell divisions in erythroid cell development is a determinant of the type of haemoglobin synthesized. This opinion, however, is not shared by other workers (22).

Homeostasis and Adaptive Regulation of Blood Cell Number.

In adult animals, the control of blood cell number well-illustrates the 'goal-seeking' behaviour of biological systems. In brief, blood cell number appears to be regulated in accordance with functional demand.

This form of control may be usefully separated into two components; that involving the restoration of 'normal' blood cell number following a transient perturbation, and that involving the adjustment of blood cell number in response to alterations of the level of functional demand.

The restoration of blood cell numbers following depletion has been thoroughly investigated. It is known that haemopoiesis is increased following blood loss and that differential regulation of erythropoiesis, granulopoiesis and thrombopoiesis occurs in response to selective perturbation of numbers of specific blood cell types. Conversely, in animals rendered polycythaemic by injections of packed red cells, erythropoiesis quickly drops to low or even zero levels. The corresponding experiments on granulopoiesis and thrombopoiesis are more difficult, technically.

Adaptive regulation of erythropoiesis is exemplified by the enhanced erythropoiesis which occurs at high altitudes in normal individuals. Teleologically, the increased red cell number partially compensates for the reduced oxygen level by improving the efficiency of the oxygen transport system. Conversely, erythropoiesis is reduced under hyperbaric conditions.

It is not quite so easy to study adaptive granulopoiesis as

adaptive erythropoiesis. However, granulocytes are certainly implicated in the defence against infections and it is undoubtedly significant that bacterial invasion, or infusion of bacterial endotoxin can stimulate granulopoiesis to a marked degree. This phenomenon is further considered in chapter 9.

The Regulation of Erythropoiesis.

That erythropoiesis is actively controlled has been known for many years. As far back as 1906, Carnot and Deflandre (23), noting the capacity of serum obtained from anaemic animals to stimulate erythropoiesis in normal animals, suggested that production of a humoral erythropoietic factor was invoked by anaemia.

This suggestion is now known to be in essence correct, but it was not until the second half of the twentieth century that experimental evidence in favour of a circulating stimulator of erythropoiesis became compelling (24 - 27). A reliable 'in vivo' assay of the stimulator - now generally known as 'erythropoietin' - became available with the use of polycythaemic mice in which endogenous erythropoiesis had been suppressed (28 - 30) but an equally reliable 'in vitro' assay system is still awaited.

Good evidence now exists that erythropoietin functions as an inducer of terminal cytodifferentiation, acting at an epigenetic level on erythroid precursor cells of uncertain morphology. Erythropoietin has been shown to stimulate iron incorporation (31) and haemoglobin synthesis in cells hitherto inactive in this respect (32 - 34). The molecular biology of erythropoietin action remains controversial. Paul and Hunter (35) observed that erythropoietin stimulated a transient burst of RNA synthesis, followed by DNA synthesis, in embryonic cells, while Ortega and Dukes (36), with adult marrow cells, found a DNA-dependant synthesis of RNA but not synthesis of DNA itself. In either case, removal of a

transcriptional block appeared to have taken place.

It has been suggested that, in high concentrations, erythropoietin may accelerate the maturation of erythroid precursor cells allowing insufficient time for the usual number of cell divisions. If, for example, erythropoietin were to control the rate of haemoglobin synthesis, and if a critical haemoglobin concentration signalled the cessation of cell division (37), copious amounts of erythropoietin might lead to large, well-haemoglobinized erythrocytes ('macrocytes') whose size exceeded normal due to the smaller than usual number of cell divisions in erythroid development (38). The observation, that anaemia (which increases erythropoietin production) shortens the mean maturation time of erythroid precursors (39), is therefore of appreciable interest.

The site of erythropoietin production was initially thought to be the kidney, and this presumption is supported by the erythropoiesis-depressing effect of nephrectomy (40,41).

It now seems however that the kidney manufactures an enzyme which activates - possibly by cleavage - an erythropoietin precursor produced elsewhere (42 - 44), possibly in the liver (45).

By and large, erythropoietin production is inversely related to functional demand for oxygen transport between the lungs and the tissues. It has for some time been supposed that renal hypoxia accelerates erythropoietin production directly, but it is becoming apparent that the viscosity of the blood (46,47) and the total red cell mass (48) may also be important.

The identity of the 'target cell' for erythropoietin is a very controversial subject which will be discussed subsequently.

Apart from erythropoietin itself, a number of factors are known to influence erythropoiesis. Iron, for example, can be a limiting factor if body stores are depleted. Cobalt ions, androgens and oestrogens all alter erythropoiesis.

Vitamin B_{12} is essential for erythroid cell development and lack of B_{12} underlies 'pernicious anaemia'. Whether any of these factors act through the intermediacy of erythropoietin remains to be established.

The Regulation of Granulopoiesis.

Granulopoiesis is less easily studied than erythropoiesis. The reasons for this include the lack of a specific label for granulocyte precursors, continuing uncertainty about the physiological function of the granulocytes and the existence of large numbers of mature granulocytes in the marrow and the tissues as well as in the vascular system.

Two points of control of granulopoiesis have been established with a fairly high degree of probability.

Firstly, it appears that the release of mature granulocytes from the marrow (where there are a large number) is not a passive process but one which involves active physiological regulation. The mode of release is said to be first-in-first-out (49) and the release rate to be controlled by functional demand for granulocytes in the blood or tissues, apparently irrespective of the size of the marrow granulocyte reserve itself (50 - 53). Recent evidence suggests that this control is mediated by a humoral factor, which has been called the 'leukocytosis inducing factor' (L.I.F.).

On the other hand, it would be surprising if the control of granulopoiesis were not to involve an inducer of cytodifferentiation, a 'granulopoietin', analogous to erythropoietin in the control of red cell production. Evidence in favour of such an inducer is accumulating (54 - 56) and it appears most probable that the marrow granulocyte population, directly or indirectly, exerts an inhibitory effect on its production (56).

The studies mentioned above are all 'in vivo' investigations.

However, 'in vitro' experiments on the growth of granulocytic colonies have revealed the existence of a 'colony stimulating factor' (C.S.F.) (57 - 61) capable of inducing granulocytic differentiation 'in vitro' and also detectable in normal human and animal plasma and urine in physiologically significant amounts (see 15).

It seems a plausible hypothesis that 'granulopoietin' and 'C.S.F.' are related, if not identical, substances. However, the induction of granulopoiesis is less well understood than the induction of erythropoiesis and further studies on the inducing factor (5), including the molecular biology of its action, are required.

The Regulation of Thrombopoiesis.

On current theory, platelet production is regulated in a qualitatively similar fashion to the production of erythrocytes and granulocytes. A humoral inducer - 'thrombopoietin' - has been postulated and evidence for its existence reported (62 - 65). As with 'granulopoietin', the characterization of the factor is at a rudimentary stage and virtually nothing is known of its production or mode of action.

An interesting feature of platelet production is the cellular nature of platelet precursors. These precursor cells are multi-nucleated giant cells termed 'megakaryocytes', and having many times the usual cellular complement of DNA. They evidently arise from the same 'stem cell' species as erythrocytes and granulocytes but their differentiation involves division only of the nucleus, not of the cytoplasm. The causes of this unusual behaviour remain unknown.

The Nature of the Haemopoietic Stem Cell.

The nature of the haemopoietic stem cell is the outstanding enigma of contemporary haematology. Stem cells, defined by Lajtha, Oliver and Gurney (66) as those cells which '...can maintain their own number and give rise to differentiated cells' have yet to be positively identified in terms of morphological criteria. Failing such identification, stem cells must be numerically assayed in terms of their capacity to fulfill the requirements of the definition given above.

A crucial question pertaining to the nature of the haemopoietic stem cell is that of 'pluripotentiality' i.e. the ability of a stem cell to give rise to different kinds of terminally differentiated cells. Taking into account the apparent ancestral convergence of at least three lines of blood cell, evidence of competition between differentiated blood cell lines for a common precursor species (67,68) and cytogenetic evidence from studies of 'in vitro' blood cell cultures (69,70) it appears highly likely that a multipotential stem cell species must exist.
However, the existence of a multipotential stem cell is no guarantee that other cell species fulfilling the requirements of a unipotential stem cell do not exist also. Starting with the report of Bruce and McCulloch in 1964 (71), considerable evidence has been presented that the target cell for erythropoietin is not identical with, though derived from, the multipotential stem cell (e.g. 72 - 74. See also 1,15). Many workers now consider that 'unipotential' or 'committed' stem cells exist for each line of differentiated

cells, in addition to the multipotential stem cell, which is presumed ancestral to them (For a review, see 75).

On the other hand, some workers (e.g. Fogh 76) consider that the 'unipotential stem cell' is merely the multipotential stem cell in a particular, temporary, physiological state. A particularly ingenious theory, designed to reconcile bodies of apparently conflicting evidence has been advanced by Okunewick (77). In essence, Okunewick suggests that cytodifferentiation could be a 'multi-hit' process involving more than one of an inducer species molecule.

'Unipotential stem cells' might then be those stem cells for which the number of 'hits' is less than that required to initiate irreversible cytodifferentiation. Discussion of this theory, which may have very important implications if true, will be resumed in the final chapter of the present study.

The Regulation of Stem Cell Number.

Whether the stem cell population consists of one or several distinct types it is well-established that the stem cell population number, as assayed, for example by the spleen colony technique of Till and McCulloch (78), is subject to homeostatic regulation. This regulation is illustrated by the restoration of stem cell number following depletion caused by drugs (79) or radiation (80).

Moreover, unless a stem cell gives rise to two dissimilar daughter cells* at mitosis (81), a concept which involves some difficulties (82) - it is evident that the demand for differentiated cells must be a continual draw on the stem cell population. The existence of such depletion has been advanced as the reason for the sensitivity of the stem cell population to prolonged irradiation at low dose rates, which otherwise seems to be anomalous (83).

In chapter 7, a model will be proposed of the control of stem cell number. It is only fair to note now however, that experimental evidence which directly bears on the subject is extremely sparse and the control mechanisms involved are largely a matter of conjecture.

* i.e. a stem cell and a cell destined for differentiation.

Microenvironmental Regulation of Haemopoiesis.

Humoral regulation of haemopoiesis is probably studied more easily than regulation processes which involve interactions on a purely microscopic scale. There is strong evidence however that the local (micro-) environment of a stem cell or early developing cell critically influences its behaviour or fate.

The erythroid:granuloid (E:G) ratio differs significantly between spleen and marrow. Moreover, it has been shown that this ratio remains the same whether endogenous or exogenous stem cells are responsible for haemopoiesis in a given experimental situation (84,85). This suggests that local environment has an important role in determining the pattern of differentiation induced in resident stem cells. The mechanism of the presumed influence remains a matter for speculation.

On the basis of these and other results, it has been suggested that in spleen (and presumably marrow also) there exist small localities which induce a single type of blood cell differentiation (84 - 86). By implanting marrow into spleen it has been demonstrated that the E:G pattern of the implant remained of marrow type and that where colonies bridged the marrow-spleen junction, part of the colony in spleen was of spleen type and the part in marrow was of marrow type (84,85,87,88).

If each organ were composed of a mosaic of microenvironments the recognized tendency of colonies to convert from 'pure' to 'mixed' type with increasing size (84,88 - 90) would be

explicable - assuming stem cell replication took place as well.

TABLE 2.1 : MEAN SIZES AND MEAN CONCENTRATIONS OF PRINCIPAL BLOOD CELLS IN PERIPHERAL BLOOD

CELL	SIZE	CONCENTRATION
Erythrocyte	7.2 μ	$7.1 \times 10^{6}/(\text{m.m.})^{3}$
Granulocyte	13 μ	$6.0 \times 10^{3}/\ \text{mm}^{3}$
Platelet	3 μ	$2.5 \times 10^{4}/\ \text{mm}^{3}$
Monocyte	18 μ	$5.0 \times 10^{2}/\ \text{mm}^{3}$
Small Lymphocyte	10 μ	$2.5 \times 10^{3}/\ \text{mm}^{3}$
Large Lymphocyte	16 μ	

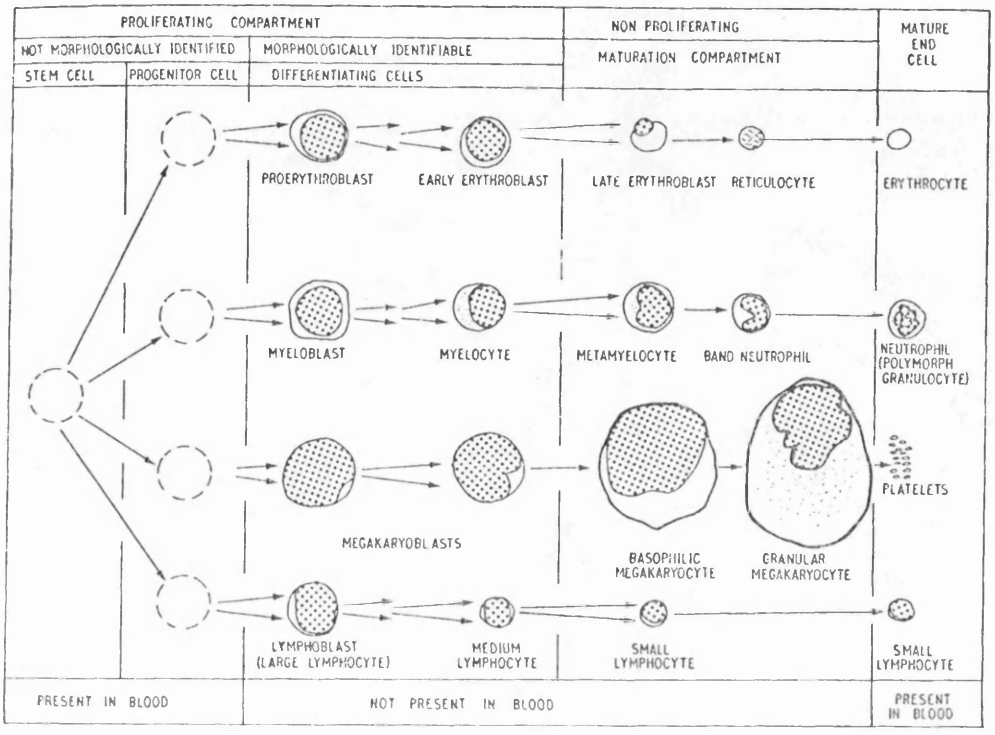

Fig. 1.1. Schematic diagram indicating the nomenclature, morphology and some properties of the various blood cells.

FIG. 2.1

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3. DISORDERS OF THE CONTROL OF BLOOD CELL PRODUCTION.

3. DISORDERS OF THE CONTROL OF BLOOD CELL PRODUCTION.

Introduction.

The pathology of haemopoiesis is a wide and active field and a comprehensive review will not be attempted here. In this chapter, attention will be focussed on those disorders involving overproduction or underproduction of red cells or granulocytes and on disorders which cast some light on these production processes. This implies a concentration of attention ondisturbances of homoestasis of red cell and granulocyte numbers to the exclusion of disorders involving other cell types, immunological disease and metabolic abberations.

Disturbances of cell number and anomalies of cell structure and function are rarely separable. However, structural and functional defects will be considered primarily in the light of their relation to cellular production.

The Leukaemias.

The identification of leukaemia as a cause of death is of surprisingly recent origin. Damesheck and Gunz (1) suggest that the first documented case was that reported by Vepleau (2) in 1825. The malady concerned had rendered the blood 'like gruel, resembling in consistency and colour the yeast of red wine'.

However, leukaemia seems not to have been recognized as a distinctive entity until about 1845 when Bennet (3) and Virchow (4) each reported cases. Thereafter, the existence of a disorder in which the blood altered remarkably (it was said to resemble pus rather than blood) was widely confirmed. The name 'leukaemia', meaning 'white blood' came to be applied.

A variety of clinical symptoms were found in association with leukaemia, but a high ratio of white to red cells in the peripheral blood was considered to be the distinguishing feature. As is not uncommon in medicine, closer study soon revealed that leukaemia was not a unitary entity but a group of diseases characterized by a relative or absolute overproduction of leukocytes. Classification and investigation of the individual disorders comprising the group was obviously an important task.

Classification of the Leukaemias.

Broadly speaking, there are two main ways of classifying the leukaemias. These ways correspond to the different viewpoints of the physician and the cytologist.

Clinically, some types of leukaemia follow a rapid course leading to the death of the patient in a matter of weeks or months. These types are called 'acute leukaemias'. On the other hand, some types follow a relatively protracted course, the patient commonly surviving for several years. The latter types are called 'chronic leukaemias'. The two categories of leukaemia can usually be distinguished by examination of the blood and marrow. A high proportion of primitive, poorly differentiated 'blast' cells is a common feature of acute leukaemia.

The other classification is based on the type of leukocyte which predominates. Hence in addition to being classified as 'acute' or 'chronic', leukaemias may be classed as 'granulocytic', 'lymphocytic' and 'monocytic'. In the case of the poorly differentiated acute leukaemias where blast cells predominate, it may not be easy to determine unequivocally the cellular type involved. However, it has been claimed that such classification is possible in the great majority of cases, provided sufficiently detailed investigations are carried out (5).

The relation of the leukaemias to various solid tumours such as chloroma, lymphosarcoma and Hodgkins' disease has proved to be a controversial issue. The view expressed by Damesheck and Gunz (1), that these latter disorders represent local

manifestations of a process which in the leukaemias proper has become spatially distributed, appears to have gained favour. Such tumours may therefore be classed with the leukaemias as 'lymphoproliferative' or 'myeloproliferative' disorders - the latter being of interest here. Despite its merits, this nomenclature has the possible disadvantage of obscuring what may be crucial differences in cell motility and adhesion, and the interpretation of 'positional information' (6).

In part because of this, attention is here fixed on the spatially distributed varieties of myeloproliferative disease, while admitting that these may be only the late stages of initially localized tumours of the marrow.

The Nature of Leukaemia.

Nowadays, most workers are in agreement that all forms of leukaemia are 'neoplastic' i.e. they are forms of cancer, albeit of varying degrees of 'malignancy'. However, the implications of this consensus are obscured by the difficulty of providing a precise definition of cancer.

Some workers, accepting that malignant cells typically exhibit inheritance of the malignant property (i.e. daughters of a malignant cell are usually malignant) have supposed that an alteration in the nucleotide sequences of the genetic DNA must be involved in oncogenesis*. This, however, seems a premature conclusion. As highlighted by the work of Braun (7) and Pierce (8,9), amongst others, oncogenesis may involve modification in the pattern of gene activity or protein synthesis, rather than a change in the integrity of the genome itself.

In addition, adaptive regulatory mechanisms which display or mimic characteristics of heritability have been proposed by Dean and Hinshelwood (10) in connection with adaptation in bacteria, and may have implications for higher cells also.

Finally, it seems difficult to express in exact terms what is implied by the widespread agreement that leukaemias are 'cancers of the blood'. Perhaps the main implication is that the understanding of leukaemia is on the same conceptual level as understanding 'fundamental' biological problems, like embryogenesis or ageing.

* Or the integration of a viral genome into the genome of the cell.

Research on such problems, and on oncogenesis and leukaemia, are to a considerable extent complementary endeavours.

Acute Granulocytic (Myeloid) Leukaemia.

As previously mentioned, the unambiguous cytological classification of acute leukaemias may be difficult. However, a number of features of the principal types are generally agreed.

In acute granulocytic leukaemia (A.G.L.), examination of blood and marrow commonly reveals a high proportion of morphologically abberrant, immature granulocyte precursors, often at the myeloblast level. The disorder is usually accompanied by anaemia and sometimes thrombocytopenia.

Cytogenetic studies have revealed a host of chromosomal malformations in A.G.L. (11). Unfortunately, no single type predominates and it is difficult to draw conclusions. In some cases, there is evidence for a number of different leukaemic 'stem lines' in a single patient (12).

Somewhat surprisingly, the mitotic rate of the blast cells in A.G.L. is substantially less than that of normal myeloblasts (13 - 16). It has been suggested that the basic disturbance consists in an impaired maturation of granulocyte precursors (15 - 17) which retain proliferative potential beyond the chronological age at which it is normally lost.

An important recent observation is that 'post-mitotic' leukaemic cells retain the capacity for division. Such 'resting cells' can be triggered into cycle by administration of cytocidal agents (18 - 21) or irradiation (22). This suggests that such leukaemic cells are not totally unresponsive to physiological mechanisms responsible for

mitotic homeostasis.

Killman (23) has further suggested that virtually all blood cells in A.G.L. are derived from a leukaemic stem line with retained capacity for pluripotential differentiation. The observation that the low mitotic rate of leukaemia myeloblasts in A.G.L. is shared by the erythroblasts (in the same patient) lends support to this hypothesis.

The capacity of certain acute leukaemic cells to generate differentiated progeny has been borne out by observations both 'in vivo' (24 - 26) and 'in vitro' (27,28). Taken together, these reports suggest that the primary defect in A.G.L. is more subtle than the emergence of a wholly autonomous mutant clone.

Chronic Granulocytic Leukaemia.

Chronic granulocytic leukaemia (C.G.L.) has attracted great cytogenetic attention since the 1960 discovery of a distinctive chromosome anomaly in the great majority of cases (29 - 31). This defect - the 'Philadelphia Chromosome (Ph')' - is an acquired defect of somatic cells (not inheritable in the Mendelian sense) which is found in untreated as well as treated patients (11).

Interestingly, it has been claimed that the minority of cases of Ph' -negative C.G.L. have a particularly bad clinical prognosis (32).

It is generally accepted that the Ph' chromosome is found in erythroid as well as granulocytic precursors in C.G.L. (33,34). The implication is that the leukaemic defect arises in a pluripotential stem cell capable of differentiating into (at least) erythroid as well as granulocytic cells.

Granulocytes in C.G.L. have also attracted attention from biochemical investigators. Although various abnormalities can be found, the consistently low levels of leukocyte alkaline phosphatase (L.A.P.) are of particular interest (see 35). As is the case with the Ph' chromosome, the significance of the low L.A.P. levels in C.G.L. remains in doubt.

It appears, however, that the enzyme itself is qualitatively normal in C.G.L. but its rate of synthesis by individual cells is reduced. Moreover, under certain circumstances, synthesis of L.A.P. can be either initiated or accelerated in leukaemic granulocytes (36 -38). These observations are in

agreement with a hypothesis advanced by Teplitz (37); that the low L.A.P.. levels result, not from deletion of structural genes coding for the enzyme itself, but from the deletion of 'modifier' genes, leading to the reversible blocking of structural gene expression.

The physiological function of L.A.P.. remains unknown. It has been suggested that L.A.P. (39) or a related enzyme (40) is involved in mitotic regulation, but evidence is lacking.

Typically, C.G.L.. terminates in 'blastic crisis' with increasing numbers of poorly differentiated cells and new 'stem lines' often having bizzare chromosome derangements, with the Ph' chromosome no longer the unique observable defect of karyotype. Some theories of the nature of 'blastic transformation' in C.G.L. will be discussed in chapter 10.

Polycythaemia Vera.

The clinical symptoms associated with polycythaemia vera (P.V.) result largely from the elevated erythrocyte count which is the characteristic feature of the disease. However, increased numbers of granulocytes and platelets are also common and Wintrobe has commented that (typically) 'the whole bone marrow is hyperactive rather than the erythropoietic tissue alone' (41)..

True P.V. is not a consequence of oxygen starvation although hypoxia does in fact give rise to erythroid hyperplasia, with raised erythropoietin levels. In P.V.. however, plasma erythropoietin levels are normal or reduced (42,43).

Moreover, the defect responsible for P.V. appears to be intrinsic to marrow (44) and does not consist of an increased stem cell sensitivity to erythropoietin (45).

The simplest hypothesis compatible with these observations is that due to Morley (46): that P.V.. involves an absolute increase in the number of stem cells in the bone marrow. The stem cells are presumed normal in all respects other than the number present. On such a view, P.V.. bears the same relation to leukaemia as do the 'benign' tumours of various tissues to the corresponding 'malignant' neoplasms.

Interestingly, it has been reported that leukaemia incidence in P.V.. patients may be increased.. This, however, may be due - at least in part - to the practice of using radio-phosphorus in the treatment of P.V.. (see Kelemen 35).

survey is required to firmly establish its absence.

If modulation can be shown convincingly to be absent, it would suggest that the granulocyte population in C.G.L. is almost entirely leukaemic, as supported by the ubiquity of the Ph^1 chromosome in the marrow.

(d) The published evidence is insufficient to firmly establish the association between oscillatory granulopoiesis and oscillatory thrombopoiesis in C.G.L. Some of the reports, however, suggest an increased cycle length for the platelet oscillation also (19,22). If genuine, this phenomenon may provide additional clues to the nature of the stem cell defect in C.G.L. However, as Morley has pointed out, granulocyte oscillations could perturb the available blood platelets in cyclic fashion through cyclic modulation of spleen size. In that event, the apparent platelet cycle in some cases of C.G.L. would be of no intrinsic interest.

Di Gugliemo's Syndrome and the Mixed Leukaemias.

A relatively uncommon disorder, but one of considerable theoretical interest is the so-called Di Gugliemo syndrome or 'erythroleukaemia'. As the latter name suggests (though entymologically it is a contradiction in terms), this disorder consists of the neoplastic proliferation of red blood cell precursors.

In fact 'pure' erythroleukaemia seems very rare indeed and little of certainty can be said of it. (see Damesheck and Gunz 1). However, an 'impure' form, though still rare, occurs sufficiently frequently to be assessable. This form is a 'mixed' erythroleukaemia in which erythroid, granulocytic and (sometimes) thrombocytic precursor cells proliferate together in a disordered fashion characteristic of malignant neoplasia. Atamer (47) reports that 71% of all cases terminate as granulocytic leukaemia, usually of the acute variety.

In general, it would appear that whereas red cells and platelets are usually diminished in number in the granulocytic leukaemias, there exist variants of 'granulocytic leukaemia' in which these other cell types are increased in number and may (temporarily) dominate the blood or marrow. This appears most plausible on Killman's hypothesis (23) that most blood cells in acute leukaemia are clonally derived from a defective pluripotential stem cell.

Two questions which arise are: why does anaemia rather than erythrocytosis accompany granulocytic leukaemia in the majority of cases and why do granulocytic

precursors usually come to dominate in myeloproliferative disorders initially characterized by erythrocytosis or thrombocytosis?

The special position occupied by the granulocyte in the myeloproliferative disorders evidently warrants further study.

Cyclical Disorders of Haemopoiesis.

This class of blood diseases is particularly instructive from the viewpoint of control theory and cybernetics. Because of this, cyclical haemopoiesis is discussed in detail in chapter 5 and in subsequent chapters as appropriate.

Consideration of cyclical haemopoiesis is therefore omitted from the present chapter.

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4. MATHEMATICAL REPRESENTATION OF CELLULAR MATURATION AND PROLIFERATION.

4. THE MATHEMATICAL REPRESENTATION OF CELLULAR MATURATION AND PROLIFERATION.

Introduction.

Hitherto, all discussion has been qualitative in form. In this chapter, a mathematical representation of cellular maturation and proliferation, framed in terms of observable quantities, is proposed. Although crude in many respects, the representation leads to a set of equations which do not appear to have been previously derived.

Originally, the motivation for the derivation of these equations was the necessity for the analytic formulation of cellular maturation in kinetic (non-causal) terms, to serve as a framework for the causally-based models and theories which form the subject of later chapters.

However, the representation lends itself to an iterative method of calculation of cellular maturation times in non-steady-state situations, previously considered intractable. To illustrate such applications, an extract is presented from a kinetic analysis of erythroid cell maturation in the mouse embryo. The full analysis, however, has been witheld as inappropriate in a dissertation whose primary subject is biocybernetics.

Consider a population comprising $N(t)$ cells at time t. We suppose the cells to be undergoing some kind of maturation process, with or without cell division. If $n(t,a)\,\delta a$ represents the number of cells whose chronological age (i.e. since onset of maturation) lies between a and $a+\delta a$,

$$N(t) = \int_0^\infty n(t,a)\,da \qquad (4.1)$$

If $\psi(t)$ is the rate of gain of cells to the population and $E(t)$ the rate of loss of cells from it, the conservation equation is

$$\frac{dN}{dt} = \frac{d}{dt}\cdot\int_0^\infty n(t,a)\,da = \psi(t) - E(t) \qquad (4.2)$$

Now, if $\psi(t)$ is dissociated into an age-specific cellular immigration term $\epsilon(t,a)$ and a proliferation rate term $\rho(t,a)$

$$\psi(t) = \int_0^\infty \left\{ \epsilon(t,a) + \rho(t,a)\cdot n(t,a) \right\} da \qquad (4.3)$$

Similarly, $E(t)$ may be expressed in terms of $\lambda(t,a)$, the loss rate function for cells aged a.

Thus,

$$\int_0^\infty \lambda(t,a)\cdot n(t,a)\,da = E(t) \qquad (4.4)$$

and

$$\int_0^\infty \left\{ \frac{d}{dt}\cdot n(t,a) + \left(\rho(t,a) - \lambda(t,a)\right) n(t,a) + \epsilon(t,a) \right\} da = 0 \qquad (4.5)$$

Now since equation (4.5) must hold for all times and all ages,

$$\frac{dn}{dt} + (\rho - \lambda) n + \epsilon = 0 \qquad (4.6)$$

Also, since

$$\frac{dn}{dt} \equiv \frac{\partial n}{\partial t} + \frac{\partial n}{\partial a} \cdot \frac{\partial a}{\partial t} \qquad (4.7)$$

and, with age and time measured on the same scale,

$$\frac{\partial a}{\partial t} \equiv 1$$

we have

$$\frac{\partial n}{\partial t} + \frac{\partial n}{\partial a} + (\rho - \lambda) n + \epsilon = 0 \qquad (4.8)$$

Equation (4.8) is the fundamental equation of cell population kinetics on the continuous representation. Similar equations have been introduced by Von Foerster (1), Trucco (2,3) and Rubinow (4).

Although conceptually elegant, this formulation encounters the difficulty that neither chronological age nor 'maturity' (as in Rubinow's equation 4) can be assessed continuously, nor even at evenly spaced intervals. Representations closer to those employed by experimentalists are necessary for the analysis of experimental data.

A Compartmental Representation of Cell Population Kinetics.

As discussed in chapter 2, developing cells are usually classified as belonging to one or another of a sequentially related set of stages of maturity. The definition of the stages and criteria for assignment of a particular cell to a particular stage are not free from subjective components.

Consider a series of identifiable stages of cellular maturation, the linear order of which reflects the supposed temporal order of maturation (fig. 4.1). This representation incorporates the abstraction that all cells considered to be at a particular stage of maturation are homogeneous in terms of maturity. The transition from one stage to another is conceived as being sharp, irreversible and temporally uneven. These improbable idealizations are the necessary penalty for a representation geared to observables, rather than one based on 'a priori' considerations.

It is convenient to regard maturational stages as being akin to 'compartments' as used in the theory of tracer kinetics. It should be noted however that maturational compartments are not defined in either space or time, and that no assumptions as to 'mixing' are of relevance in such a context.

Consider some defined maturational compartment. Let $N(t)$ denote the cellular content of the compartment, $S(t)$ the rate of entry of cells maturing from a previous compartment, and $E(t)$ the rate of exit of cells entering a subsequent compartment, all at time t .

Suppose that all cells undertake division whilst in the compartment (Homogeneity of proliferation is an important

assumption - see later sections). If t_c is the mean cell cycle time - assumed constant over any time-interval of interest, then it may be shown (appendix 4.1) that the cellular proliferation rate is

$$\alpha N(t) = \frac{\ln 2}{t_c} \cdot N(t)$$

Then, provided cellular death within the compartment is negligibly small,

$$\frac{d}{dt} \cdot N(t) = \xi(t) + \alpha N(t) - E(t) \qquad (4.9)$$

The value of this representation depends on obtaining an expression for the exit rate $E(t)$. As a first approximation to reality, suppose that maturation is a first-in-first-out process in which all cells reside within the compartment for a fixed 'maturation time', τ , here assumed time-independent.

Then, the exit rate $E(t)$ will be the entry rate time-delayed by τ and amplified by a factor representing the number of descendants of a single cell, produced in time τ . Thus,

$$E(t) = e^{\alpha\tau}\, \xi(t-\tau)$$

so that

$$\frac{dN}{dt} = \xi(t) + \alpha N(t) - e^{\alpha\tau}\, \xi(t-\tau) \qquad (4.10)$$

As is proved in appendix 4.II, equation (4.10) may be

alternatively written in integral formulation:

viz.

$$N(t) = \int_0^{\tau} e^{\alpha\theta}\, \xi(t-\theta)\, d\theta \qquad (4.11)$$

or

$$N(t) = e^{\alpha t} \int_{t-\tau}^{t} e^{-\alpha\psi}\, \xi(\psi)\, d\psi \qquad (4.12)$$

The alternative forms (4.10), (4.11) and (4.12), though mathematically equivalent, are useful for dealing with different kinds of problem.

Two other kinds of situation require attention. In the first place, post-mitotic cells mature without division e.g. reticulocytes maturing to become erythrocytes. This means that the proliferation constant, α , is zero. Then equations (4.10), (4.11) and (4.12) become

$$\frac{dN}{dt} = \xi(t) - \xi(t-\tau) \qquad (4.13)$$

$$N(t) = \int_0^{\tau} \xi(t-\theta)\, d\theta \qquad (4.14)$$

$$N(t) = \int_{t-\tau}^{t} \xi(\psi)\, d\psi \qquad (4.15)$$

respectively.

A more difficult problem is presented by a maturational stage which contains both dividing and non-dividing cells. This problem is deferred until some applications of the above equations have been described, and is considered in a later section.

An Iterative Method for the Calculation of Maturation Times.

Writing the basic maturation equation in the form of (4.12), viz.

$$N(t) = e^{\alpha t} \int_{t-\tau}^{t} e^{-\alpha\psi} \xi(\psi)\, d\psi \qquad (4.16)$$

it is sometimes possible to compute the value of τ.

Suppose that estimates of $N(t)$, $\xi(t)$ and α are available, so that τ is the only unknown quantity in equation (4.16). Such an equation, with the unknown appearing as one of the limits of an integral, has been little studied in the field of pure mathematics. Consequently, no analytical methods of solution are known, nor have any computational algorithms been devised.

Here, an iterative method of solution is described, which, subject to some restrictions, permits the evaluation of to within any desired margin of accuracy. This method of solution was proposed by the author, and the necessary algorithm and computer program was written by Dr. James Kirk.

The method is as follows; let $\Delta\tau$ be a small quantity at least one order of magnitude less than the time-scale appropriate to the cellular maturation process under consideration.

Then, since, $\tau \gg \Delta\tau$

$$\int_{t-\tau}^{t} e^{-\alpha\psi} \xi(\psi)\, d\psi \gg \int_{t-\Delta\tau}^{t} e^{-\alpha\psi} \xi(\psi)\, d\psi$$

because $e^{-\alpha\psi}\, \xi(\psi) \geq 0$ for all values of ψ.

Then, from (4.16) it follows that

$$N(t) \gg e^{\alpha t} \int_{t-\Delta\tau}^{t} e^{-\alpha\psi}\, \xi(\psi)\, d\psi$$

so that

$$\left\{ N(t) - e^{\alpha t} \int_{t-\Delta\tau}^{t} e^{-\alpha\psi}\, \xi(\psi)\, d\psi \right\} \ll 0$$

However

$$\left\{ N(t) - e^{\alpha t} \int_{t-\tau}^{t} e^{-\alpha\psi}\, \xi(\psi)\, d\psi \right\} = 0$$

These relationships allow the possibility of evaluating τ by an iterative procedure. Let us define the function $I(k,t)$ as

$$I(k,t) = e^{\alpha t} \int_{t-k\Delta\tau}^{t} e^{-\alpha\psi}\, \xi(\psi)\, d\psi \qquad (4.17)$$

Then if

$$I(k,t) \leq N(t)$$

but

$$I(k+1,t) \geq N(t)$$

it follows that

$$k\Delta\tau \leq \tau \leq (k+1)\Delta\tau$$

Thus, by starting with a small step $\Delta\tau$ which is built up sequentially until I reverses sign, a first approximation to τ can be obtained. Closer approximations may be obtained by using a smaller step, say $\Delta\tau^*$, to repeat the procedure over the narrow range $[\, k\Delta\tau,\ (k+1)\Delta\tau \,]$ within which τ is known to lie.

A useful refinement may be introduced to avoid local

irregularities associated with turning points and inflections in $N(t)$ or $\xi(t)$.

Let $J(k, t_1, t_2)$ be defined as

$$J(k, t_1, t_2) = \int_{t_1}^{t_2} dt \; e^{\alpha t} \int_{t - k\Delta\tau}^{t} e^{-\alpha\psi} \xi(\psi) \, d\psi \qquad . \quad (4.18)$$

and $N^*(t_1, t_2)$ as

$$N^*(t_1, t_2) = \int_{t_1}^{t_2} N(t) \, dt$$

Then if

$$J(k, t_1, t_2) \leq N^*(t_1, t_2)$$

and

$$J(k+1, t_1, t_2) \geq N^*(t_1, t_2)$$

the inequalities

$$k\Delta\tau \leq \tau \leq (k+1)\Delta\tau$$

yield an approximation to the value of τ over the interval (t_1, t_2).

The algorithmic representation of this method of solution is depicted in fig. (4.2).

It should be noted that the assumed time-invariance of is a necessary assumption in the derivation of the basic maturation equations. The results of applying the method described above may be accepted only if the value of τ over adjacent intervals of the form $[(t_1, t_2) ; (t_2, t_3)]$ changes sufficiently slowly that a constant τ is a good approximation

within either interval.

A deficiency of the method is the absence of any analytical means of guaranteeing the convergence of the iteration, or, if convergent the uniqueness of the solution.

These problems have not given rise to practical difficulty in applications so far completed but the investigation of this topic and derivation of analytical criteria for convergence and uniqueness remains an important task.

The extension of this method to evaluate the maturation times of cells in post-mitotic compartments involves merely setting the proliferation constant, α , to zero.

The Status of Heterogeneous Transitional Compartments.

Throughout, homogeneity of proliferative activity has been assumed i.e. all cells in a compartment are in cycle with cycle time t_c, or are not in cycle at all. Of course, not all cycling cells will really have the same cycle time; however, t_c may be considered the mean of a statistical distribution of cycle times, without too great a loss of realism, for most types of compartment encountered.

However, a more basic difficulty arises when a particular compartment type - a transitional compartment - is encountered. As blood cells mature, they eventually become post-mitotic (with the exception of certain lymphoid cells, of no interest here). It would be convenient if this transition, from proliferative to post-mitotic cell, were a morphologically distinct change permitting a proliferative compartment to be clearly distinguished from the succeeding post-mitotic compartment. Unfortunately, present techniques do not allow such a distinction.

Consequently, some defined compartments such as polychromatic erythroblast (in erythroid development) or myelocyte (in granulocytic development) are heterogeneous in terms of proliferation i.e. they contain both proliferating and post-mitotic cells. The analysis of maturation within a heterogeneous transitional compartment constitutes a difficult problem.

Under steady-state conditions, it is possible to formulate and experimentally evaluate simple models of the mechanism of transition (5 - 7) but the non-steady-state situation

presents additional difficulties. Probably, it will be necessary to return to a continuous 'a priori' representation and to formulate models of the transition mechanism in terms of this. The relation of the continuous representation to the experimental data then poses a problem which will require to be solved by imposing a piecewise continuous transformation to represent the assignment of members of the continuously-varying population to discrete categories. This is evidently a task for the future.

For the present, very approximate estimates may be obtained by noting that the true proliferative status of a heterogeneous transitional compartment is intermediate between that of a homogeneously proliferative and a homogeneously post-mitotic compartment.

To obtain bounds on τ, the 'true' maturation time, let τ^* be the solution to

$$N(t) = e^{\alpha t} \int_{t-\tau^*}^{t} e^{-\alpha\psi} \xi(\psi)\, d\psi \qquad (4.20)$$

and τ^{**} the solution to

$$N(t) = \int_{t-\tau^{**}}^{t} \xi(\psi)\, d\psi \qquad (4.21)$$

Provided equations (4.20) and (4.21) may be numerically solved by the iteration method described above, the inequation

$$\tau^* < \tau < \tau^{**}$$

provides bounds on τ, and hence gives a rough idea of its magnitude.

Despite its limitations, the proposed representation and the associated iteration method permit an approach to be made to the analysis of cellular maturation in certain classes of non-steady-state system. To compute maturation times, it is necessary to assume that neither the cell cycle time, nor maturation time, of the cells concerned vary too rapidly. However, the input rate and cell number of a defined compartment can vary in any way at all.

Hence, although some restrictions have been imposed, the formal steady-state requirements of most other analytical procedures have been relaxed.

A situation where relaxed steady-state requirements are highly advantageous is presented by erythropoiesis in the embryo. As discussed in chapter 2, embryonic erythropoiesis in higher species exhibits a dynamic pattern, including a shifting of erythropoiesis from one organ to another. It follows that the early phases of embryonic erythropoiesis are intrinsically non-steady-state and hence resistant to analytical methods of interpretation for which rigidly steady-state conditions are required.

Recently, the hepatic phase of erythropoiesis in the mouse embryo has been a focus of attention. Experimental data furnished by two groups of workers; Paul, Conkie and Freshney (8) and Tarbutt and Cole (9) provide information as to the numbers of different kinds of erythroid cells, their cell cycles (where appropriate) and (indirectly) the input rates to certain compartments - all at different gestational ages.

The analysis of a section in this data - that pertaining to the basophilic erythroblast compartment - is presented here, in illustration of the possible utility of the representation described. A detailed analysis of hepatic erythropoiesis in the mouse embryo is presented elsewhere (10).

Analysis of Maturation of Embryonic Basophilic Erythroblasts.

The basophilic erythroblasts belong to the last homogeneous proliferative compartment of the erythroid series (see fig. 2.1). It directly follows the proerythroblast compartment, which is also homogeneously proliferative. Now, provided a reasonable amount of amplification through cell division occurs in the proerythroblast compartment, the input rate to it will be small in comparison with the exit rate from it. Therefore,

$$\text{EXIT RATE} \approx \alpha' N' - \frac{dN'}{dt} \qquad (4.22)$$

where N' denotes the number of proerythroblasts and α' the rate constant of proliferation. This exit rate, which is identical with $\xi(t)$, the entry rate to the basophilic erythroblast compartment, can be calculated from the published data, for different stages of gestation.
Then, using the usual notation,

$$N(t) = e^{\alpha t} \int_{t-\tau}^{t} e^{-\alpha\psi} \xi(\psi)\, d\psi \qquad (4.23)$$

which, provided N and α are also available from the data, allows calculation of τ for the basophilic erythroblast compartment. Values α', N', α and N are available from the data of Tarbutt and Cole (9) and, independently, values of N' and N are also available from the data of Paul, Conkie and Freshney (8).
The iteration method described was applied to these two sets of data, to obtain estimates of τ at different stages of gestation (the murine gestation time is 18 - 19 days -

hepatic erythropoiesis is evident from 12 - 13 days). The results of the computation are presented in table 4.1.

Gestation Time (Days)	Maturation Time (Days)	
	Paul et. al.	Tarbutt + Cole
12 - 13	1.22	0.16
$12\frac{1}{2} - 13\frac{1}{2}$	0.44	0.23
13 - 14	I.T.*	0.28
$13\frac{1}{2} - 14\frac{1}{2}$	0.26	0.33
14 - 15	0.34	0.40
$14\frac{1}{2} - 15\frac{1}{2}$	0.31	0.44
15 - 16	0.33	0.42
$15\frac{1}{2} - 16\frac{1}{2}$	0.29	0.38
16 - 17	0.29	0.35
$16\frac{1}{2} - 17\frac{1}{2}$	0.25	0.36
17 - 18	0.24	0.32

* I.T.: Iteration terminated because of failure to converge.

TABLE 4.1 : MATURATION TIME OF EMBRYONIC BASOPHILIC ERYTHROBLASTS.

Two points are worthy of note; firstly, in the results obtained from the data of Paul et al., there is a rapid change from 1.22 days maturation time (12 - 13 days gestation) to 0.44 days ($12\frac{1}{2} - 13\frac{1}{2}$ days gestation). So rapid

a change violates the assumption that τ is steady, or changing only slowly, so the first two results are probably unacceptable. Secondly, at 13 - 14 days gestation time, the iteration inexplicably failed to converge. These two points emphasize the desirability of a more rigorously analytic treatment of the iteration procedure, than has been given here.

However, from about $13\frac{1}{2}$ days gestation onwards, the maturation time, as calculated from both sets of data, changes only slowly, and appears to be of the order of about 8 hours (one third of a day) which is appreciably shorter than the steady-state result obtained for adult rats ($\simeq$ 23 hours).

Two sources of error should be mentioned. Firstly, the neglect of input of precursors to the proerythroblast compartment underestimates the input to the basophilic erythroblast compartment and leads to an overestimate of the maturation time. Secondly, death of developing cells has been neglected. Should this occur to the same degree in all compartments, little error would result from neglecting it, but a differential death rate in different compartments complicates the problem. However, death rate is also (usually) neglected in steady-state analysis.

In summary, the analysis given provides sufficiently good estimates of the basophilic maturation time to establish two main conclusions.

From about $13\frac{1}{2}$ days gestation time, both sets of data yield results showing a slight initial rise in maturation time, peaking around 14 - 15 days and followed by a slow progressive drop. The mean value of the maturation time is

evidently around a third of a day. (Fig. 4.3)
As more data becomes available, the method given should allow a progressive refinement of the kinetics of embryonic erythropoiesis, a situation not otherwise amenable to analysis. Other non-steady-state situations involving cellular maturation should be open to analysis in a similar manner.

APPENDIX 4 I

For any cell, mitosis is a discrete, quantized, event. Nevertheless, a population of dividing cells can be treated by continuous analysis, provided the population is sufficiently large, all cells have (at least roughly) the same cycle time, t_c, and the members of the population are randomly distributed over the phases of the cell cycle.

Let there be $N(t)$ cells in such a population at time t. There will then be $N(t)\ 2^{\left(\frac{\delta t}{tc}\right)}$ cells at time $t + \delta t$, neglecting losses. Denoting the lossless increment due to mitosis during the interval $[t,\ t + \delta t]$ as δN_m we obtain

$$\delta N_m = N(t)\ 2^{\frac{\delta t}{tc}} \tag{4.I.1}$$

$$= N(t)\ e^{\alpha\, \delta t} \tag{4.I.2}$$

where $$\alpha = \frac{\ln 2}{t_c} \tag{4.I.3}$$

Expanding $e^{\alpha\, \delta t}$ in a Taylor series, we have

$$\delta N_m = N(t) \sum_{j\,=\,1}^{\infty} \frac{(\alpha\, \delta t)^j}{j!} \tag{4.I.4}$$

$$\frac{\delta N_m}{\delta t} = N(t)\, \alpha + \sum_{j=2}^{\infty} \frac{\alpha^j\, (\delta t)^{j-1}}{j!} \tag{4.I.5}$$

As $t \to \infty$ $\frac{\delta N_m}{\delta t} \to \frac{dN_m}{dt}$ and $\sum_{j\,=\,2}^{\infty} \frac{\alpha^j\, (\delta t)^{j-1}}{j!} \to 0$

Thus,

$$\frac{dN_m}{dt} = \alpha\, N(t) \tag{4.I.6}$$

APPENDIX 4 II

We wish to establish the equivalence of equations (4.11), (4.12), (4.13). Equation (4.12) states

$$N(t) = \int_0^{\tau} e^{\alpha\theta} \xi(t-\theta)\, d\theta \tag{4.II.1}$$

Put $\psi = t - \theta$ so that $\theta = t - \psi$ and $d\theta = -d\psi$. Then,

$$N(t) = -\int_t^{t-\tau} e^{\alpha(t-\psi)} \xi(\psi)\, d\psi \tag{4.II.2}$$

$$= e^{\alpha t} \int_{t-\tau}^{t} \bar{e}^{\alpha\psi} \xi(\psi)\, d\psi \tag{4.II.3}$$

which establishes the equivalence of equations (4.12) and (4.13)

Differentiating,

$$\frac{dN}{dt} = \frac{d}{dt} \cdot e^{\alpha t} \int_{t-\tau}^{t} \bar{e}^{\alpha\psi} \xi(\psi)\, d\psi \tag{4.II.4}$$

$$= \alpha\, e^{\alpha t} \int_{t-\tau}^{t} \bar{e}^{\alpha\psi} \xi(\psi)\, d\psi + e^{\alpha t} \frac{d}{dt} \cdot \int_{t-\tau}^{t} \bar{e}^{\alpha\psi} \xi(\psi)\, d\psi \tag{4.II.5}$$

$$= \alpha N(t) + e^{\alpha t} \left\{ \int_{t-\tau}^{t} \frac{d}{dt} \cdot \left(\bar{e}^{\alpha\psi} \xi(\psi) \right) d\psi + \left[\bar{e}^{\alpha\psi} \xi(\psi) \right]_{\psi = t} \frac{d}{dt} \cdot (t) \right.$$

$$\left. + \left[\bar{e}^{\alpha\psi} \xi(\psi) \right]_{\psi = t-\tau} \frac{d}{dt} \cdot (t-\tau) \right\} \tag{4.II.6}$$

$$= \alpha N(t) - e^{\alpha\tau} \xi(t-\tau) + \xi(t) \tag{4.II.7}$$

Since equation (4.II.7) is identical with equation (4.11), we have shown that (4.12) and (4.13) are equivalent forms which each satisfy (4.11).

Now (4.11) is an ordinary linear differential equation of the first order. The general solution of such an equation can be written (See (11))

$$x(t) = Ay(t) + z(t) \qquad (4.II.8)$$

where $y(t)$ is the 'complementary function', $z(t)$ the 'particular integral' and A a constant of integration.

We have shown that, for equation (4.11),

$$z(t) = \int_0^{\tau} e^{\alpha\Theta}\xi(t-\Theta)\, d\Theta = e^{\alpha t}\int_{t-\tau}^{t} e^{-\alpha\psi}\xi(\psi)\, d\psi \qquad (4.II.9)$$

The 'complementary function' for (4.11) is the solution to the homogeneous equation

$$\text{viz} \qquad \frac{dx}{dt} = \alpha\, x(t) \qquad (4.II.10)$$

$$\therefore \qquad x(t) = A\, e^{\alpha t} \qquad (4.II.11)$$

where A is a constant.

Hence, the general solution of equation (4.11) is

$$N(t) = A\, e^{\alpha t} + e^{\alpha t}\int_{t-\tau}^{t} e^{-\alpha\psi}\xi(\psi)\, d\psi \qquad (4.II.12)$$

To evaluate A, we must impose conditions appropriate to the problem. Here, the appropriate condition is that $N(t)$ be a continuous function of τ and that $N(t) \to 0$ as $\tau \to 0$ ie Instantaneous maturation gives zero maturing cells.

Now $$e^{\alpha t}\int_{t-\tau}^{t} e^{-\alpha\psi}\xi(\psi)\, d\psi \longrightarrow 0 \quad \text{as} \quad \tau \to 0$$

$$\therefore \qquad N(t) \to Ae^{\alpha t} \quad \text{as} \quad \tau \to 0$$

But we require

$$N(t) \to 0 \quad \text{as} \quad \tau \to 0$$

Hence $A = 0$

It follows that

$$N(t) = e^{\alpha t}\int_{t-\tau}^{t} e^{-\alpha\psi}\xi(\psi)\, d\psi \qquad (4.II.13)$$

and its equivalent forms, comprises the solution of (4.11).

APPENDIX 4 III

Evaluation of Maturation Times of Post-Mitotic Cells By a Graphical Method

This method is restricted in applicability to a rather special class of cellular maturation processes. However, it provides, for such processes, certainly the quickest and the easiest method available for estimating maturation times.

Consider two post-mitotic maturation compartments. The cell content of the earlier compartment is denoted by N_o (t), that of the later compartment by N_1 (t) and that of the composite compartment, obtained by joining both, by N_2 (t). Let $\xi(t)$ be the entry rate into the earlier compartment. Then, if τ be the maturation time of the earlier compartment, $\xi(t-\tau)$ will be the exit rate from this compartment, which is identical to the entry rate to the next.

The following restrictive assumptions are introduced :

a) The pathway of maturation is irreversible and unbranched.

b) The maturation time of the earlier compartment is effectively constant over times of interest.

c) No cells leave the second compartment once they have entered. (ie its maturation time, or the cellular lifetime is infinite).

d) No cell death occurs in either compartment.

e) No cell division occurs in either compartment.

Granting these assumptions, the Kinetic equations for the ~~earlier~~ later and the composite compartment are

$$\frac{dN_1}{dt} = \xi(t-\tau) \qquad (4.III.1)$$

$$\frac{dN_2}{dt} = \xi(t) \qquad (4.III.2)$$

$$\therefore N_1(t) - N_1(0) = \int_o^t \xi(\theta-\tau)\, d\theta \qquad (4.III.3)$$

$$N_2(t) - N_2(0) = \int_0^t \xi(\theta)\, d\theta \qquad (4.III.4)$$

Setting $\psi = \theta - \tau$, the integral in eqn (4.III.3) becomes

$$\int_0^t \xi(\theta - \tau)\, d\theta = \int_{-\tau}^{t-\tau} \xi(\psi)\, d\psi \qquad (4.III.5)$$

$$= \int_0^{t-\tau} \xi(\psi)\, d\psi + \int_{-\tau}^{0} \xi(\psi)\, d\psi \qquad (4.III.6)$$

Hence $$N_1(t) = N_1(0) + \int_0^{t-\tau} \xi(\psi)\, d\psi + \int_{-\tau}^{0} \xi(\psi)\, d\psi \qquad (4.III.7)$$

Suppose now that t_1 and t_2 are times such that

$$N_1(t_1) = N_2(t_2)$$

$$\underline{\text{ie}} \quad N_1(0) + \int_0^{t_1-\tau} \xi(\psi)\, d\psi + \int_{-\tau}^{0} \xi(\psi)\, d\psi = N_2(0) + \int_0^{t_2} \xi(\theta)\, d\theta \qquad (4.III.8)$$

Now,

$$N_2(t) - N_1(t) = N_o(t) \qquad (4.III.9)$$

but, in particular,

$$N_2(0) - N_1(0) = N_o(0) \qquad (4.III.10)$$

Also $$N_o(t) = \int_{t-\tau}^{t} \xi(\psi)\, d\psi \qquad (4.III.11)$$

and $$N_o(0) = \int_{-\tau}^{0} \xi(\psi)\, d\psi \qquad (4.III.12)$$

Hence, equation (4.III.8) becomes

$$\int_0^{t_1-\tau} \xi(\psi)\, d\psi = \int_0^{t_2} \xi(\theta)\, d\theta \qquad (4.III.13)$$

from which it follows that

$$t_1 - \tau = t_2 \qquad (4.III.14)$$

or

$$\tau = t_1 - t_2 \qquad (4.III.15)$$

Equation 4.III.15 permits a simple graphical estimate of τ for maturation processes which fulfill the necessary conditions (see fig. 4.4)

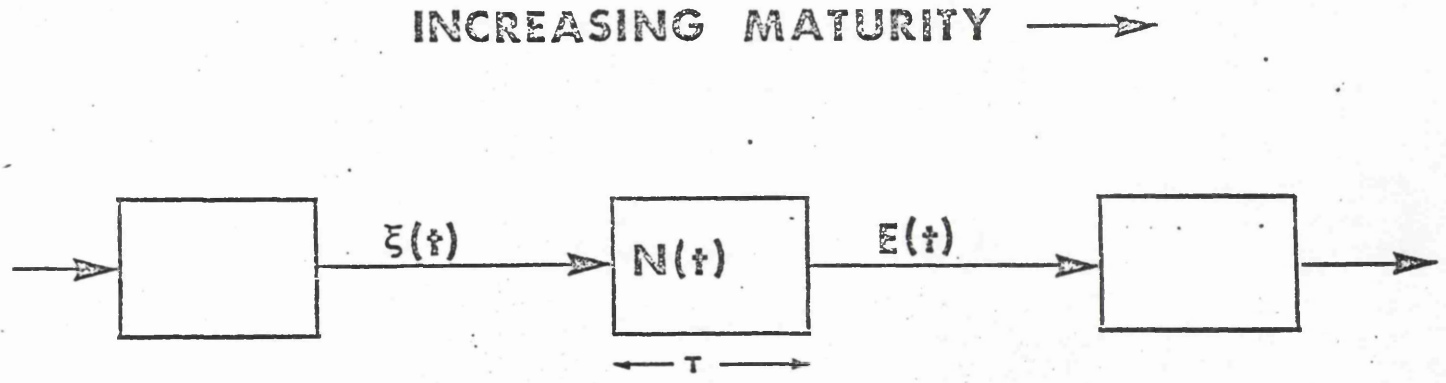

FIG. 4.1

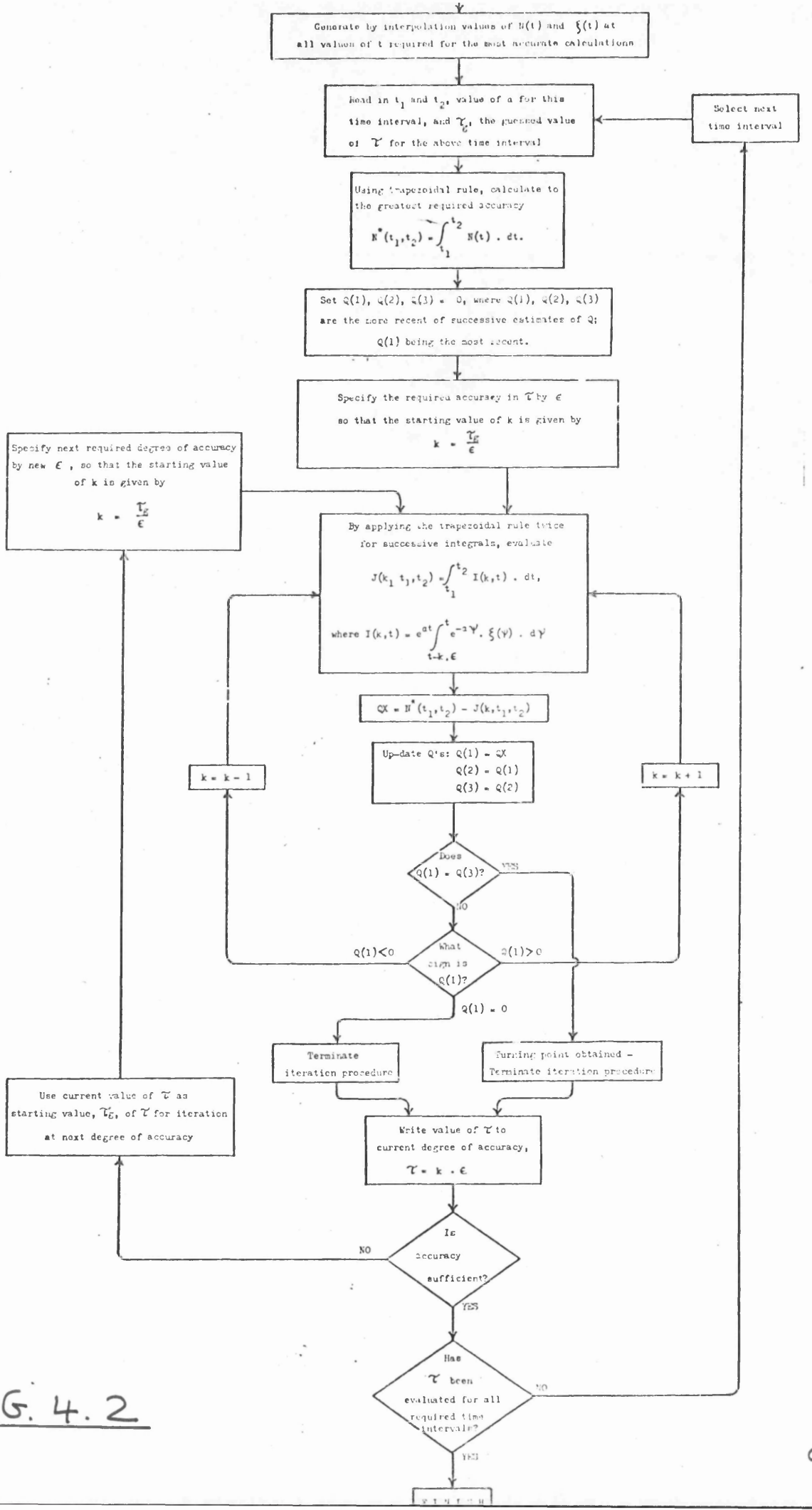
Generate by interpolation values of N(t) and ξ(t) at all values of t required for the most accurate calculations
Read in t_1 and t_2, value of a for this time interval, and τ_g, the guessed value of τ for the above time interval
Select next time interval
Using trapezoidal rule, calculate to the greatest required accuracy
$N^*(t_1,t_2) = \int_{t_1}^{t_2} N(t)\,.\,dt.$
Set Q(1), Q(2), Q(3) = 0, where Q(1), Q(2), Q(3) are the more recent of successive estimates of Q; Q(1) being the most recent.
Specify the required accuracy in τ by ϵ so that the starting value of k is given by
$k = \frac{\tau_g}{\epsilon}$
Specify next required degree of accuracy by new ϵ, so that the starting value of k is given by
$k = \frac{\tau_g}{\epsilon}$
By applying the trapezoidal rule twice for successive integrals, evaluate
$J(k_1\ t_1,t_2) = \int_{t_1}^{t_2} I(k,t)\,.\,dt,$
where $I(k,t) = e^{at}\int_{t-k,\epsilon}^{t} e^{-a\gamma}.\,\xi(\gamma)\,.\,d\gamma$
$\delta X = N^*(t_1,t_2) - J(k,t_1,t_2)$
Up-date Q's: Q(1) = δX, Q(2) = Q(1), Q(3) = Q(2)
k = k - 1
k = k + 1
Does Q(1) = Q(3)?
YES
NO
What sign is Q(1)?
Q(1)<0
Q(1)>0
Q(1) = 0
Terminate iteration procedure
Turning point obtained - Terminate iteration procedure
Use current value of τ as starting value, τ_g, of τ for iteration at next degree of accuracy
Write value of τ to current degree of accuracy, $\tau = k\,.\,\epsilon$
Is accuracy sufficient?
NO
YES
Has τ been evaluated for all required time intervals?
NO
YES

FIG. 4.2

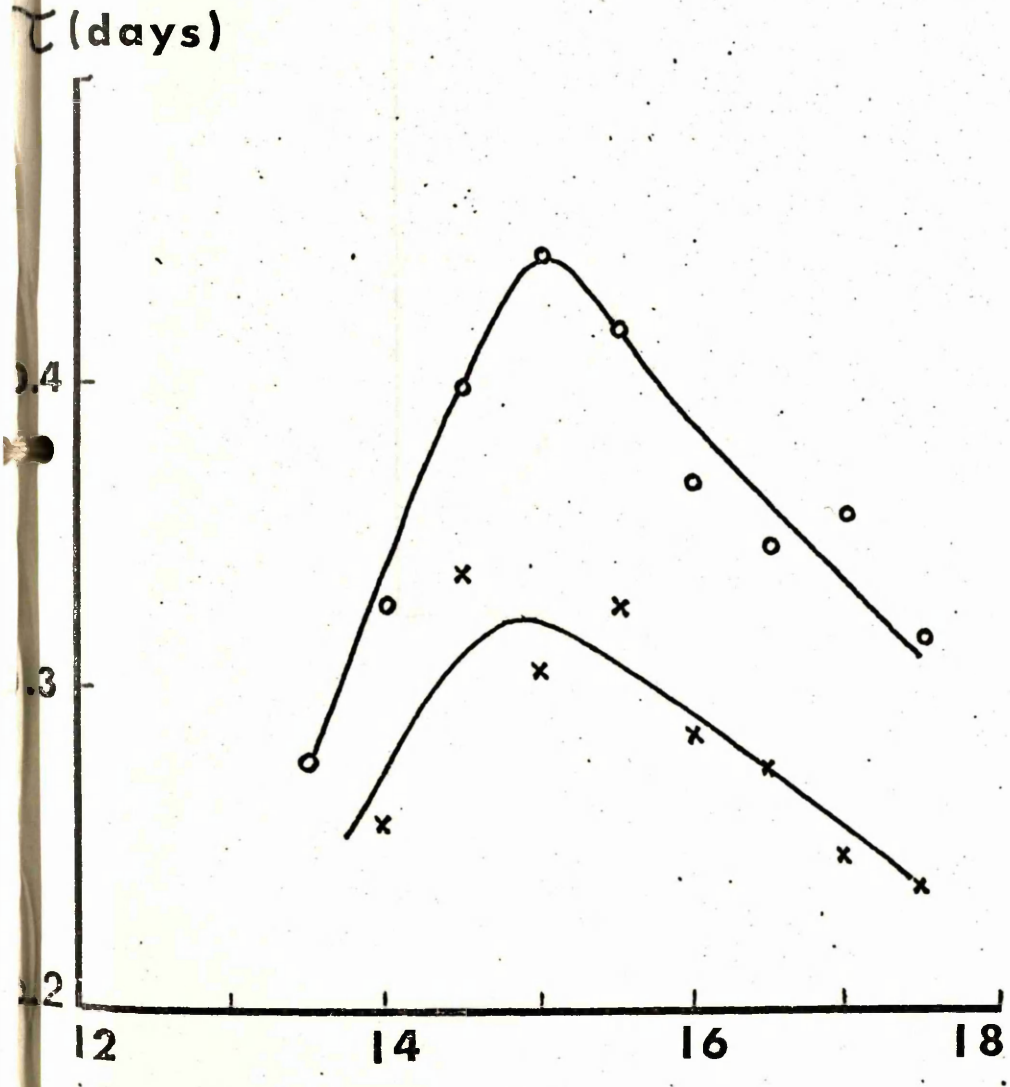

FIG. 4.3

× - Paul et al.
o - Tarbutt and Cole

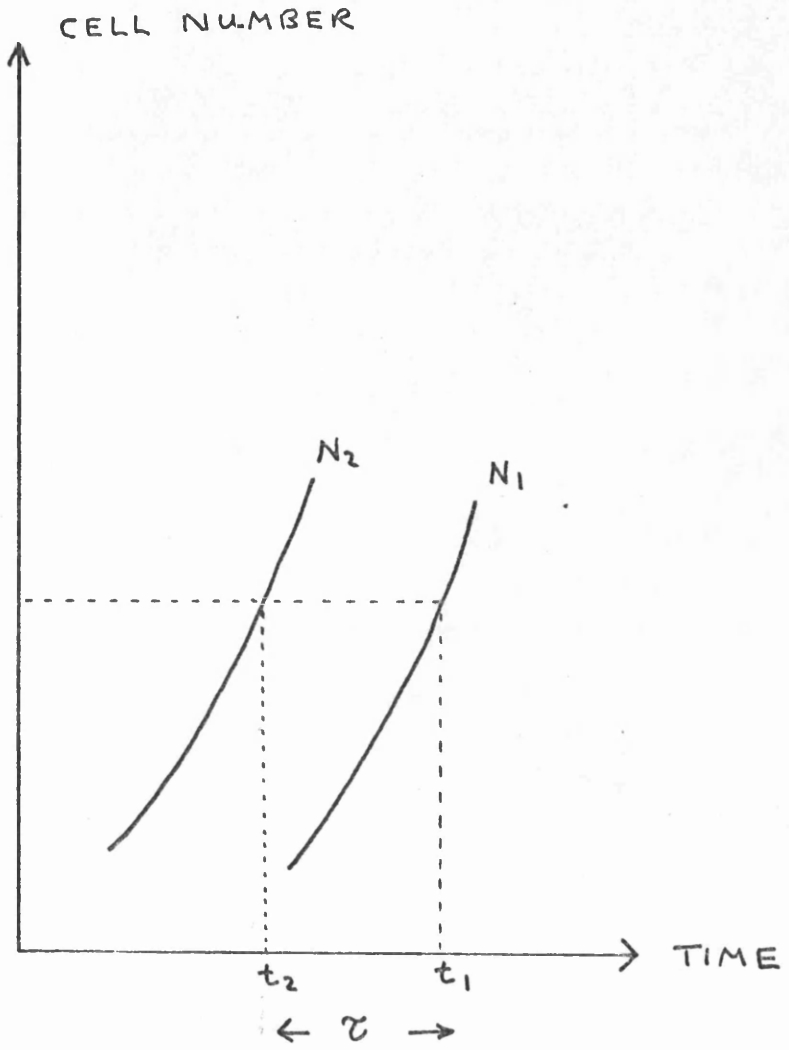

FIG. 4.4

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5. CYCLICAL HAEMOPOIESIS.

5. CYCLICAL HAEMOPOIESIS.

Steady-States and Stability.

Until recently, it has been generally assumed that a biological system (e.g. the haemopoietic system of an experimental animal) known to be stable over long periods of time would, if undisturbed, be in a 'steady-state' – its kinetic behaviour being independent of time. This assumption has been almost ubiquitous throughout biological science and provides the conceptual basis for methods of interpretation of a vast quantity of experimental data.

Theoretically, stability and steady-state are not synonymous concepts. Stability, in the present context, means the property of a system free from external disturbances, greater than some non-zero threshold, to maintain its integrity over 'long' periods of time. Of course, 'long' is ill-defined; if the time concerned were taken to be several centuries, few systems of any biological organism could be considered stable. In practice, however, confusion is seldom likely to arise.

A 'steady-state' is a theoretically tighter concept, requiring temporal invariance of the system behaviour, again over 'long' intervals of time. Now, a system in a steady-state condition is obviously in a state of stability. However, a stable system is not necessarily in a steady-state condition. For stability, it is only necessary that the system, if it alters with time, never does so in such a way as to impair its integrity.

Conceptually, there is no reason why particular states of the

system should recur at regular intervals, or indeed recur at all, but, as will be discussed below, repeating states are a very commonly encountered natural phenomenon. The essential first point is that freedom from external disturbance does not suffice to establish that a stable system is in, or approaching, a steady-state condition, nor even that the temporal evolution of the system is occuring slowly.

Feedback, Time-Delays and Limit Cycles.

Systems of particular interest here are those biological control systems involving feedback. If the system is a stable one, at least one section of the system must involve negative rather than positive feedback, otherwise the 'amplification' effect produced by positive feedback would proceed without limit(but see chapter 9).

In chapter 1, a negative feedback system was described in terms of a mechanism which coupled a measure of some generalized 'distance' to another mechanism capable of increasing or decreasing this 'distance'. Omitted from consideration was the time between the measurement of the 'distance' and the change of 'distance' induced in response to the measurement. Since, the velocity of signal transmission is always finite, this time cannot be zero.

The possible effect of such a time-delay on a negative feedback control system is intuitively obvious. If a delay ensues before the system reacts to an increasing 'distance', the 'distance' will increase further before its direction of change is reversed. Conversely, the measurement of this reversal will not induce an immediate response and the control mechanism is liable to 'overcorrect' by causing a greater decrease of the 'distance' than would suffice to restore the system to its former state. In other words, an oscillation can occur.

Threetypes of oscillation are of interest here. Divergent oscillations exhibit increasingly large swings with time and cannot occur in truly stable systems. Damped, or convergent,

oscillations die away with time and are a common mode of a systems' return to a steady-state condition following a transient perturbation.

The third type of oscillation, a sustained oscillation whose amplitude remains the same with time, is a form of stable behaviour whose likelihood of occurrence has not been widely appreciated. In fact, Gontcharoff (1) and Rubin and Sitgreaves (2) have shown that as the number of couplings between the elements of a system becomes larger, (strictly, the number of degrees of freedom increases) the likelihood of a stable sustained oscillation being established becomes larger too. This argument has been developed by Goodwin(3) in his theory of temporal organization in cells.

Historically, sustained oscillations were first studied in the context of the 'physics of vibrations', the harmonic oscillator being the prototype of the class of oscillators under consideration. Mathematically, however, the harmonic oscillator is an implausible kind of device because it exemplifies 'neutral stability' i.e. its amplitude of vibration is determined for all time by its starting condition. Physically realistic systems do not exhibit neutral stability (Quantum-mechanical systems are not under consideration here). Those which at all resemble the harmonic oscillator are either unstable (divergent oscillation) or react to perturbations with an oscillatory response whose amplitude decays with time. In general, models based on the harmonic oscillator are of little value in studying sustained oscillations in biological systems.

A class of non-linear oscillations more appropriate

to the present context is the class of 'limit cycles' originally discussed by H. Poincaré as the asymptotic solutions to certain non-linear differential equations (4) and later by B. Van der Pol (5,6) in the field of electronics. Limit cycle oscillators exhibit dissipative rather than neutral stability, which means that they return to a stable mode of oscillation after transient perturbations. A limit cycle oscillator resembles a damped harmonic oscillator except that the equilibrium state is a periodic oscillation of constant amplitude rather than a steady-state. Time-delayed control systems are especially prone to oscillate in the limit cycle mode. Limit cycles are therefore to be anticipated in biological systems.

Oscillatory Phenomena in the Control of Haemopoiesis.

Physiological control systems, like those regulating haemopoiesis, may be described mathematically in terms of sets of differential-difference or integral equations. Associated with such equations will usually be various coefficients and constants which together form a set of parameters uniquely defining the system in the context of the specified equations.

The work of Gontcharoff (1) and Rubin and Sitgreaves(2) establishes that, as a set of equations becomes (loosely speaking) more complicated, the probability that a random choice of the parameters leads to a state of limit cycling approaches unity. However, in physiological systems, the choice of parameters cannot be considered at random. In general, evolution will select against features (as represented here by the parameters) which are biologically detrimental. Now, it is generally true that the sensitivity of a control system is linked to its liability to oscillate and an evolutionary compromise between sensitivity and steady behaviour would probably result in a selection of a set of parameters corresponding to a 'border-line' situation between steady-state and limit cycling (see 7).

Recent evidence in the field of haematology - much of it due to Morley and his colleagues - suggests that the control systems regulating blood cell production are operating close to this border-line. For the main pathways of blood cell development, production is controlled by a negative feedback loop linking the size of the appropriate mature cell

population to the induction of cytodifferentiation in receptive stem cells. Typically, the maturation of the developing cells is a process taking several days and which introduces a substantial time-delay into the control system. Oscillatory behaviour is therefore very liable to occur, but may be offset by the evolutionary selection of sets of parameters conferring stability or the evolution of compensatory mechanisms, should the occurrence of violent oscillation be physiologically detrimental.

However, not every individual of a population or species need have identical sets of parameter values and, if such values are very critical, a statistical spread could result in the occurrence of steady-state behaviour in some individuals of the population and limit cycling in others.

Again, the effective parameter values may be altered from one characteristic state to another by experimental manipulation. The evocation of limit cycle behaviour in previously steady-state control systems is a possible intervention to which the name 'stress cycle analysis' has been applied (8).

Apart from experimental interference, disease processes may have the effect of shifting one or other of the system parameters in such a way as to alter its behaviour in this regard. Such diseases may be quite difficult to understand in classical physiological terms, and may require the application of control theory for their comprehension and rational therapy.

Cyclical Granulopoiesis in Normal Subjects.

The first definite report of cyclical granulopoiesis in normal human subjects was that of Morley (9) in 1966. Since then, the qualitative features of this report have been confirmed by the unpublished observations of the present author and others.*

In eight of eleven healthy adult males, Morley found an oscillation of the peripheral blood neutrophil count having a period between 14 and 23 days (averaging 20 days) and a somewhat variable amplitude. The assessment of cyclical phenomena in female subjects is obviously complicated by the existence of the female menstrual cycle but there is no reason to suppose that the control of granulopoiesis differs between the sexes. Observations on pre-pubertal and post-menopausal female subjects would resolve the point but such observations have not yet been reported.

Morley and his colleagues have interpreted cyclical granulopoiesis in normal subjects as a manifestation of a time-delayed control system regulating granulopoiesis(10,11). The mean period of the oscillation, 20 days, would be that resultant from a time-delay of the order of 10 days, which is consistent with experimental estimates of the granulocytic maturation time in man (12).

* Late note : see criticism by Dale et. al. Brit. J. Haemat. 24, 57 (1973

Cyclical Neutropenia.

The observation of cyclical granulopoiesis in normal subjects casts new light on cyclical neutropenia, a pathological disorder of granulopoiesis in children, which has been recognized for many years.

The clinical features of cyclical neutropenia, though varied, may usually be referred to an absolute neutrophil shortage which recurs at intervals of about 20 days.

Typically, the blood neutrophil count is almost zero at the nadir of the oscillation, rising to between 20% and 50% of the normal count at its zenith. The oscillation is not due to a cyclic variation in granulocyte margination or marrow release but is apparent at the level of myeloblastic precursors.

A review of publications relating to this disorder has been given by Page and Good (13), who also report the case of a 14 year old girl, studied since early childhood. The disorder exhibits no obvious relationship to the menstrual cycle in girls or to recognized hormonal function in either sex.

Riemann (14) advanced the view that cyclical neutropenia evidenced a class of metabolic oscillation not specific to haemopoiesis. However, since the realization that the haemopoietic control systems may be rather prone to oscillate, cyclical neutropenia has been more plausibly interpreted as a limit cycle mode of granulopoiesis resulting from alterations in the parameters of the granulopoietic control system.

Two features are of particular interest; the period is normal but the mean level is abnormally low. From the latter observation, an increased destruction rate of granulocytes may be suspected, and is consistent with the observation that autoimmune phenomena are sometimes in evidence (15,16) and that cyclical neutropenia can be induced in dogs by the administration of cyclophosphomide (17). However, as cyclical neutropenia has been reported in a patient who was unable to manufacture antibodies (aggamaglobuniema) (18), this is probably not the universal cause of this disorder.

The variation in periphal neutrophil count in a 3 year old boy suffering from cyclical neutropenia is shown in fig. 5.1. As may be seen, the period is close to 20 days and the mean neutrophil count well below normal (Dr. Michael Willoughby, personal communication 1971).

Cyclical Granulopoiesis in Chronic Granulocyte Leukaemia.

In 1967, Morley, Carew and Baikie (19) discovered the existence of cyclical granulopoiesis in patients suffering from chronic granulocytic leukaemia (C.G.L.). Since then, the phenomenon has been confirmed by three additional different groups (20 - 22) and it seems likely that it exists in at least a proportion of all cases of C.G.L.

The observations contained in the four reports so far published* are summarized in table 5.1.

Report	No. Of Cases Observed	Mean Period of Oscillation	Reference
Morley et. al. (1968)	4	≈ 50 days	19
Kennedy (1970)	5	≈ 40 days	20
Shadduck et. al. (1972)	1	~ 60 days	21
Vodopick et. al. (1972)	2	≈ 60 days	22

TABLE 5.1. CYCLICAL GRANULOPOIESIS IN C.G.L.

From table 5.1. it may be seen that the period of the granulocyte oscillation in C.G.L., though variable from one patient to another, is generally at least double that which would be expected for a normal maturation time and observed in normal individuals (9,11). Also, the mean granulocyte

level is not stationary (as it is in normal granulopoiesis and in cyclical neutropenia) but progressively rises with time.

These observations provide valuable information on the kinetics of granulopoiesis in C.G.L. which may assist in the understanding of this disease. In chapter 9, the significance of cyclical granulopoiesis in (at least some) C.G.L. patients will be further considered in the light of the mathematical models of the control of stem cell proliferation and granulocyte production which are described in chapters 6,7 and 8.

However, some comments can be made without the assistance of particular mathematical models:-

(a) In C.G.L., control of granulopoiesis is not abolished. The growth of the granulocyte population is neither exponential nor Gompertzian, but at least partly responsive to mechanisms regulating the production of granulocytes.

(b) The most straightforward interpretation of the increased cycle length in C.G.L. is to suppose that leukaemic granulocytic precursors mature abnormally slowly (10,23). It is noteworthy that the increased cycle length is not consistent with the co-existence of a normally maturing granulocyte population and a leukaemic population absolutely incapable of maturation.

(c) Should a normally maturing population co-exist with a slowly maturing leukaemic population, a modulated oscillation resulting from the combination of the two frequencies would be expected. None of the published reports exemplify such modulation, although a more detailed analysis is required.

Cyclical Erythropoiesis.

Despite occasional reports in the literature (24,25), cyclical erythropoiesis does not appear to have been recognized as a distinct phenomenon until quite recently.

In 1968, Orr, Kirk, Gray and Anderson (26) reported distinctive cyclical erythropoiesis in rabbits subjected to constant dose injections of red cell iso-antibody, two or three times weekly, to simulate autoimmune haemolytic anaemia. The period of the oscillation was typically about 18 days and thus unlikely to be directly related to the much shorter time-intervals between consecutive injections of iso-antibody.

The oscillation was clearly present in both the haemoglobin and reticulocyte levels.

Discontinuation of the injections depressed reticulocyte levels, increased haemoglobin and seemingly abolished the oscillation (fig. 5.3).

Should similar phenomena accompany true haemolytic anaemia in man, the assessment of randomly-timed blood sampling may be a hazardous procedure.

Subsequently, Morley and Stohlman (27) have examined the kinetics of the reticulocyte population in normal dogs, undisturbed except for blood sampling, and reported the existence of cyclical erythropoiesis in six of eleven animals studied.

Interestingly, the phase of the oscillation could be altered by bleeding followed by retransfusion. This provides evidence that the observed oscillation is intrinsic to the

erythropoietic system rather than being a consequence of some other cycle arising from elements external to the marrow.

Cyclical haemopoiesis has also been reported by Morley (28) in two polycythaemia patients. In one, a 15 day neutrophil cycle was found, co-existing with a 27 day platelet cycle. (The dissociation of cyclical granulopoiesis and cyclical thrombopoiesis is of interest). The second patient displayed a marked reticulocyte cycle with a period of approximately 17 days. In the first patient, however, no reticulocyte cycle could be found and in the second no neutrophil or platelet cycle could be found.

Cyclical Thrombopoiesis.

As previously mentioned, cyclical thrombopoiesis has been observed together with cyclical granulopoiesis. In addition, Morley (29) has reported a platelet cycle with a period of 21 - 35 days in four of eleven normal individuals. A platelet disorder analogous to cyclical neutropenia - cyclical thrombocytopenia - exists and exhibits an oscillation with a similar period.

The similarity of the periods of cyclical granulopoiesis and cyclical thrombopoiesis, both in normal individuals and in C.G.L. patients, suggests that the oscillation is occurring at the level of a multipotent stem cell population. If this is so, it might be expected that a cyclicity in any haemopoietic pathway of development would be transmitted to any other pathway for which the stem cell pool is common.

However, it is of interest that platelet oscillations are not in fact a universal accompaniment of cyclical neutropenia. This means either that the level of the stem cell pool is not greatly affected in this condition or that compensatory mechanisms have damped out the oscillation before it appeared as a fluctuation in the platelet level.

Such mechanisms could be, for example, active control over megakaryocyte production rate by regulation of mitotic rate, or a 'death control' mechanism regulating the degree of 'ineffective thrombopoiesis'. By using Se-methianonine to measure the production rate of megakaryocytes at different times. it may be possible to test these possibilities.

A Mathematical Model of the Control of Erythropoiesis.

Improving information on the kinetics of haemopoiesis, especially that regarding cyclical haemopoiesis, has stimulated interest in mathematical models of the control of haemopoiesis. The first such model, motivated by the observation of cyclical erythropoiesis in rabbits, was described by Kirk, Orr and Hope in 1968 (25) and in a number of subsequent publications (7,8,30,31).

Models of the control of haemopoiesis differ from the models of the kinetics of haemopoiesis described in chapter 4 in that the former models incorporate assumptions as to the exchange of messages between groups of cells resulting in increased, decreased or maintained levels of cellular production according to the physiological conditions which prevail. Models of control need not specify the physical nature of the presumed intercellular signals, although they may do so.

In the model described by Kirk and his colleagues, assumptions as to the nature of the signals are made explicit. The structure of the model is that depicted in fig. 5.4. Two cybernetic loops are postulated, one controlling the rate of production of erythropoietin and one regulating the size of the stem cell population. Erythropoietin production is taken to be inversely proportional to the instantaneous haematocrit, while erythropoietin itself is presumed to act at the stem cell level, producing mature erythrocytes after a time-delay imposed by the erythroid maturation time.

For the regulation of the stem cell numbers, a stem cell mitotic inhibitor or 'chalone' is proposed.
The model is explicitly formulated in terms of time-lagged differential equations. It has the property of exhibiting oscillatory erythropoiesis when the life-span of the erythrocytes is reduced below normal, as it would be, for example, by the iso-antibody infused into rabbits in the experiments of Gray and Anderson.
A characteristic feature of the model is the absence of sustained oscillations for either the stem cell loop or the erythropoietin loop acting in isolation. Limit cycling can occur only when both loops operate together. An interesting conclusion derived from the model is that an extremely non-linear relationship between a change in haematocrit and adjustment of erythropoietin must be postulated (see 8). Subsequently, experiments by Adamson have provided a degree of confirmation that the magnitude of the non-linearity is in the range suggested (32).

The King-Smith - Morley Model of the Control of Granulopoiesis.

Following the observations of Morley and his colleagues on cyclical granulopoiesis under normal conditions and in C.G.L., King-Smith and Morley (10) proposed a computer model of the regulation of granulopoiesis.

The essence of the model is depicted in fig. 5.5. The rate of production of mature granulocytes is taken inversely proportional to the blood granulocyte level, although the physical intermediaries (e.g. 'granulopoietin') are not explicitly postulated.

An active control over the rate of release of granulocytes from the mature cell store in the marrow is postulated and this rate is also taken inversely proportional to the blood granulocyte level, although no time-delay is here involved.

No stem cell loop is introduced and the stem cell population (which is not a component of the model) is, in effect, taken to be constant.

This model was not formulated by King-Smith and Morley in analytic mathematical terms. Instead, the model was physically simulated using the electronic circuits of an analogue computer to represent the biological components of the model. Explicit mathematical equations are nowhere introduced in this form of study which is appreciably different from the approach to modelling employed by Kirk and his colleagues with the model of control of erythropoiesis described above and by the present author with the several models of the control of granulopoiesis to

be described in subsequent chapters. The present approach has the advantage of unambiguously displaying all built-in assumptions in complete detail as well as permitting the use of analytic theorems in some situations.

However, the studies of King-Smith and Morley led to some interesting conclusions : that the loop controlling de novo granulopoiesis was inherently oscillatory but was stabilized by the action of the loop controlling the release rate of mature granulocytes from the marrow store. Depletion of the marrow store, e.g. due to decreased production rate, might underlie disorders such as cyclical neutropenia. This is in agreement with the observation that cyclical neutropenia could be induced in dogs by the administration of the anti-mitotic drug cyclophosphomide. King-Smith and Morley did not advance a detailed interpretation of cyclical granulopoiesis in C.G.L., though they noted that the increased cycle length was consistent with an extended maturation time of leukaemic granulocytic precursors.

This model is open to criticism more on the grounds of its omissions than of its content. King-Smith and Morley did not (a) express their assumptions in analytic form, which would have allowed them to systematically investigate the effects of varying each parameter entering into the equations; (b) investigate the consequences of postulating control loops different from those incorporated in the model; (c) incorporate a stem cell loop (which surely must exist in reality); (d) investigate all the ways in which control of granulopoiesis could, theoretically, break down.

Of course, any pioneering study may be criticised for its

incompleteness; it is likely to be the prototype in the field rather than the last word. The above considerations, however, do suggest that a more comprehensive study of mathematical models of the control of granulopoiesis is warranted. Such a study is described in the chapters which follow. Of course, this investigation too has its omissions -- some glaring - and should be regarded as merely the next logical step in what may be a very protracted undertaking.

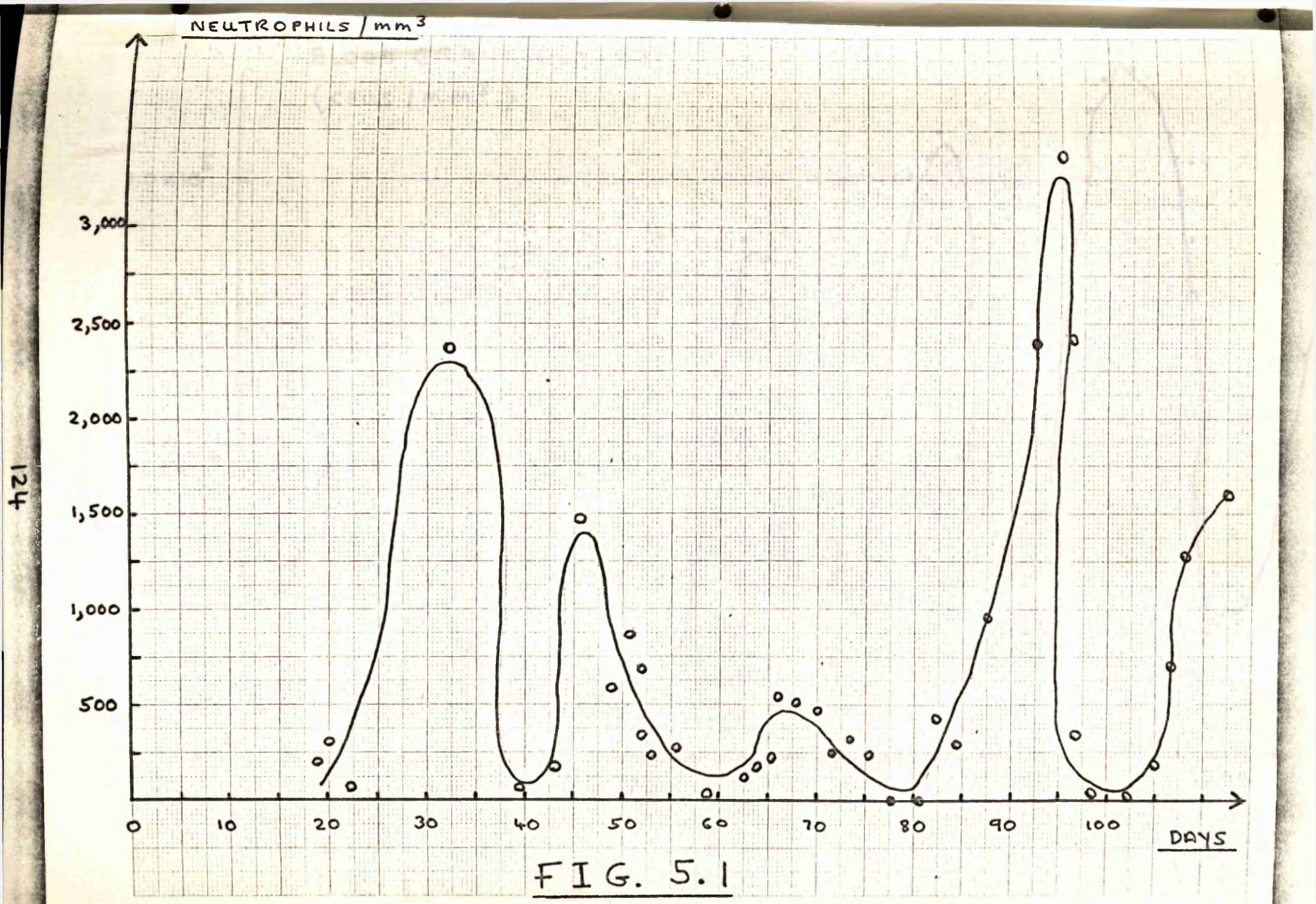
NEUTROPHILS / mm 3
3,000
2,500
2,000
1,500
1,000
500
0
10
20
30
40
50
60
70
80
90
100
DAYS

FIG. 5.1

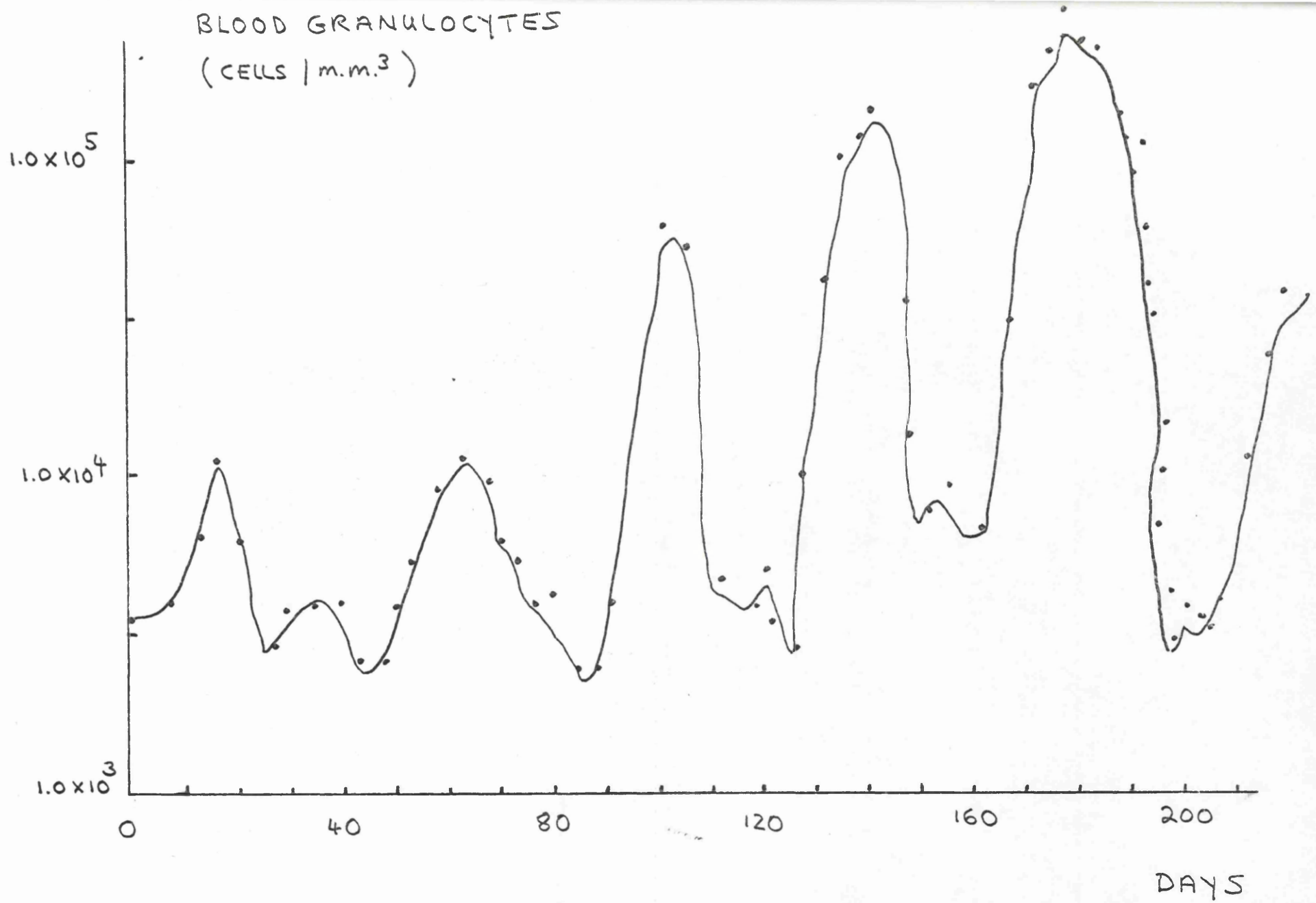

FIG. 5.2 : CYCLICAL C. G. L.

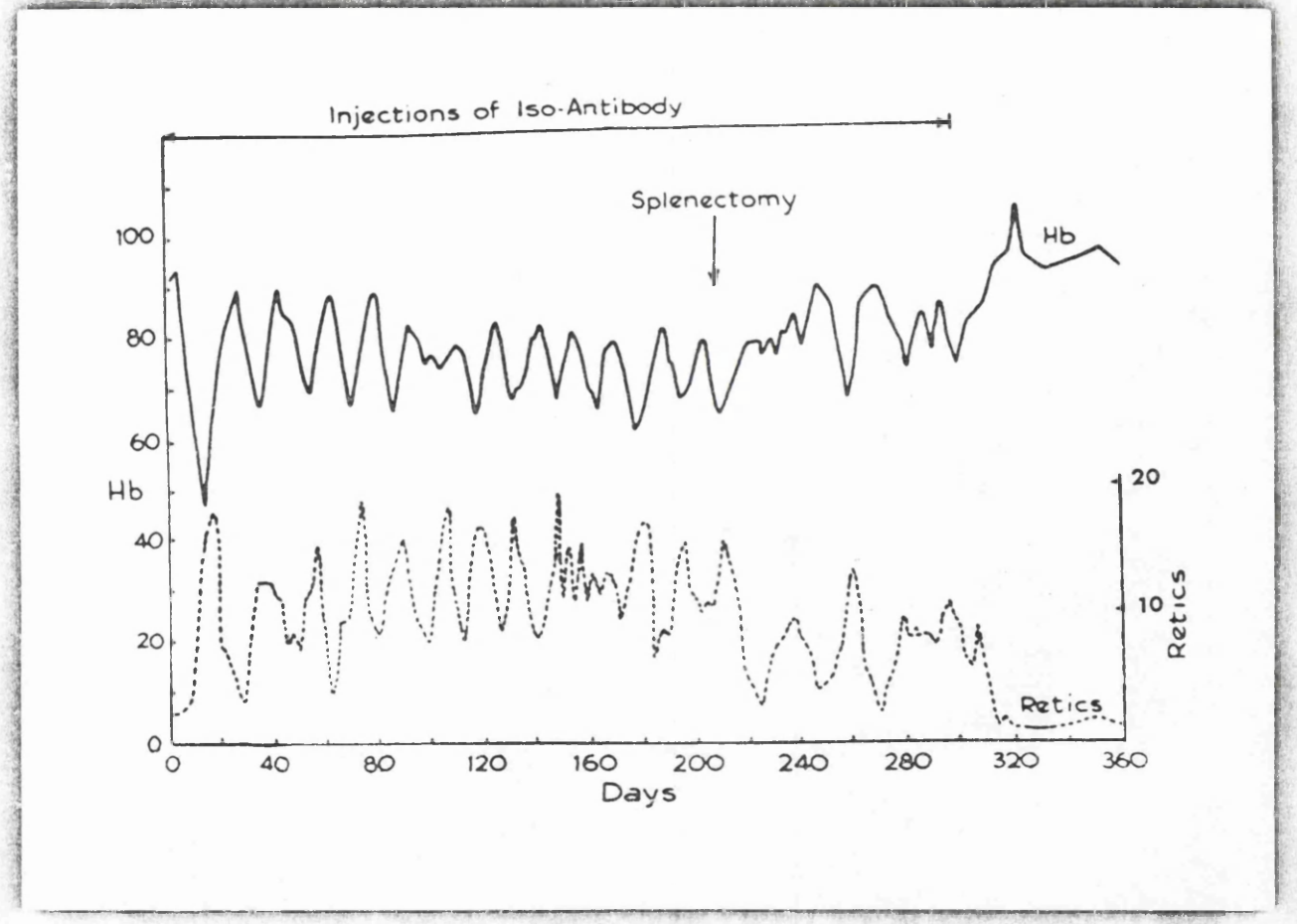
Injections of Iso-Antibody
Splenectomy
Hb
Retics
100
80
60
40
20
0
Hb
20
10
Retics
0
40
80
120
160
200
240
280
320
360
Days

FIG. 5.3

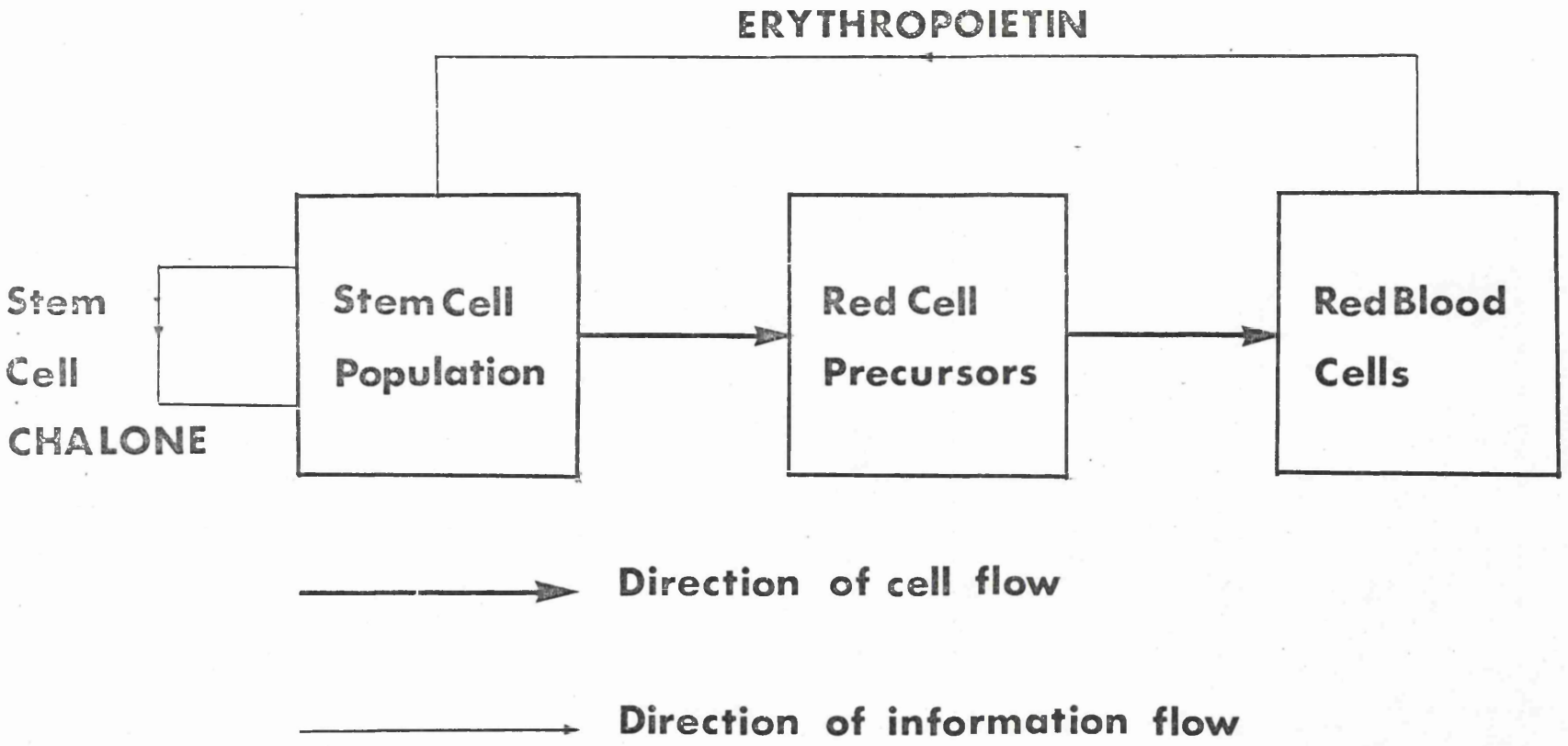

FIG 5.4 STRUCTURE OF THE KIRK-ORR-HOPE MODEL OF THE CONTROL OF ERYTHROPOIESIS

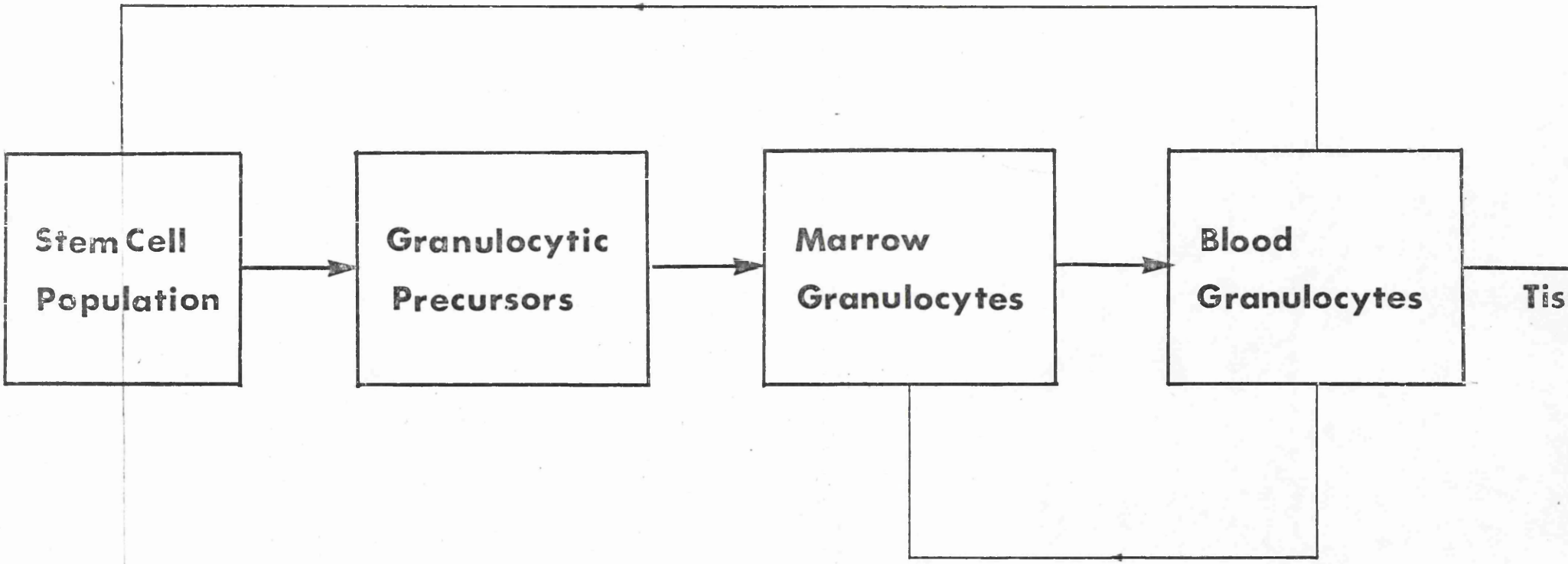

FIG 5.5 THE KING-SMITH-MORLEY MODEL OF THE CONTROL OF GRANULOPOIESES

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6. A TWO-LOOP MODEL FOR THE CONTROL OF GRANULOPOIESIS.

6. A TWO-LOOP MODEL FOR THE CONTROL OF GRANULOPOIESIS.

Introduction.

As discussed in chapters 2 and 5, current evidence favours the existence of at least two distinct points of control for the system regulating granulopoiesis i.e. control of production of granulocytes from primitive precursors (stem cells?) and control of marrow release.

These control points are unlikely to be the only ones which exist. An obvious omission is that of control of stem cell number. The question, which is fairly involved, is considered in chapters 7 and 8. In the present chapter it is assumed that stem cell number remains effectively constant, which implies that any simulated perturbations should not be excessively severe.

Some other possibilities, however, have been excluded from consideration altogether. These include the mitotic inhibition (chalone) control of proliferation rate of granulocytic precursors by mature granulocytes (1) and the active regulation of intramedullary cell death (2).

Neither of these suggestions is unreasonable and their exclusion from consideration reflects only the limited objectives of the present study. As will be seen, analysis of the responses of two and three-loop models is complicated enough.

Formulation of the Model in Logical Terms.

The model to be considered is logically similar to that examined by King-Smith and Morley (3) : (see chapter 5, pp 121 — 123). However, the following differences merit attention:

(a) King-Smith and Morley assumed that the 'sensed variable' of the loop regulating 'de novo' granulopoiesis was the blood granulocyte number - as it was for the loop regulating marrow release. However, there exists evidence that marrow regeneration following injury can precede changes in the blood granulocyte number (4).

Accordingly, the marrow granulocyte number is here taken to be the sensed variable of the primary production loop giving a model whose logical structure is that depicted in fig. 6.1.

(b) Although granulocyte passage through marrow is allegedly first-in-first-out (5), there is a difficulty about the analytic representation of this which is related to the problem of time-varying maturation (Appendix 6.I). As a first approximation to reality, marrow transit has been taken at random rather than first-in-first-out.

(c) Where a choice exists, explicit analytical representation is greatly preferable to a 'black box' description using function generators on an analogue computer (3). In this case, an explicit representation using delay-differential equations is adopted. This permits simulation using both analogue and digital computational techniques and enables the effect on system behaviour of changes in parameter values to be more closely studied.

Formulation of the Model in Mathematical Terms.

Let $G_m(t)$ be the number of mature granulocytes resident in the marrow at time t . Then, assuming random egress

$$\frac{d}{dt}\cdot\left[G_m(t)\right] = \xi(t) - \lambda G_m(t) \qquad (6.1)$$

where $\xi(t)$ is the precursor input rate and λ the loss rate parameter.

Now, if granulocyte production is regulated by stimulation of development of early precursors, the strength of this stimulus must be inversely related to the number of marrow granulocytes present at time t . However, a signal of infinite intensity should not give an infinite response (i.e. $\xi(t)$ must be bounded above). Also, allowing for maturation of duration τ , a pulse of signal should give a pulse of response time-delayed by the factor τ .

A plausible expression for $\xi(t)$ is therefore

$$\xi(t) = \frac{\alpha}{1+\beta\left[G_m\right]^{\varkappa}_{t-\tau}}$$

where α , β and $\varkappa$ are positive constants and $\left[G_m\right]_{t-\tau}$ is a symbolism for $G_m(t-\tau)$, which will be found convenient in later applications. Hence equation (6.1) becomes:

$$\frac{dG_m}{dt} = \frac{\alpha}{1+\beta\left[G_m\right]^{\varkappa}_{t-\tau}} - \lambda G_m \qquad (6.2)$$

i.e. a non-linear delay-differential equation in G_m .

However, in order to take account of the action of the second postulated loop, that controlling marrow release, the

release rate parameter λ should be related inversely to $G_B(t)$, the blood granulocyte number at time t. Again, the release rate should be bounded above but (since random kinetics is postulated), no further time-delay is involved. Thus

$$\lambda \rightarrow \frac{\lambda}{1+\mu\,[G_B]^{\gamma}}$$

where μ and γ are positive constants. Equation (6.2) then becomes

$$\frac{dG_m}{dt} = \frac{\alpha}{1+\beta\,[G_m]^{\kappa}_{t-\tau}} - \frac{\lambda G_m}{1+\mu\,[G_B]^{\gamma}} \qquad (6.3)$$

The rate of change of $G_B(t)$ must then be the difference of

$\frac{\lambda G_m}{1+\mu\,[G_B]^{\gamma}}$ (i.e. rate of input of marrow granulocytes)

and a term expressing loss of blood granulocytes from the vascular system. As vascular loss is mainly random (6), and there is little evidence for its active regulation, a suitable equation is

$$\frac{dG_B}{dt} = \frac{\lambda G_m}{1+\mu\,[G_B]^{\gamma}} - \omega G_B \qquad (6.4)$$

where $\omega = \frac{\ln 2}{T}$ and T is the half-lifespan of vascular granulocytes.

Equations (6.3) and (6.4) together, define the model in analytic terms.

Relation of Model Parameters to Kinetics of Granulopoiesis.

A reasonable choice of parameter values for the model - at least in the first instance - would be a set giving model properties close to the kinetic properties of granulopoiesis in normal man.

Some estimates of the latter properties are given in table 6.1. However, it is neither necessary nor desirable that such estimates should be accepted unquestioningly in choosing the model parameters for normal granulopoiesis. Firstly, the parameters can be chosen in more than one way i.e. the chosen set is not unique. Secondly, the estimates given in table 6.1 are very approximate, they represent means of distributions showing very large dispersions and depend on various assumptions in the interpretation of the raw experimental data. In such a situation, any attempt to derive quantitative insights from the model simulations would be rightly considered naïve.

The criteria for choosing the parameters may best be viewed in light of the objectives of the study. Since few of the data can be considered precise, a more reasonable stratagem would be to accept any parameters which are within a range of normal kinetics and to pursue qualitative rather than quantitative insights.

It will be appreciated therefore that no great significance need be attached to particular parameter values, provided the kinetic quantities which they determine are not so far from the estimates given in table 6.1 as to be deemed unrealistic. Of greater importance (in the present study) will be effect

of changes (in each direction) of parameter values on the form of the model response to a given class of perturbations.

This objective corresponds to that mentioned in chapter 1; the use of a mathematical model to obtain qualitative insights into system organization and behaviour.

Computational Procedures.

Simulation studies of the model were carried out using both analogue and digital techniques to integrate equations (6.3) and (6.4). Each of these techniques has particular advantages and corresponding drawbacks.

Analogue computation is particularly convenient when continuous variation of parameters over comparatively small ranges is required, but is less suitable when marked changes in parameter values are being made, because of problems of scaling the analogue computer for a limited range. On the other hand, no scaling problems arise with digital methods, but continuous parametric variation is impossible. Accordingly, each mode of computation was used as appropriate in a given situation.

In the case of analogue simulation, the EAI-680 analogue computer at Y-ARD (Glasgow) was used. The procedure was fairly straightforward for an analogue computation but, when time-delays were involved, a fourth order Padé approximation* was employed. This approximates a delayed function $F(t-\tau)$ in the form

$$F(t-\tau) \approx F(t) - \tau \frac{d}{dt}\cdot\left[F(t)\right] + \frac{\tau^2}{2!}\frac{d^2}{dt^2}\cdot\left[F(t)\right] - \frac{\tau^3}{3!}\frac{d^3}{dt^3}\cdot\left[F(t)\right] \qquad (6.5)$$

i.e. A Taylor expansion truncated at the fourth term. The truncation 'rounds' the delay and probably improves rather than depreciates the physical realism of the representation (because a 'hard' delay neglects any variation in the

* This may be more realistic than a 'pure' delay in any case.

maturation times of individual cells or clones).

The simulations were carried out by Mrs. Helen M. Finlay, Control Section (Y-ARD), in conjunction with the author and could be displayed visually on a C.R.O. as well as being recorded using a graph plotter.

The digital integrations were carried out on two different computers; the ICT-1905 digital computer owned by the Western Regional Hospital Board and the PDP-15 digital computer at Y-ARD (Glasgow).

In the former case, the integration procedure utilized a programme written in FORTRAN by Dr. James Kirk, using a modified Runge-Kutta integration procedure for time-delayed differential equations. In the latter case, the programme utilized a modified DES-50 integration procedure written in FORTRAN by Mr. Tony Griffin (Control Section, Y-ARD) and executed 'on line' by the author, with the technical assistance of members of the staff of Y-ARD. In both cases, a 0.1 day integration step was used.

In a substantial number of cases (about one third) analogue and digital simulations of the same problem were directly compared and in all cases excellent agreement was observed.

The results of these studies are presented in the remainder of the chapter. The presentation does not follow the chronological order of the simulations carried out which have been re-arranged with a view to clarity of description. Each of the diagrams shown is of a digital simulation. In rather more than half of the cases, the digital computation merely confirmed a preceding analogue simulation. The digital results were, however, the easier to present in diagramatic form

The Basic Parameters for the Model.

To facilitate presentation, consider a set of 'basic parameters' with which all other sets of parameters may be compared. The basic parameters are given in table 6.2. The kinetic features of the model which they generate are set out in table 6.3. Comparing tables 6.1 and 6.3 it will be seen that the basic parameters generate kinetic properties close to, but not identical with, the presumed kinetic properties of granulopoiesis in normal man.

The basic parameters were not derived from 'a priori' consideration but by a process of trial and error using many different combinations of parameter values. In the process, many interesting results were encountered prior to the choice of the basic parameters; these are presented later, reversing the actual order of events.

The criteria used to evaluate the basic parameters for normal granulopoiesis were

(a) They should generate kinetic properties not drastically different from those specified in table 6.1;

(b) Simulated recovery from transient perturbations should resemble that found experimentally (in animals) i.e. a series of damped oscillations exhibiting 'moderate' overshoot for 'moderate' perturbations (10 - 12). However, curve-fitting was not attempted as reliable human data is not available.

(c) The parameters should be a stable set in the sense that small changes (e.g. $\sim$ 20%) in their assigned values should generate only small changes in the kinetic properties of the model. Thus, values close to instabilities in either direction were rejected.

Response of the Model with Basic Parameters to Impulsive Perturbations.

The model with basic parameters is taken to be representative of normal granulopoiesis. It is therefore necessary that the model responds adequately to impulsive perturbations, as does the real system to transient insults e.g. perturbing blood and marrow granulocyte numbers by brief exposure to ionizing radiation or short-term infusions of cytotoxic drugs (10 - 12).

On the model, this was simulated by choosing different initial conditions for integration of equations (6.3) and (6.4). As figures 6.2 and 6.3 illustrate, the model returns to its steady-state value when perturbed either above (fig. 6.2) or below (fig. 6.3) this value.

Lacking adequate human data, rigorous comparisons are impossible, but the model does seem to give a fair representation of the recovery from impulsive perturbations of the granulopoietic system in experimental animals (10 - 12).

Effect of Modulation of Parameters.

As previously stated, the values assigned to the basic parameters are not accurate or unique. It is reasonable therefore to examine the qualitative effect of modulating the basic parameters in both directions.

This was done using a facility available on the EAI-680 analogue computer viz The resistance of a potentiometer may be set proportional to a parameter value and the resistance varied continuously over a defined range. The effect of such modulation was assessed by choosing a fixed initial condition (relative to the steady-state defined by the parameters) and noting the effect of modulation of each parameter on the damped oscillation (of the form of fig. 6.3) displayed on a cathode ray oscilloscope.

The observations were noted at the time of the simulations and are presented in qualitative form in table 6.4. As may be seen from this table, increasing the parameters α , β , μ enhances stability while increasing parameters λ , ω , τ x and γ reduces it.

The biological interpretation of the results presented in table 6.4 can also be presented in tabular form viz table 6.5., which indicates how the presence or absence of particular elements would be likely to affect the stability of the control system. This question will be further considered in chapter 10 in relation to the pathological regulation of granulopoiesis.

Sensitivity of the Model Behaviour to Changes in the Indicial Parameters α and γ .

Since α and γ occur as indices in equations (6.3) and (6.4) it is reasonable to expect them to be very influential in determining the form of system behaviour. The effects of changing α and γ through a range of values can be seen from figs. 6.2 - 6.8 .

The model is particularly sensitive to changes in α , but as figs. 6.2 – 6.8 illustrate α and γ act synergistically in promoting first limit cycling and then overt instability.

However, with the structure of the present model and parameter values close to those given in table 6.2 it seems unlikely that stability is possible with a primary loop gain much greater than that represented by $\alpha = 2$.

Should higher loop gains be indicated by future 'in vivo' studies in man, the above investigations should be considered evidence for the existence of one or more control loops additional to those postulated in the model described above.

It is of interest that the gain of the marrow-release loop, besides that of the primary loop, is likely to lead to unstable behaviour. On the other hand, increasing the coupling co-efficients (β and μ) of each of the two loops promotes stability.

Hence, the marrow-release loop may contribute to stabilizing the system, as King-Smith and Morley have suggested (3) but only if the gain of this loop is rather low.

Model Response to Time-Periodic Perturbations of Granulocyte Production Rate and Death Rate.

One virtue of mathematical modelling is that it may permit the examination of response patterns to more complex types of experimental interference than would normally be considered amenable to analysis. The type of complex interference being referred to consists of a temporally-varying perturbation of the system, the duration of the perturbation being finite but appreciable (i.e. not an impulse).

The type of perturbation employed depends on the objectives of the investigation. Conceptually, the simplest type of time-varying perturbation is a periodic one, preferably of sinusoidal form. On the other hand, Wiener and his associates (13) developed a method of synthesis of non-linear models which requires knowledge of the real-system response to perturbations having the form of Gaussian (white) noise. Unfortunately, this method is not designed to provide conceptual understanding regarding the integration of system elements, but only a 'black box' model whose purely phenomenological behaviour mirrors that of the system. Wiener's theory is therefore inappropriate in the present context. The same is true of some simpler methods of non-linear synthesis, for example that recently proposed by Eisner and Milne (14). Finally, methods based on Fourier analysis of response patterns to periodic inputs can be of great value in dealing with linear systems (15) but problems of interpretation become severe when (as here) the system is highly non-linear.

In spite of the difficulties mentioned, the model response to sinusoidal variations in granulocyte production rate and death rate was thought to be of some interest, particularly as the corresponding experiments should be practically feasible. For example, a sinusoidal modulation of a daily dose of radiation should readily provide a sinusoidal driving of cellular production. On the other hand, removal of granulocytes on a periodic basis, using leukapheresis techniques (16,17) could similarily modulate the loss rate of mature granulocytes.

Each of these situations was simulated for simusoids having different periods. The modulation of production rate was represented by modifying equation (6.3) to read

$$\frac{dG_m}{dt} = \frac{\frac{1}{2}\alpha\{1+\sin\theta t\}}{1+\beta\left[G_m\right]^{x}_{t-\tau}} - \frac{\lambda G_m}{1+\mu\left[G_B\right]^{y}} \qquad (6.6)$$

and the modulation of loss rate by modifying equation (6.4) to read

$$\frac{dG_B}{dt} = \frac{\lambda G_m}{1+\mu\left[G_B\right]^{y}} - \frac{\omega}{2}\left(1+\sin\theta t\right) \qquad (6.7)$$

The two modifications were made independently so that the model with modulated production rate was described by equations (6.6) and (6.4) and that with modulated loss rate by equations (6.3) and (6.7). In all cases the basic parameters of table 6.3 were used, θ being the only one to vary.

The results are depicted in figs. 6.9 - 6.14 with the corresponding situations to which they refer specified in table 6.6.

As these diagrams show, the perturbations to the granulocytic death rate, $\omega \rightarrow \frac{\omega}{2}(1+\sin\theta t)$, generate broadly similar damped-oscillation responses for different periods of the perturbation.

On the other hand, the perturbation of granulocytic production rate $\alpha \rightarrow \frac{\alpha}{2}(1+\sin\theta t)$ generates a more complex oscillation with indications of reasonance phenomena as the natural period of the system ($\approx$ 20 days) is approached.

In principle, the death (loss) rate perturbation could be carried out experimentally using cyclically modulated leukapheresis, and of the production rate by using cyclically modulated irradiation or drug infusion. It would be of interest to see whether the two perturbations generate qualitatively different oscillations, as suggested by the simulation studies presented here.

It seems probable that simulation of biological system responses to time-varying perturbations may permit differences to be discerned in the predictions which result from different sets of assumptions. The present work merely indicates this possibility and suggests that further investigations of this topic would be warranted.

Appendix 6.I.

Representation of Ordered Transit with Variable Transit Time.

The representation of first-in-first-out cellular maturation, where the maturation time is itself variable, poses a more complex problem than any of those considered so far.

Suppose that a scalar quantity, μ , can be defined as an 'index of maturation' and that $n(\mu,t)\delta\mu$ represents the number of cells, at time t , having maturity between μ and $\mu+\delta\mu$.

Then, if no cells are gained or lost in the course of maturation (no division, no death), we have

$$n(\mu+\delta\mu,t) = n(\mu, t-\delta t) \qquad (6.1.1.)$$

Expanding (6.1.1) as a Taylor series

$$n(\mu,t) + \delta\mu \frac{\partial n}{\partial \mu} + \sum_{j=2}^{\infty} \frac{(\delta\mu)^j}{j!} \frac{\partial^j n}{\partial \mu^j}$$

$$= n(\mu,t) - \delta t \frac{\partial n}{\partial t} + \sum_{j=2}^{\infty} (-1)^j \frac{(\delta t)^j}{j!} \frac{\partial^j n}{\partial t^j} \qquad (6.1.2)$$

Then,

$$\frac{\delta\mu}{\delta t} \cdot \frac{\partial n}{\partial \mu} + \frac{\delta\mu}{\delta t} \sum_{j=2}^{\infty} \frac{(\delta\mu)^{j-1}}{j!} \frac{\partial^j n}{\partial \mu^j} = -\frac{\partial n}{\partial t} + \sum_{j=2}^{\infty} (-1)^j \frac{(\delta t)^{j-1}}{j!} \frac{\partial^j n}{\partial t^j} \qquad (6.1.3)$$

Therefore,

$$v \frac{\partial n}{\partial \mu} + v \left\{ \lim_{\substack{\delta\mu \to 0 \\ \delta t \to 0}} \sum_{j=2}^{\infty} \frac{(\delta\mu)^{j-1}}{j!} \cdot \frac{\partial^j n}{\partial \mu^j} \right\}$$

$$= -\frac{\partial n}{\partial t} + \lim_{\substack{\delta\mu \to 0 \\ \delta t \to 0}} \sum_{j=2}^{\infty} \frac{(\delta t)^j}{j!} \frac{\partial^j n}{\partial t^j} \qquad (6.1.4)$$

where $v \equiv v(\mu, t)$, the maturation velocity, is defined as

$$v(\mu,t) \equiv \lim_{\substack{\delta\mu \to 0 \\ \delta t \to 0}} \left(\frac{\delta\mu}{\delta t} \right) \qquad (6.1.5)$$

In the limit, therefore

$$\frac{\partial n}{\partial t} + v \frac{\partial n}{\partial \mu} = 0 \qquad (6.1.6)$$

Equation (6.1.6) provides a more general continuity principle for cellular maturation (withour gains or losses) than any equation derived previously.

The number of cells, $N(t)$, in a recognizable 'compartment', of the type discussed previously, will be

$$N(t) = \int_0^{\mu_{max}} n(\mu,t)\, d\mu \qquad (6.1.7)$$

where μ_{max} is the maturity value at which compartment transit is deemed to occur.

There are two interpretations of variable transit time, viz:

(a) The maturity velocity is a function of time,

(b) The maximum maturity, μ_{max} , is a function of time.

In either case, there exist different classes of mechanism which may be postulated to give different functions v or μ_{max} . The investigation of the properties of different models is certainly worthwhile but lies beyond the scope of the present study.

The author cannot accept as valid the treatment given by King-Smith and Morley (43) and some others in attempting to avoid postulating specific mechanisms in their (alleged) computer simulation of first-in-first-out maturation with variable maturation time.

In the present instance, the equation (6.4), representing random transit, has been accepted as an approximation to the first-in-first-out kinetics which probably exist in reality.

TABLE 6.1 : SOME ESTIMATES OF THE KINETIC PROPERTIES OF GRANULOPOIESIS IN NORMAL MAN

Quantity	Estimate	Reference
Total Blood Granulocyte Number	6.2×10^8 cells/kg	(7)
Vascular Granulocyte Half-Lifespan	6.7 hours	(6)
Marrow Granulocyte Number	2.6×10^9 cells/kg	(7)
Normal Steady-State Rate of Granulopoiesis	1.63×10^9 cells/kg/day	(8)
Maximum Rate of Granulopoiesis in Normal Man	9.78×10^9 cells/kg/day	(9)
Granulocytic Maturation Time (Total)	10 days	(7)
Mean Transit Time for Mature Marrow Granulocytes	2.5 days	(5)

TABLE 6.2 : BASIC PARAMETER VALUES FOR GRANULOPOIESIS IN NORMAL MAN

PARAMETER	VALUE
α	1.0×10^{10} cells/kg/day
β	1.0×10^{-12} $(\text{cells/kg})^{-x}$
λ	10.0/day
μ	4.0×10^{-8} $(\text{cells/kg})^{-\gamma}$
ω	2.43/day
$\approx$	7 days
x	1.25
γ	1.00

TABLE 6.3 : KINETIC PROPERTIES OF THE MODEL GENERATED BY THE BASIC PARAMETERS FOR NORMAL GRANULOPOIESIS

Kinetic Property	Mathematical Representation	Value
Steady-State Value of G_B^*	$G_B^{(0)}$	1.1×10^9 cells/kg.
Steady-State Value of G_m	$G_m^{(0)}$	1.1×10^{10} cells/kg.
Steady-State Rate of Granulopoiesis	$\frac{\alpha}{1 + \beta \left[G_m^{(0)} \right]^{\chi}}$	2.1×10^9 cells/kg. day
Maximum Rate of Granulopoiesis	α	1.0×10^{10} cells/kg.day
Mean Transit Time of Mature Granulocytes in Marrow	$\ln 2 \left\{ \frac{1 + \mu \left[G_B^{(0)} \right]^{\gamma}}{\lambda} \right\}$	3.1 days
Mean Transit Time through Vascular System	$\frac{\ln 2}{\omega}$	6.7 hours

* With $G_B^{(0)} = 1.1 \times 10^9$ cells/kg. a 70 kg man with a blood volume of 5l and a margination fraction of 0.50 would have a blood granulocyte concentration of 7.7×10^3 cells/mm^3.

TABLE 6.4 : MODULATION OF BASIC PARAMETERS

Parameter	Direction of Modulation	Effect on Maximum Overshoot of Damped Oscillation
α	↑ ↓	↓ ↑
β	↑ ↓	↓ ↑
μ	↑ ↓	↓ ↑
λ	↑ ↓	↑ ↓
ω	↑ ↓	↑ ↓
ζ	↑ ↓	↑ ↓
x	↑ ↓	↑ ↓
γ	↑ ↓	↑ ↓

TABLE 6.5 : BIOLOGICAL INTERPRETATION OF EFFECTS OF PARAMETRIC MODULATION

Biological Quantity	Effect of Increasing Biological Quantity
Maximum Rate of Granulopoiesis (α)	Stabilizing
Coupling of Primary Loop to changes in G_m (β)	Stabilizing
Coupling of Marrow-Release Loop to changes in G_B (μ)	Stabilizing
Loss of Marrow Granulocytes to Blood (λ)	Destabilizing
Loss of Blood Granulocytes to Marrow (ω)	Destabilizing
Maturation Time (τ)	Destabilizing
'Gain' of Primary Loop (x)	Destabilizing
'Gain' of Secondary Loop (γ)	Destabilizing

TABLE 6.6 : SIMULATIONS OF MODEL RESPONSES TO SINUSOIDAL PERTURBATIONS

Perturbed Quantity	Period of Perturbation $T = \frac{2\pi}{\theta}$	Model Response
Granulocyte Production Rate (c.f. equation (6.6))	10 days	Fig. 6.9
	15 days	Fig. 6.10
	30 days	Fig. 6.11
Granulocyte Loss Rate (c.f. equation (6.7))	10 days	Fig. 6.12
	15 days	Fig. 6.13
	30 days	Fig. 6.14

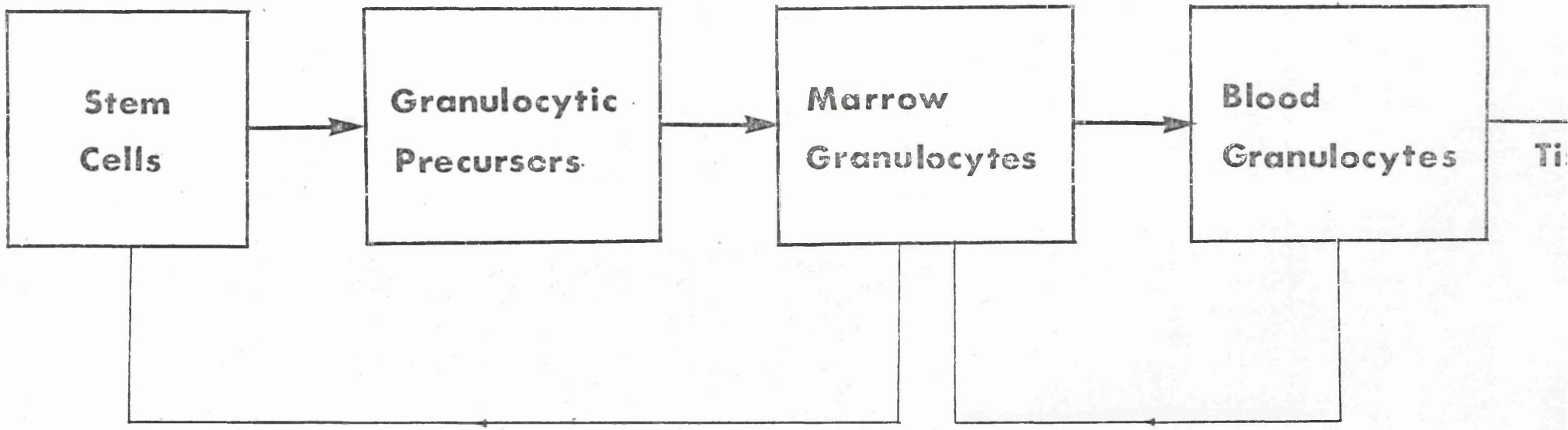

FIG. 6.1 TWO-LOOP MODEL

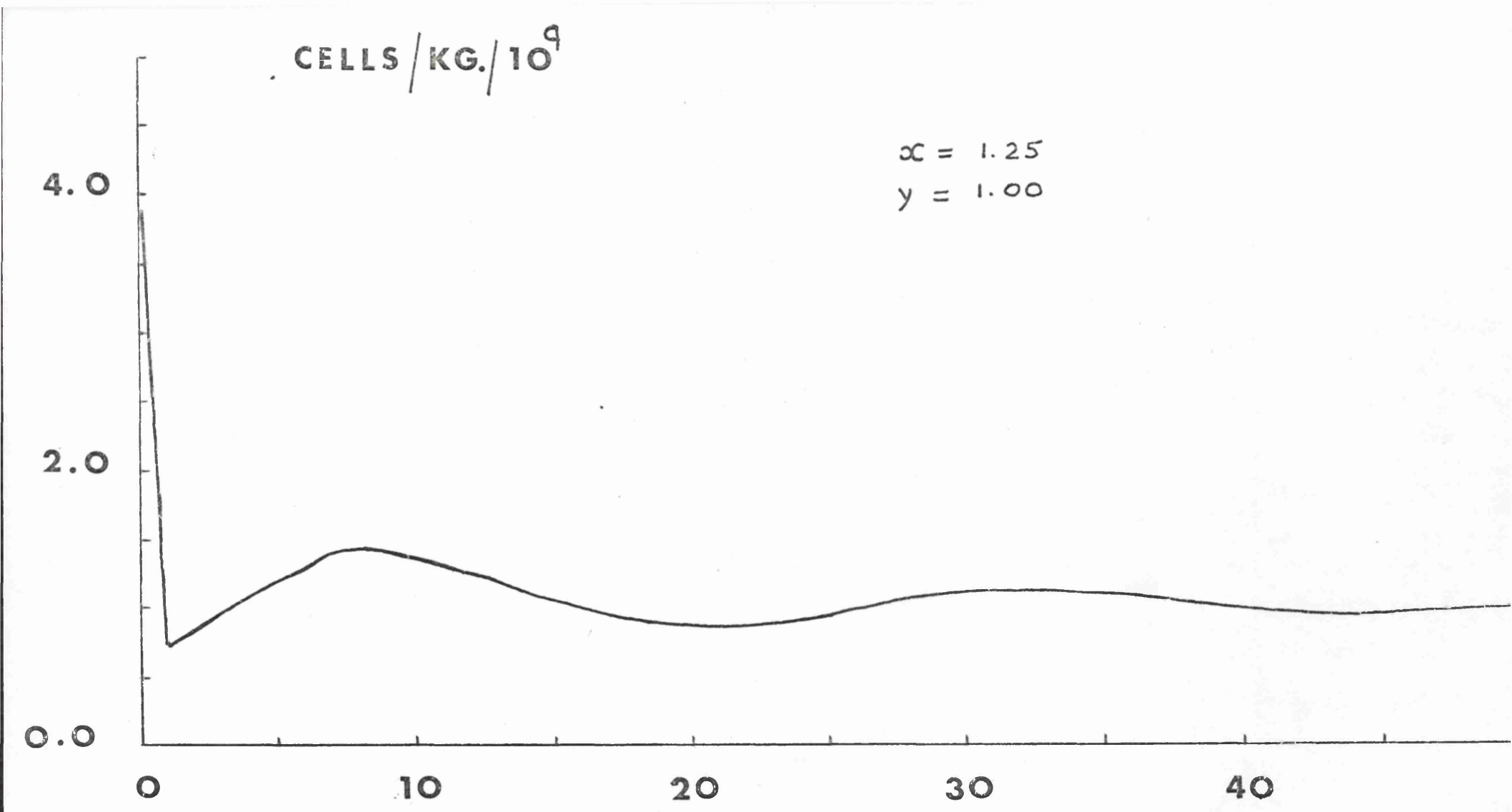

FIG. 6.2

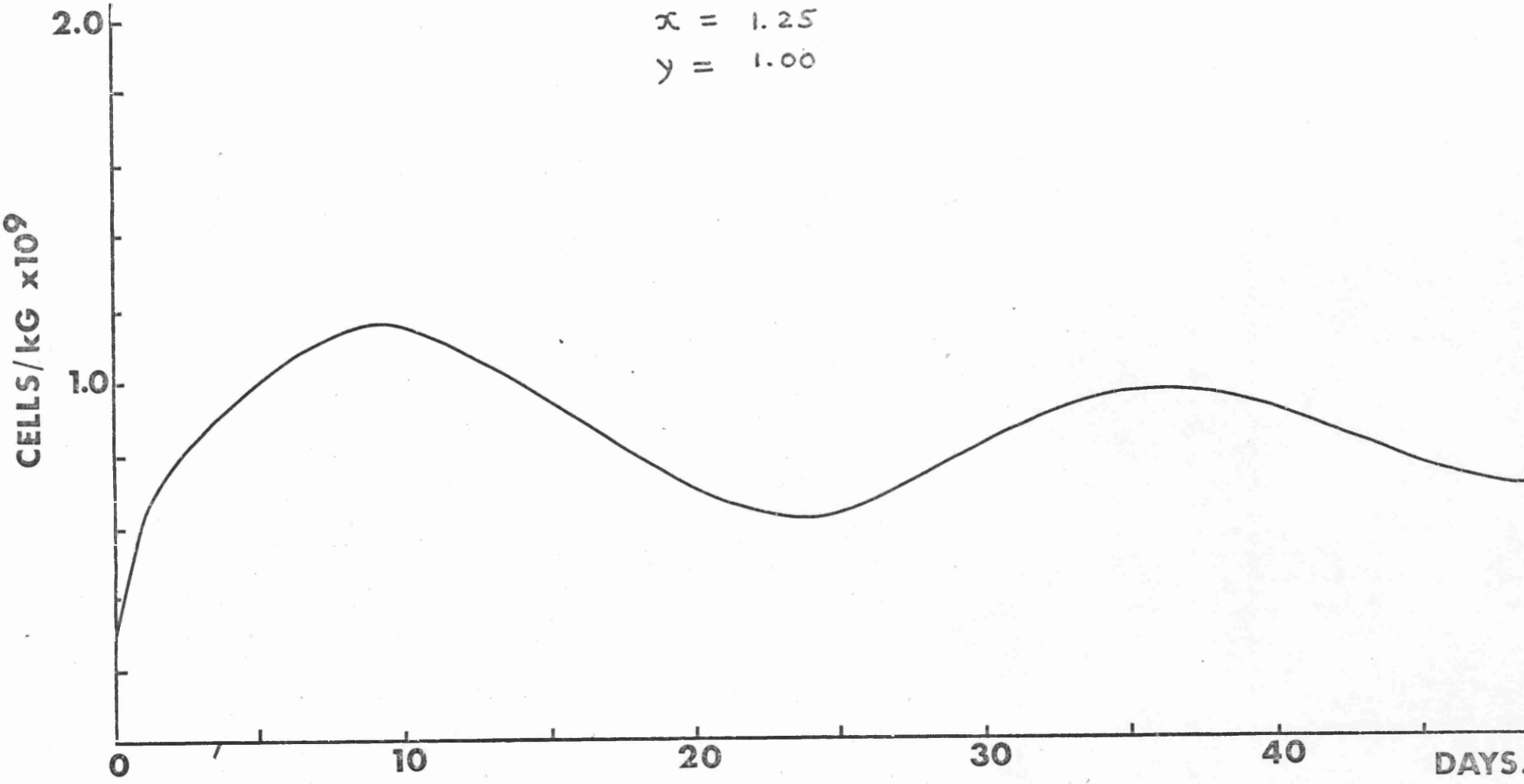

FIG. 6.3

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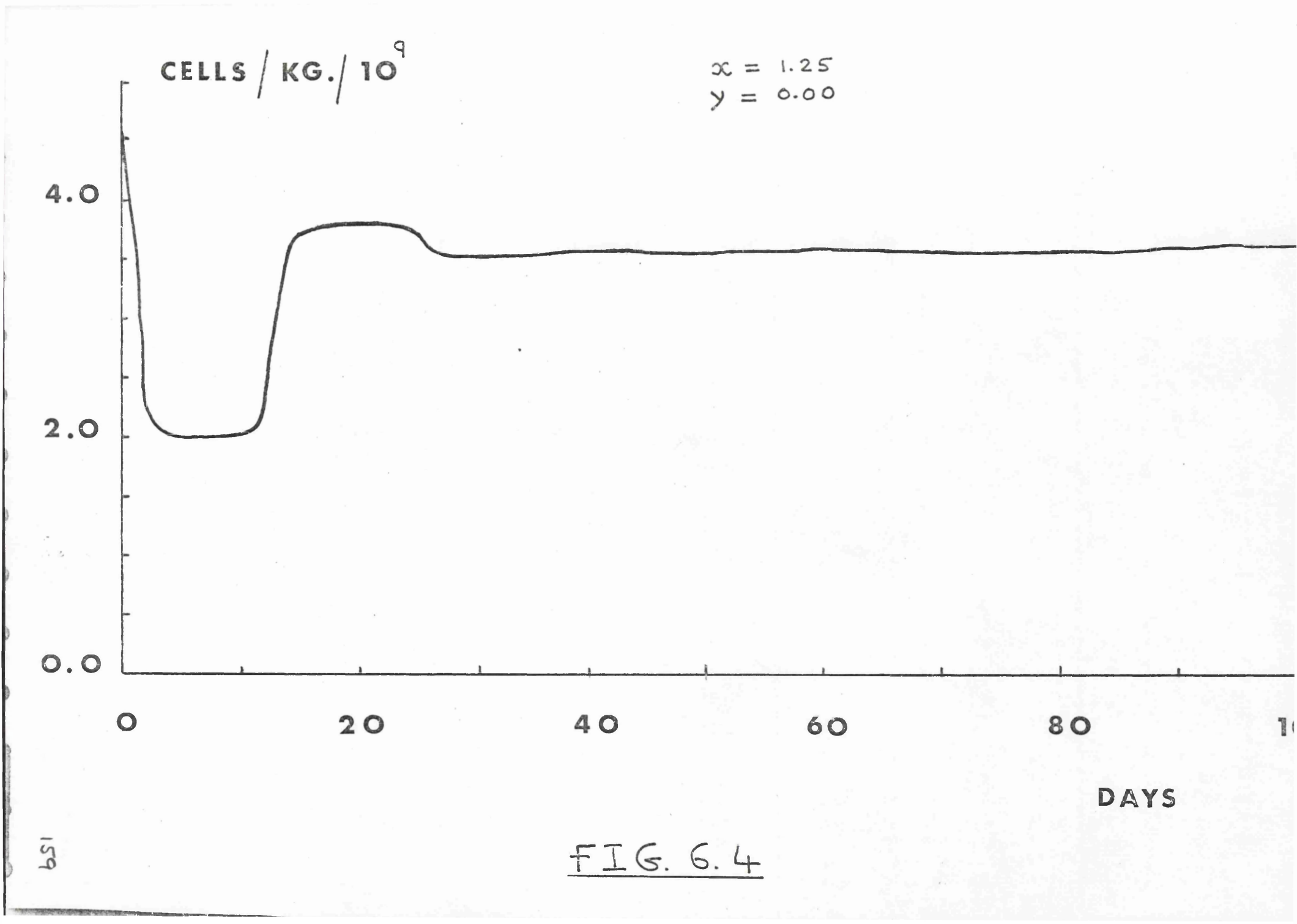

FIG. 6.4

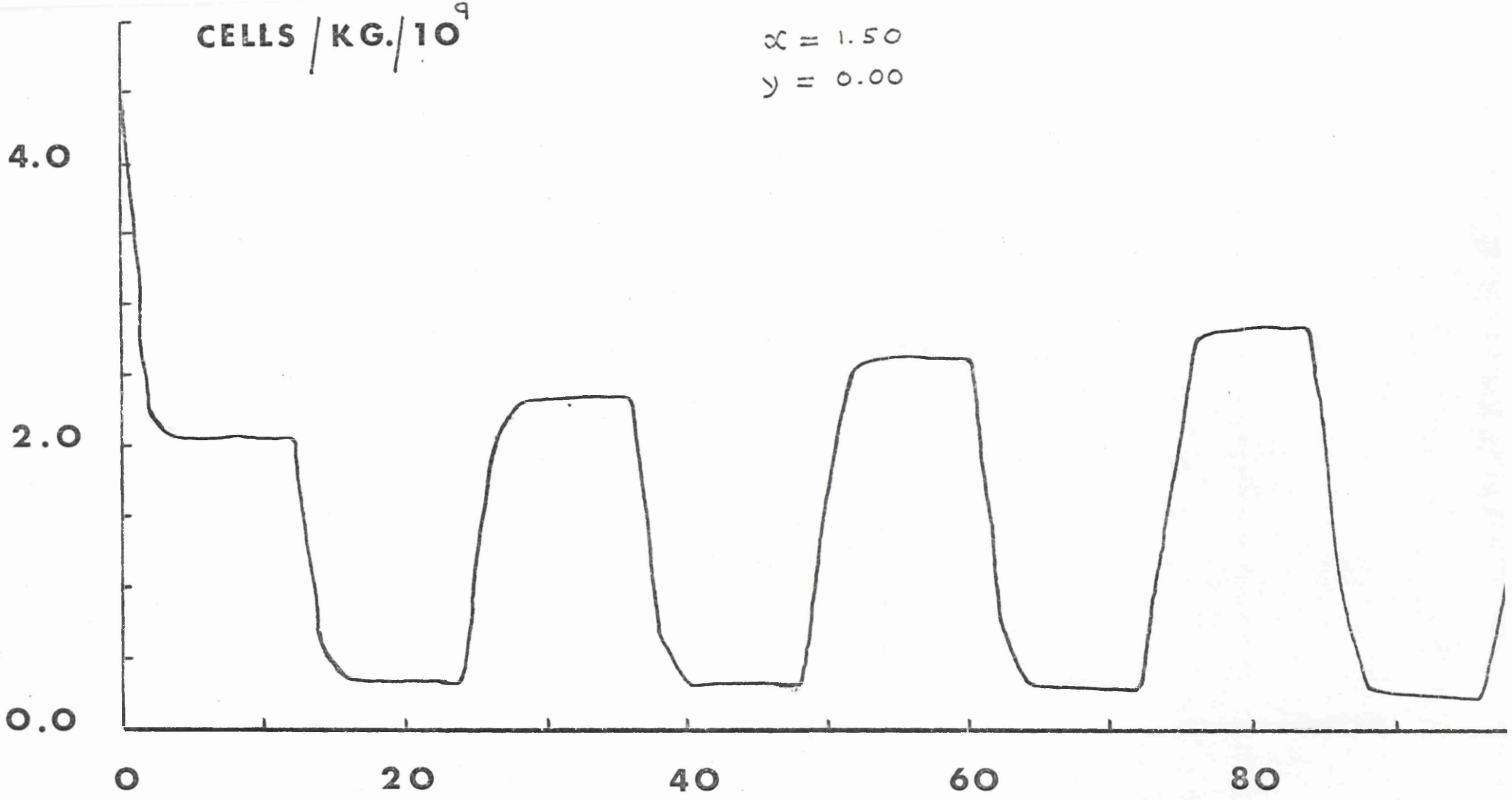

FIG. 6.5

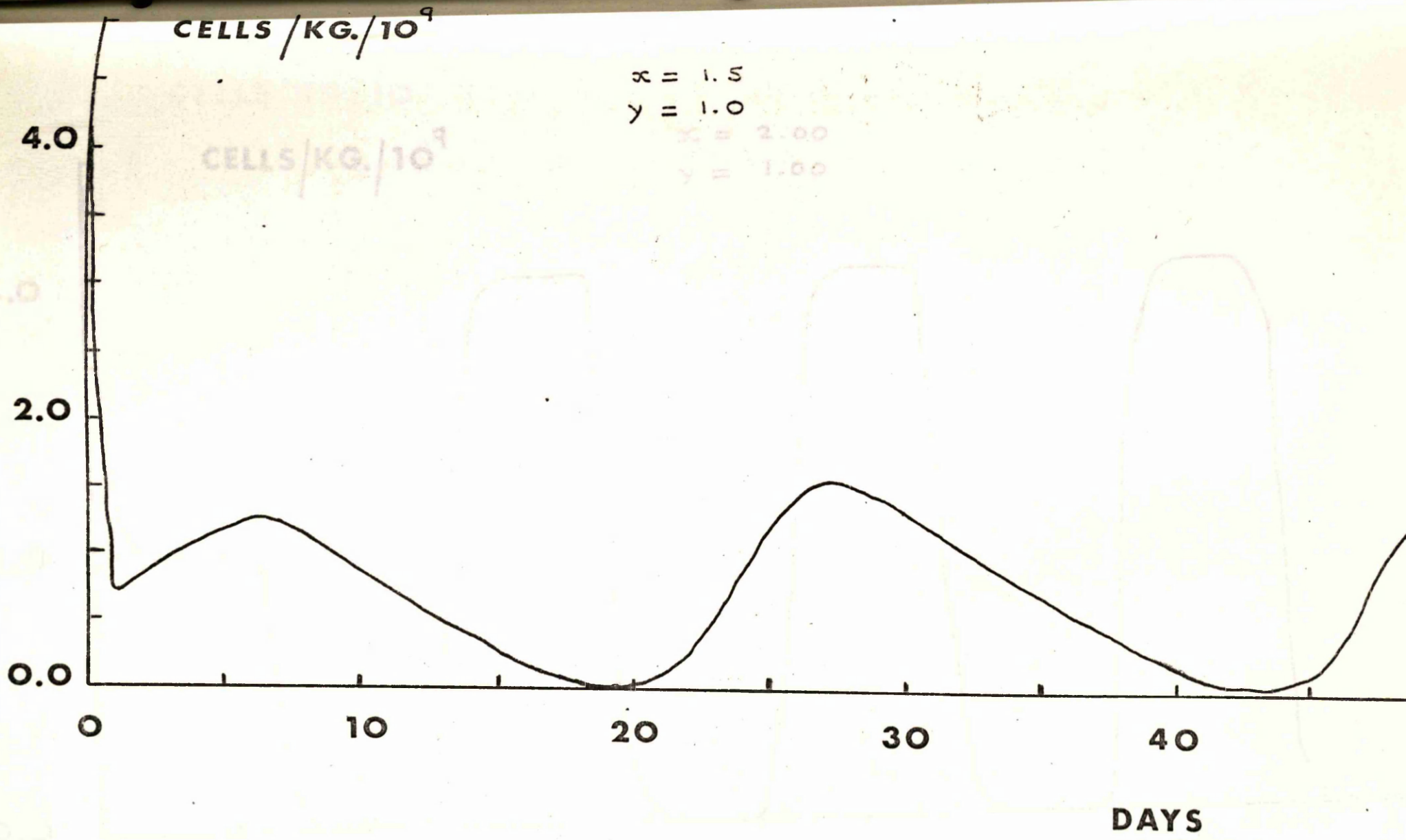

FIG. 6.6

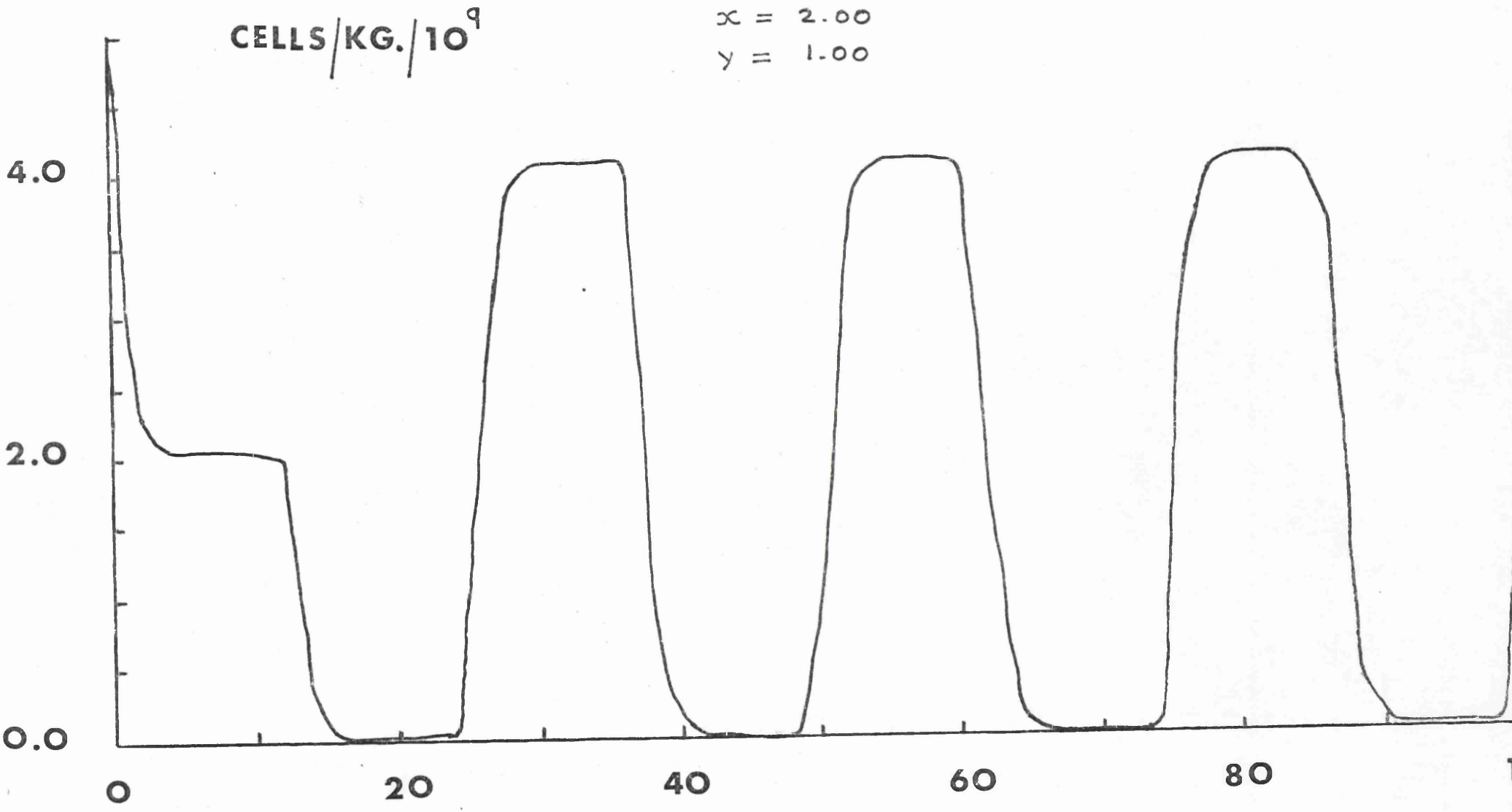

FIG. 6.7

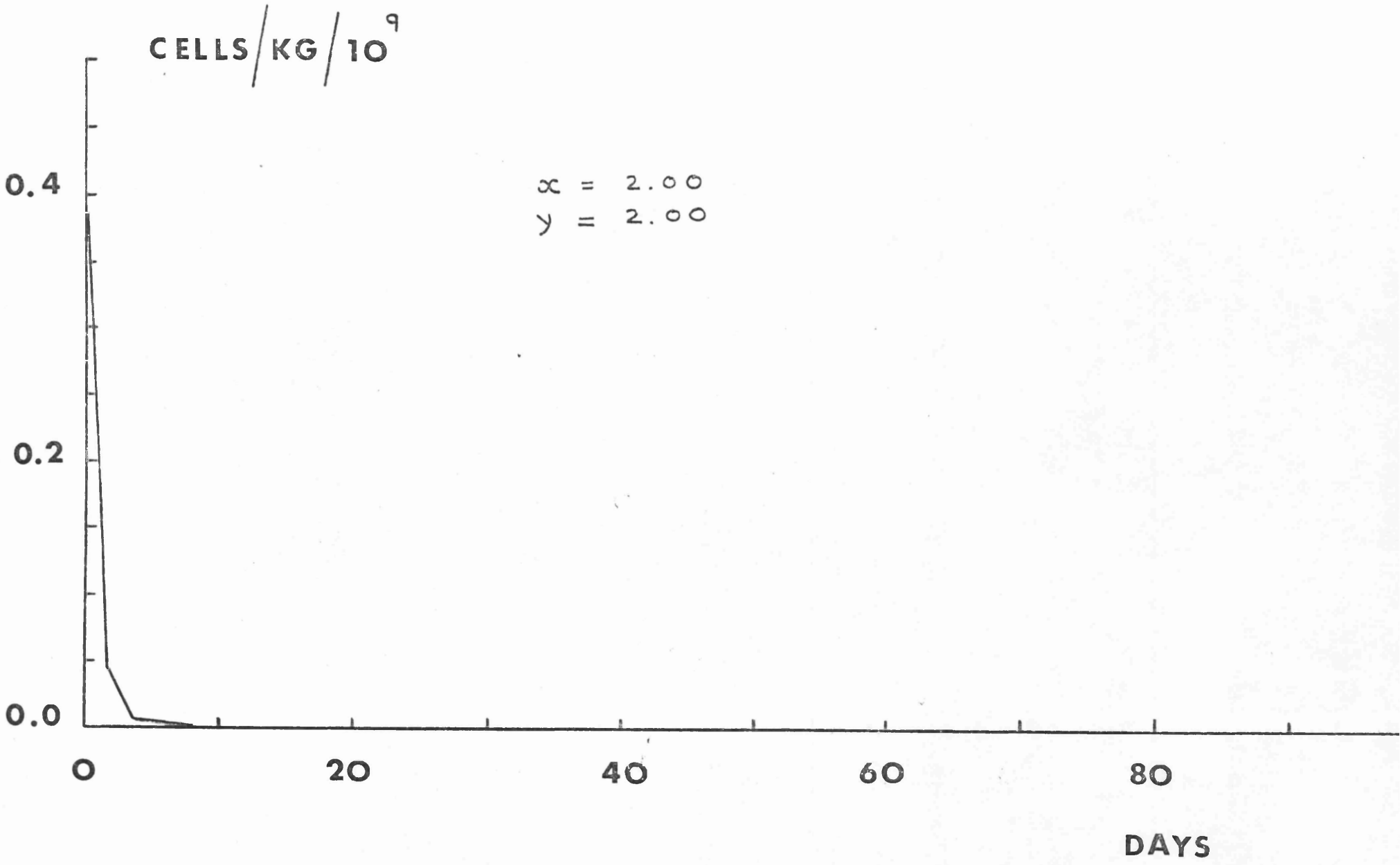

FIG. 6.8

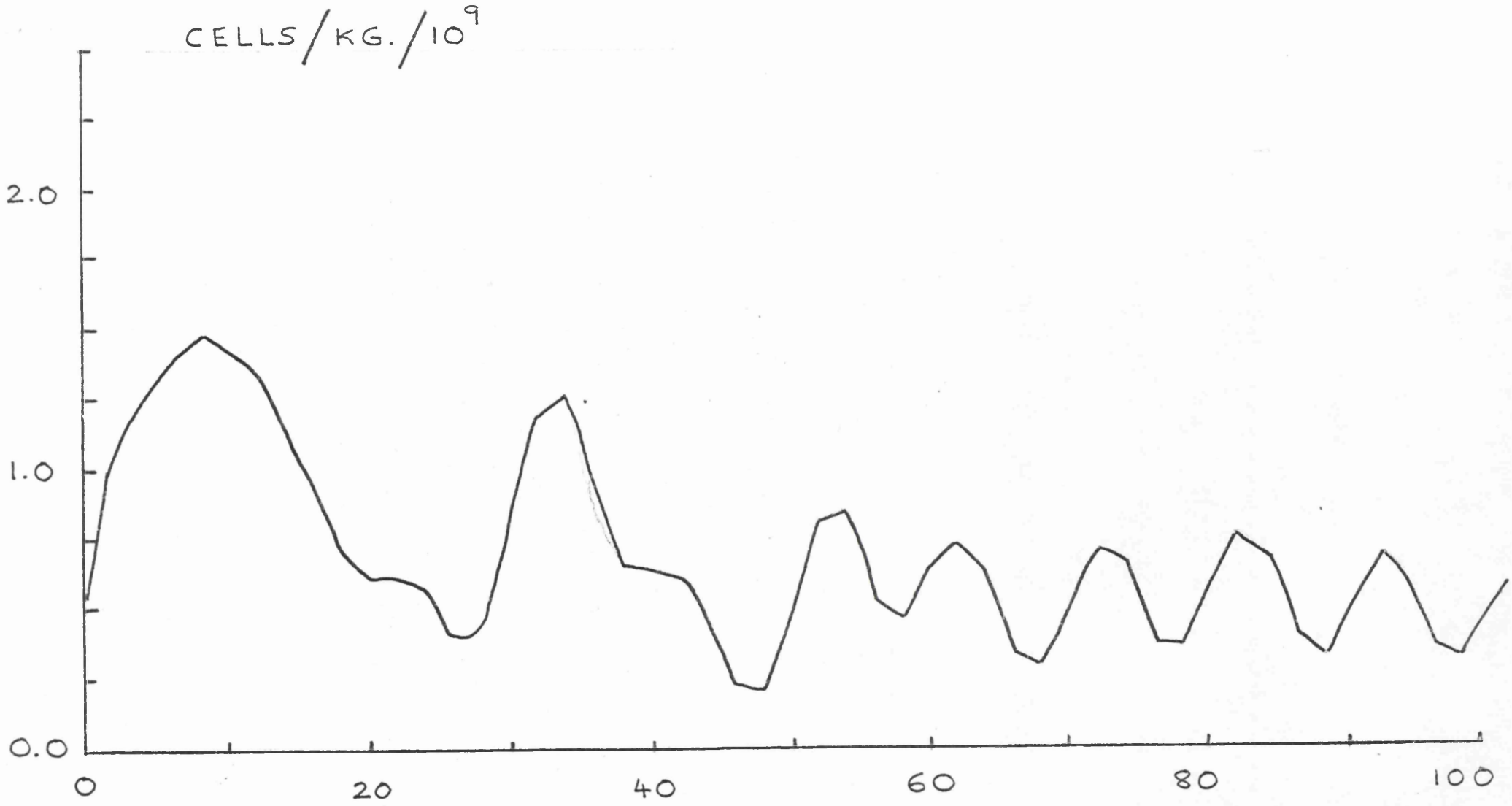

FIG. 6.9

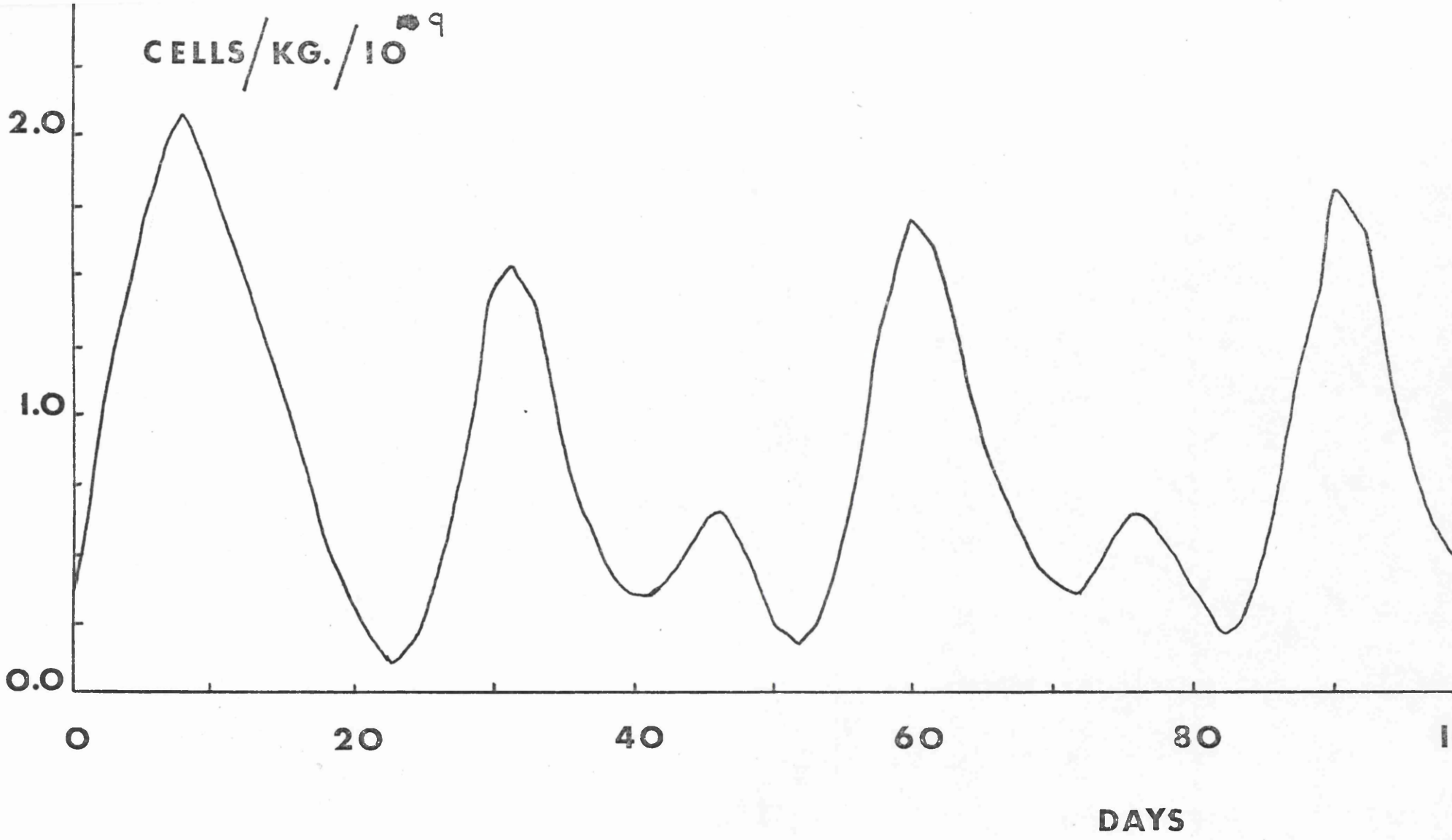

FIG. 6.10

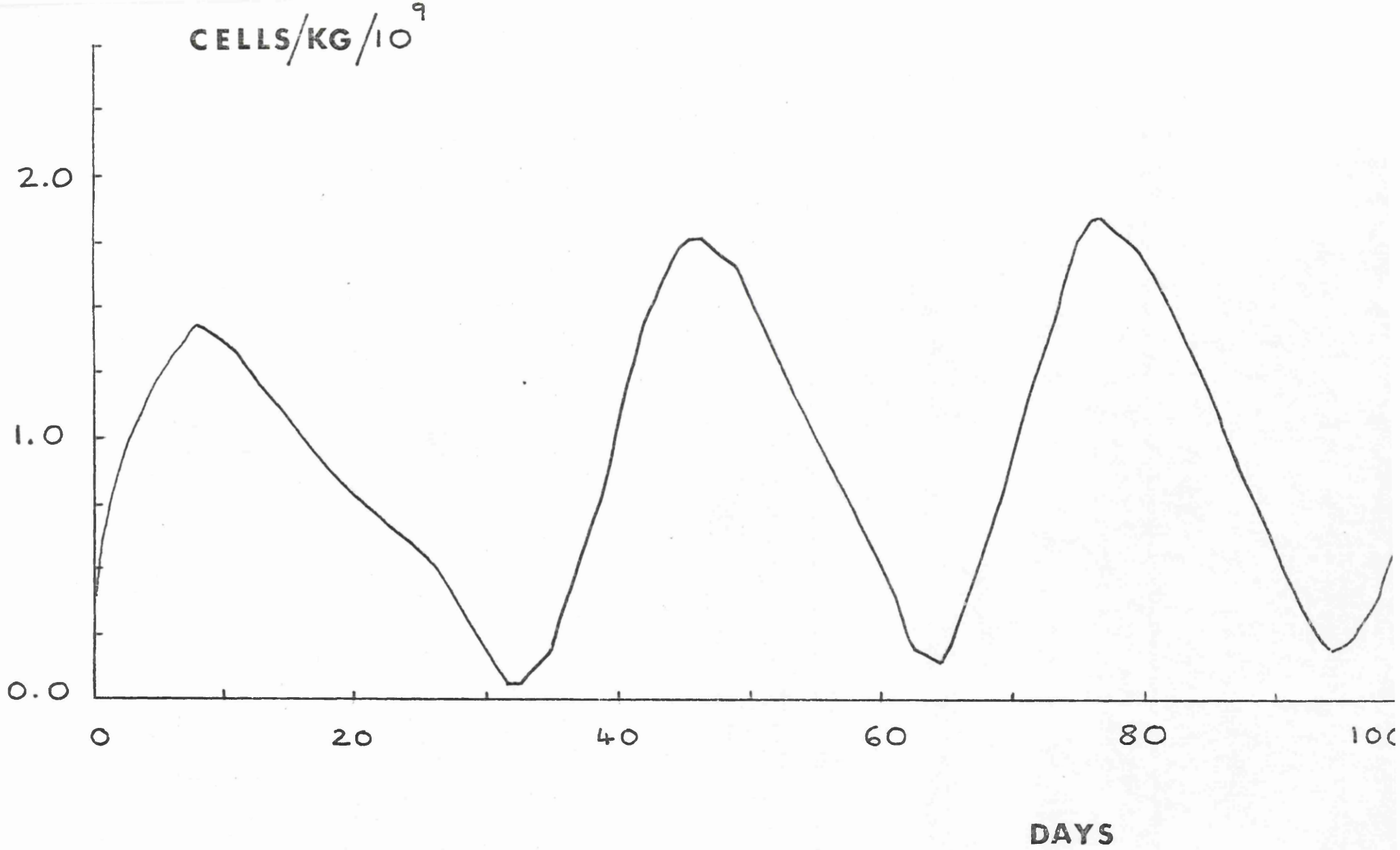

FIG. 6.11

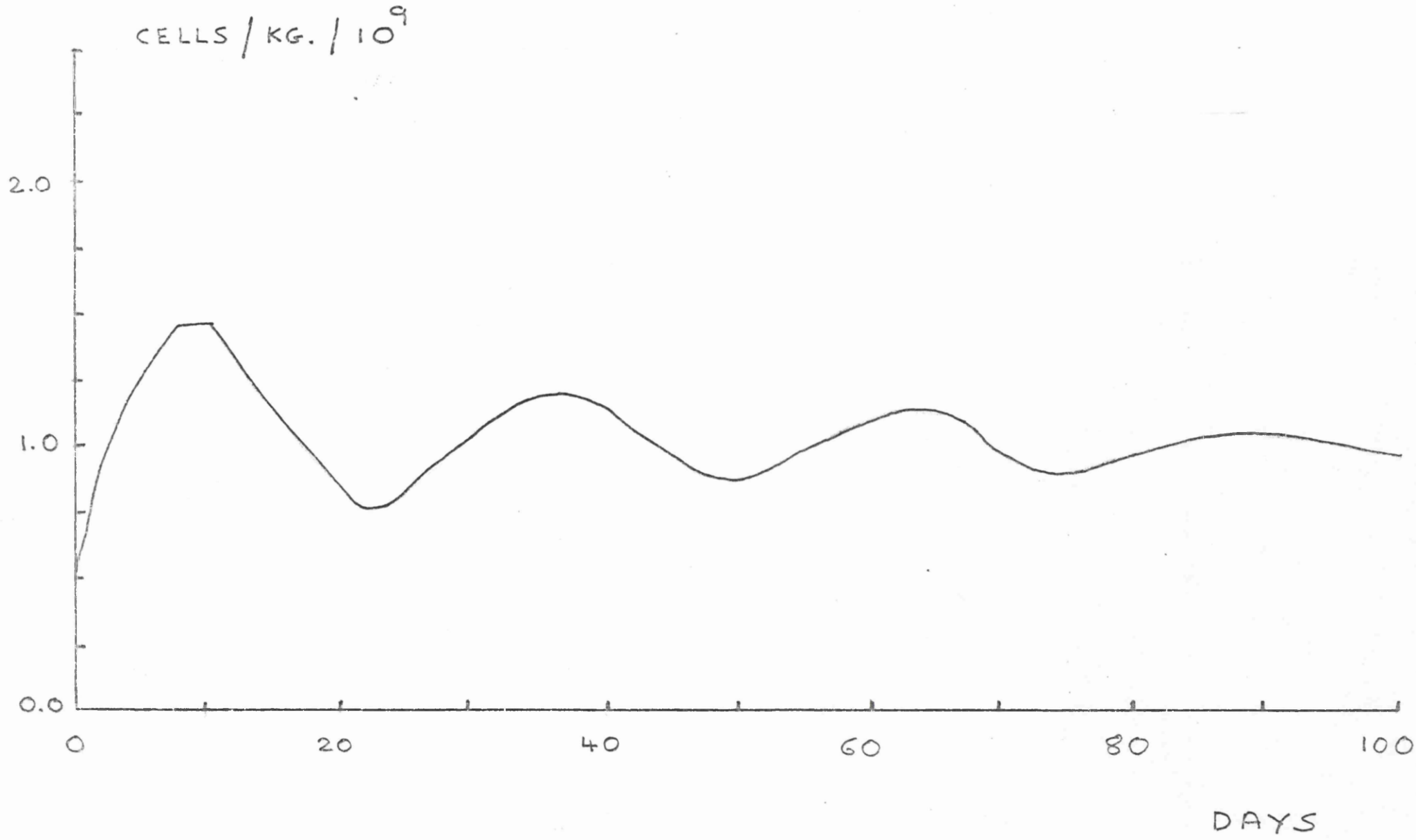

FIG. 6.12

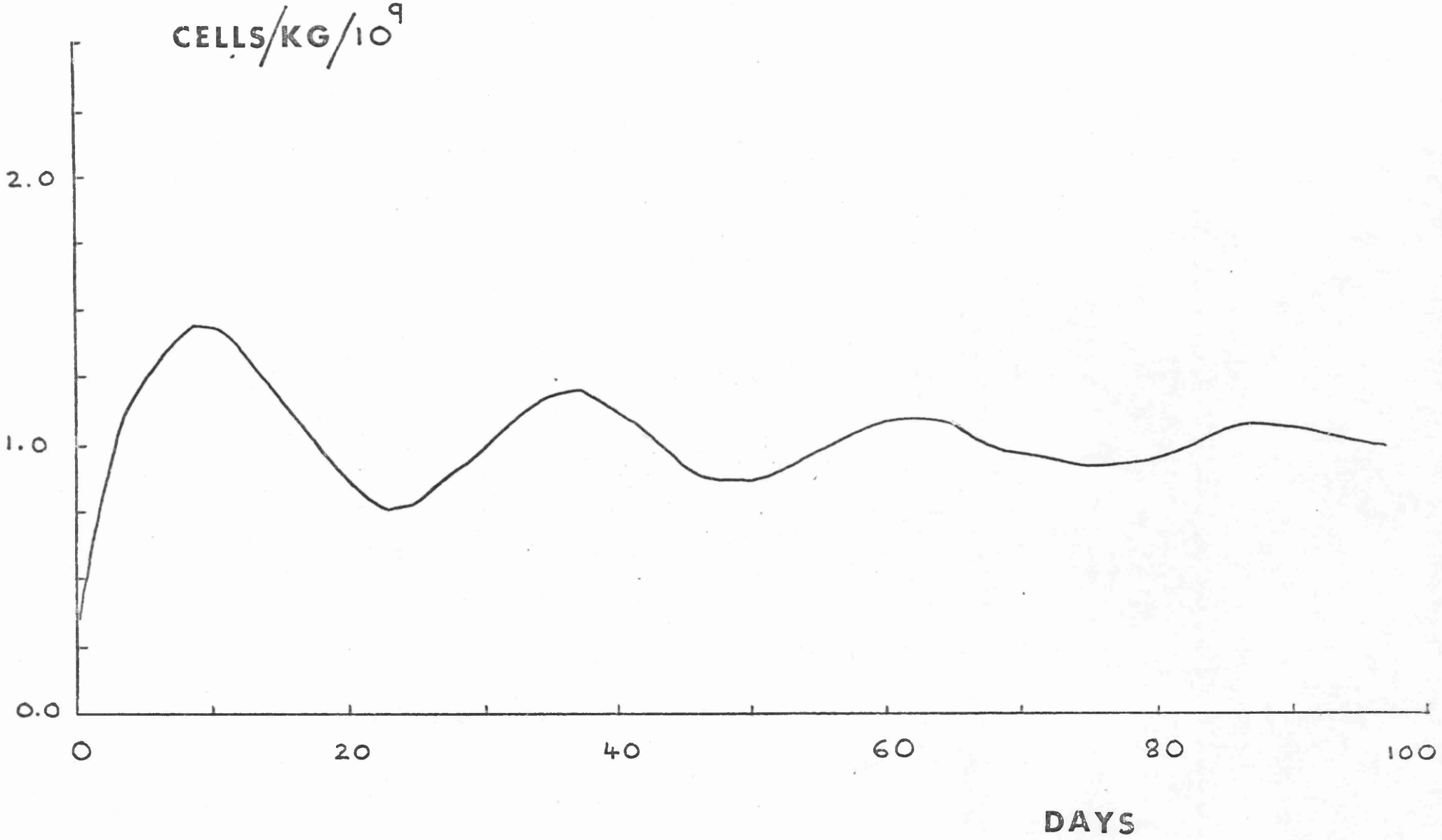

FIG. 6.13

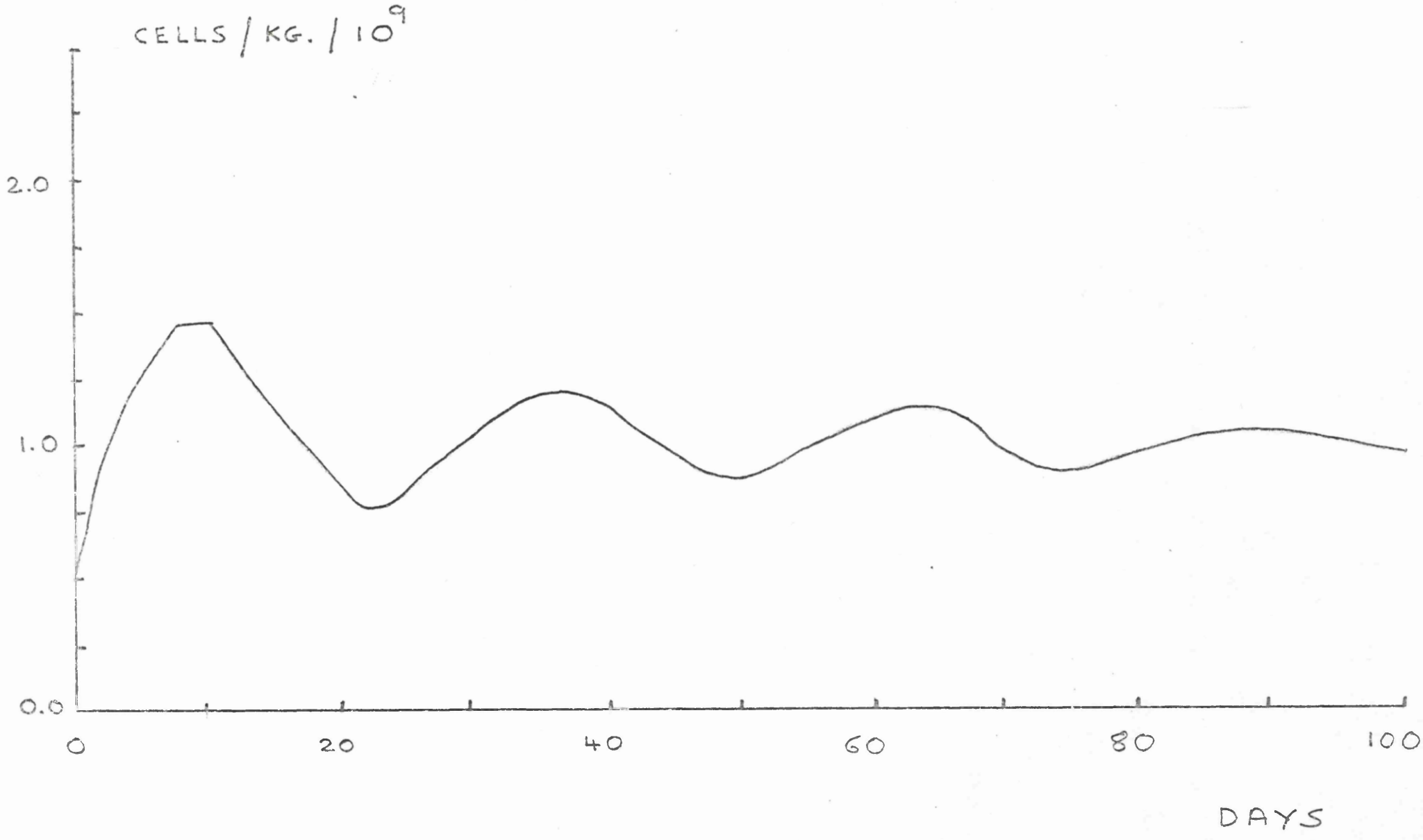

FIG. 6.14

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7. THE CONTROL OF STEM CELL NUMBER.

7. THE CONTROL OF STEM CELL NUMBER.

Introduction.

Until now, attention has been focussed on the teleonomic mechanisms which ensure a balance between the physiological requirements of the organism and the rate of supply of terminally differentiated cells capable of meeting these requirements. In the preceding considerations, it has been tacitly assumed that the non-terminally differentiated stem cells which respond to the mechanisms regulating cytodifferentiation were themselves available in at least roughly constant numbers.

Evidently, this assumption is unwarranted. Stem cell populations may suffer depletion as a result of the demand for differentiated cells (1,2) or as a consequence of cell death caused by drugs (3,4) or radiation (5,6). Provided the damage remains within certain limits, it is well established that stem cells are capable of replenishing their numbers, given time to do so. Taken at face value, this property implies the existence of mechanisms controlling the size of the stem cell population, in addition to those which regulate the numbers of terminally differentiated cells.

The mechanisms controlling cytodifferentiation and cellular proliferation are evidently complementary, not least because the phenotypic expression of an epigenetic switch might involve obligatory mitosis (7). Needless to say, the problems here encountered are of a very general nature and arise in considerations of cellular regulation in a large variety of tissues. However, as will be subsequently argued,

the stem cell population is an especially appropriate one on which to focus. The presumed advantages of this population, as well as several drawbacks, should become apparent in the course of the following discussion.

Theories of Growth Control.

Within the past few years, several theories of growth control have been proposed. Such theories, often motivated by the desire to understand neoplastic abberations, suffer from the disadvantages of attempting to deal with growth control in general, whereas it may differ in individual tissues, and in considering the problem in isolation from morphogenesis, histogenesis and pattern formation.

As will be demonstrated in the following sections, growth control cannot in general be separated from mechanisms which regulate the spatial conformation of the tissues and organs under consideration. The possibility of effecting this separation in specific individual cases - of which the control of stem cell number will be advanced as one - depends upon the possibility of accepting certain simplifications in relation to the spatial arrangements of the cells concerned.

Insofar as the problem of growth control is amenable to considerations of a general character, the simplest types of hypothesis are of a 'contact inhibition' between cells of identical or related type (8 - 10) or of synthesis by each category of cell of a tissue-specific diffusible mitotic inhibiter. The latter hypothesis has been championed in recent years by the 'chalone school' of Bullough and his associates who have reported experimental evidence for and

inhibitors in epidermis (11-13), granuloid tissue (14, 15) and erythroid tissue (16).

From a theoretical standpoint, it is possible to raise objections to each of these classes of theory, notably that they neglect the problems of organ topology and the occurrence of heterogeneous cell types in normal organs (see Wolpert 17).

Nevertheless, informative conclusions may be derived from even these straightforward theories and it would seem reasonable to explore some such possibilities before considering theories of a more sophisticated kind.

Mitotic Autoregulation and Organ Topology.

Consider the problem of growth control in a cell population whose members synthesize a diffusible mitotic inhibitor. Let $C(x,y,z,t)$ denote the inhibitor concentration and $n(x,y,z,t)$ the corresponding cell density, both at the point (x,y,z) at time t. The diffusion equation for inhibitor is

$$\frac{\partial C}{\partial t} = D\nabla^2 C + P \qquad (7.1)$$

where $$\nabla^2 \equiv \frac{\partial^2}{\partial x^2} + \frac{\partial^2}{\partial y^2} + \frac{\partial^2}{\partial z^2}$$

and P represents the net production rate of inhibitor and D is the diffusion constant. If inhibitor is uniformly synthesized by all cells, the positive component of P will be of the form γn where γ is the rate constant for inhibitor synthesis. Inhibitor may be lost either by catabolization or by loss from the cellular aggregate. If both loss mechanisms exist

$$P = \gamma n - \mu C - C_0 \lambda A \qquad (7.2)$$

where μ and λ are positive constants, C_0 is the inhibitor concentration at the surface of the aggregate and A is the area of the cellular aggregate. This formulation immediately reveals a serious difficulty. If the inhibitor loss rate

the cellular aggregate. This, however has not been specified.

The problem is more strongly emphasized when the number of cells in the population is considered. As a first approximation, it seems not unreasonable to write an equation such as

$$\frac{1}{n}\frac{\partial n}{\partial t} = \frac{\alpha}{1+\beta C} - \omega$$

illustrating the inverse relationship between cellular production and inhibitor concentration. The total cell number, $N(t)$, will then be

$$N(t) = \iiint_V n(x,y,z,t)\, dx\, dy\, dz$$

i.e. the integral of the cell density function over the volume V of the cellular aggregate. Once again, it is seen that a property of the shape of the aggregate (in this case V) enters into the problem.

In fact, the theory outlined so far is fundamentally inadequate. What is required is an additional law specifying the shape of the aggregate and its mode of change when the population increases or decreases. This law cannot be deduced from the laws governing cellular proliferation because the latter omit consideration of cell movement. These considerations serve to reinforce the probable necessity of dealing with growth control in conjunction with morphogenesis and pattern formation, as has been asserted by Wolpert (17).

This difficulty is exemplified in a recent model proposed by Riley (18)

for the mitotic autoregulation of a spherical aggregate. The spherical topology enters essentially into the formulation of the problem and is not derivable from the law proposed for mitotic autoregulation.

Riley's original model is open to criticism on mathematical grounds (19); its reformulation to meet such criticisms extends the model to cover non-spherical populations but once again exhibits the intrinsic dependence of growth control on organ topology - the latter requiring independent specification in advance (20).

The Spatial Pattern of Stem Cell Distribution.

Having established the probable interdependence of organ topology and growth control, we may consider the particular problem of control of stem cell number. Focussing attention on representative marrow segments, such as that of the femur, it is apparent that the stem cell population is spatially dispersed rather than localized in some small region of the marrow. Admittedly, the dispersion is not wholly random (21), but the departure from randomness is not sufficiently important to complicate the argument.

Thus, individual stem cells will typically be separated from one another by varying numbers of cells of different types. Moreover, the numbers and proportions of the intervening cells will vary in accordance with functional demand for erythrocytes, granulocytes and other cells whose progenitor cells are marrow residents.

The simplest control mechanism appropriate to such a situation would seem to be one depending on a diffusible mitotic inhibitor. This has the advantage of not requiring absolute contiguity between individual stem cells or on a uniformity of cellular type and number between separated stem cells. The hypothesis has been advanced by several authors (22 - 25) and has received some support from the experimental work of Gidali and Lajtha (26).

Moreover, by assuming a 'plum-pudding model' of stem cell distribution throughout the marrow the difficult problem of organ topology may be circumvented. Quite clearly, such a model is a grossly simplified abstraction. The necessary

simplifications may however be rather more acceptable in the case of stem cells than in the case of cells of other kinds.

Homogeneity of the Stem Cell Population.

In the model to be presented, all stem cells are assumed to be identical, at least in regard to synthesis of and response to the supposed mitotic inhibitor. If, as discussed in chapter 2, there exist distinct populations of pluripotential and unipotential stem cells, 'stem cells' must be interpreted as the pluripotential variety, unless unipotential stem cells are presumed to be self-regulating.
The possible relations between different categories of stem cell (if such exist) have not been considered in the present study.

A Model of the Regulation of Stem Cell Number.

Consider a number, $N(t)$, of stem cells to be randomly dispersed at time t throughout some volume of constant magnitude V.

Let $i(t)$ denote the total amount of inhibitor within the volume; this is made up of $i_c(t)$, the inhibitor within the cells and $i_e(t)$ the inhibitor external to the cells (but within the volume).

If inhibitor is synthesized at a constant rate by the cells and both catabolized at a rate proportional to the amount present and lost from the enclosure at a rate proportional to its area,

$$\frac{di}{dt} = \gamma N - \alpha i - b A i \qquad (7.5)$$

where γ, α, b and A (area) are all positive constants. Then, writing

$$\mu = (\alpha + bA) \qquad (7.6)$$

$$\frac{di}{dt} = \gamma N - \mu i \qquad (7.7)$$

Let us now suppose that the mitotic rate of a 'typical' cell is inversely proportional to the intracellular concentration of mitotic inhibitor but is restrained to some maximum by the time necessary for replication of completely uninhibited cells. The intracellular concentration of inhibitor will vary from cell to cell but in no systematic way for a random dispersion of cells. This variation may therefore be ignored and the average behaviour only considered. Stem cell loss may

be due to intrinsic death or differentiation into one or other of the haemopoietic developmental pathways. Conveniently lumping these together to give an (average) constant loss probability (assumed independent of stem cell age) we have

$$\frac{1}{N}\frac{dN}{dt} = \frac{\alpha}{1+\beta\left[i_c\right]} - \omega \qquad (7.8)$$

where α , β and ω are positive constants and the symbol $[\ ]$ is employed to denote a concentration.

It is now necessary to postulate some relationship between i_c and i_e . Suppose that a rapidly-acting uptake process preserves a constant ratio between the average intracellular and extracellular concentrations of inhibitor,

Thus,

$$\frac{\left[i_e\right]}{\left[i_c\right]} = k \qquad (7.9)$$

where k is a positive constant. Then if v is the average volume of a cell (assumed constant),

$$i = \left[i_e\right](V - Nv) + \left[i_c\right]Nv \qquad (7.10)$$

$$= \left[i_c\right]\left\{k(V-Nv) + Nv\right\} \qquad (7.11)$$

Finally, we introduce an assumption regarding the relaxation time of the systems described by equations (7.7) and (7.8). By 'relaxation time' is meant the (exponential) time constant of recovery of a linear system, or of a non-linear system subjected to a perturbation which is 'small' in the sense that an approximate linear description is appropriate. In practice, the relaxation time of a system serves to

identify the time scale on which events of interest are taking place.

Now it is reasonable to assume that the relaxation time associated with equation (7.7) be considerably shorter than that associated with equation (7.8) since the former refers to production and degradation of a molecular species and the latter to reproduction and disappearance of cells. Under such circumstances, it is a fair approximation to adopt only the steady-state solution of equation (7.7) (i.e. the particular integral) the transient being neglected as it vanishes in a time shorter than other times of interest (see Goodwin 27). Then,

$$i \approx \left(\frac{\gamma}{\mu}\right) N \qquad (7.12)$$

at all times.

Substituting for $[i_c]$ in (7.8) using (7.11), and for i in this resultant equation using (7.12), we obtain

$$\frac{1}{N}\frac{dN}{dt} = \frac{\alpha}{1 + \frac{\beta(\gamma/\mu)N}{kV + (1-k)\nu N}} - \omega \qquad (7.13)$$

Rearranging and lumping constants,

$$\frac{1}{N}\frac{dN}{dt} = \frac{\phi V + \psi N}{kV + \lambda N} \qquad (7.14)$$

where

$$\phi = (\alpha - \omega)k \qquad (7.15)$$

$$\psi = (\alpha - \omega)\nu(1-k) - \frac{\omega\beta\gamma}{\mu} \qquad (7.16)$$

and

$$\lambda = \frac{\beta\gamma}{\mu} + \nu(1-k) \qquad (7.17)$$

In appendix 7.I, it is shown that equation (7.14) has the analytically closed solution.

$$t + A = \frac{k}{\phi} \ln N + \left(\frac{\lambda}{\psi} - \frac{k}{\phi}\right) \ln |\phi Y + \psi N| \qquad (7.21)$$

where A is a constant of integration, to be evaluated from the initial conditions.

Thus,

$$N^{\left(\frac{k}{\phi}\right)} |\phi Y + \psi N|^{\left(\frac{\lambda}{\psi} - \frac{k}{\phi}\right)} = \beta e^{t} \qquad (7.22)$$

where $\beta = e^{A}$ and is evidently a positive constant.

Analytic Properties of the Solution.

The modes of behaviour of (7.22) are obviously of interest. The most important question to be raised is whether the population described by (7.22) is self-limiting for all physically meaningful values of the parameters. Evidently, this is not the case.

Obviously, the R.H.S. of (7.22) is monotonic increasing with time so that, if (7.22) is to hold,

$$N^{\left(\frac{k}{\phi}\right)} \left| \phi V + \psi N \right|^{\left(\frac{\lambda}{\psi} - \frac{k}{\phi}\right)} \to \infty \qquad \text{as} \quad t \to \infty$$

Suppose that the population is self-limiting and that $N \to N_0$ as $t \to \infty$.

Then we must have that

$$N^{\left(\frac{k}{\phi}\right)} \left| \phi V + \psi N \right|^{\left(\frac{\lambda}{\psi} - \frac{k}{\phi}\right)} \to \infty \qquad \text{as} \quad N \to N_0$$

For physically meaningful values of N_0 this is only possible if the index $\left(\frac{\lambda}{\psi} - \frac{k}{\phi}\right)$ is negative - so that $\left|\phi V + \psi N\right|$ appears in a denominator and if the quantity $\left(-\frac{\phi V}{\psi}\right)$ is identified with N_0. Then (7.22) may be written in the form

$$\frac{N^{\left(\frac{k}{\phi}\right)}}{\left| N - \left(-\frac{\phi V}{\psi}\right) \right|^{\left(\frac{k}{\phi} - \frac{\lambda}{\psi}\right)}} = \beta e^{t} \qquad (7.23)$$

from which it may be seen that $N \to \left(-\frac{\phi V}{\psi}\right)$ as $t \to \infty$ is an asymptotic solution. Three conditions are involved here. Firstly,

$$\frac{k}{\phi} > \frac{\lambda}{\psi}$$

In addition, if $\left(-\frac{\phi V}{\psi}\right)$ is the population limit it must in the first place be a positive quantity and in the second be no greater than the number of cells which would completely fill the available volume (otherwise the equations cease to hold).

Thus it is necessary that

$$\left(-\frac{\phi V}{\psi}\right) \geqslant 0$$

but

$$\left(-\frac{\phi V}{\psi}\right) \leqslant \frac{V}{\nu}$$

When these inequality constraints are evaluated in terms of the original parameters, it is possible to show that the three conditions (7.24), (7.25) and (7.26) may be reduced to the single condition

$$\frac{\omega \beta \gamma}{\mu} > \nu(\alpha - \omega) \qquad (7.27)$$

(See appendix 7.II).

If this condition is satisfied, cells whose behaviour is described by (7.22) will attain a population limit when placed in an enclosure of any size. Conversely, if (7.27) is violated, cells obeying (7.22) will increase in number to completely fill an enclosure of any size (their subsequent behaviour is not predictable from the present model). It is of interest that the necessary condition (7.27) for population self-limitation is independent of the magnitude of the available volume but that any population limit which exists will be proportional to this magnitude.

Analogue Simulation Studies of the Model.

The possible modes of system behaviour appeared to be of sufficient interest to justify further study. Accordingly, the behaviour of the system was simulated using the EAI-680 analogue computer of Y-ARD (Glasgow), as described in chapter 6. As before, the analogue programming and execution of the simulation was carried out by Mrs. Helen M. Finlay.

To facilitate the simulation, equation (7.13) was rewritten in the form

$$\frac{1}{N}\frac{dN}{dt} = \frac{\alpha}{1+\left\{\frac{\theta i}{1+KN}\right\}} - \omega \qquad (7.28)$$

where $\theta = \frac{\beta}{kV}$ and $K = \frac{\nu(1-k)}{kV}$

With this symbolism, condition (7.27) assumes the form

$$K(\alpha-\omega) < \frac{\omega\theta\gamma}{\mu} \qquad (7.29)$$

Little basis exists for the selection of numerical values of the model parameters. As a result of discussion with Dr. J. Kirk, values were selected which were comparable to those used in previous analogue simulation studies of the haemopoietic stem cell control system (28,29), insofar as direct comparisons were legitimate. For convenience, the parameters α, ω, θ, γ and μ were held fixed while K was allowed to vary from one simulation to another. The values of the fixed parameters are shown in table 7.1. The system is defined by the pair of equations 7.12 and 7.28, so retaining the relaxation-time approximation used in the analytic treatment.

PARAMETER	VALUE
α	1.00
θ	1.00×10^{-4}
γ	5.00×10^{-7}
ω	0.35
μ	0.50

TABLE 7.1 : FIXED PARAMETERS IN SIMULATION STUDY

For these values, a choice of $K = 5.0 \times 10^{-11}$ satisfies condition (7.29). The system therefore responds stably and homeostatically to a perturbation, as shown in fig. 7.1. From (7.29), it can be calculated that the value $K = 5.20 \times 10^{-11}$ is the critical value above which the population fails to limit. In the course of analogue simulation, with K able to be changed continuously, a value of 5.18×10^{-11} appeared to be the upper limit of stability which, allowing for the inevitable inaccuracies of the simulation process, is in excellent agreement with the value derived theoretically.

In a further study, a value of $K = 6.00 \times 10^{-11}$ – well above the upper limit of stability – was selected. As fig. 7.2 shows, the model behaves in an unstable fashion, as anticipated. However, the detailed kinetics of the

non-limiting growth process is revealing. The kinetic behaviour was tested to ascertain whether it exemplified exponential growth by plotting the growth-curve on log-linear graph paper. As is well-known, an exponential growth mode corresponds to a straight-line graph on log-linear paper. However, this is not found when the non-limiting growth curve is plotted in this way. As fig. 7.3 shows, the graph is curvilinear in form which indicates the rate of growth becomes less as the population becomes larger, although (as follows from violation of the self-limitation condition) the growth rate is always positive definite for a finite population.

A corollary to the form of growth curve exhibited by the model population is that, on this model, a reduction in population size results in an increased proliferation rate, giving a 'homeostatic' response to perturbations. Unlimited growth need not therefore be equated with the total absence of homeostatic responsiveness.

Local and Global Aspects of Cellular Organization.

Despite its limitations, the model suggests a general principle concerning the relation between local and global properties of cellular assemblies. As described above, the model population exhibits self-limiting and non-self-limiting modes of behaviour, the mode realized being dependent on the numerical relationships between the parameters of the model.

Now, these parameters refer to properties of the population as a whole. Clearly, such global properties must result from the integration over the population of relevant properties of single cells, taking due note of intercellular interactions and interactions with the non-cellular environment. A change in a global parameter must be interpreted as resulting from a change in the properties of one or more cells, the non-cellular environment, or both.

Some such changes could result from a primary change in a single cell whose daughter cells both perpetuated this change and possessed a proliferative advantage (i.e. a mutant clone). Strictly speaking, the present model is inadequate to represent such possibilities. However, it is not difficult to see that a shifting clonal composition of a cell population could give rise to an apparent temporal evolution of the average parameters referring to the population as a whole. This allows a more complicated behaviour than dealt with here, although it is likely that the properties here described would still exist.

These considerations reinforce the argument that macroscopic properties require attention, if an understanding of biological organization (30,31) and disorganization (32) is to be achieved.

Possible Implications for the Understanding and Control of Cancer.

A fairly straightforward biological interpretation of the different growth modes of the model population is to identify the limiting growth mode with the homeostatic behaviour of many normal tissues and the non-limiting growth mode with the proliferation of malignant cells. The non-exponential form of the non-limiting mode is then of interest in the light of reported evidence for 'teleonomic' control of cellular proliferation in human acute myeloid leukaemia (33 - 36), murine plasmacytoma (37) and some lung tumours of animals and man (38).

It has also been claimed that tumours of epidermal, melanocytic and granuloid tissue retain - at least in part - the capacity to synthesize and respond to the appropriate tissue chalone (39 - 41) although either the cellular export (39) or uptake (19) of the chalone appeared to be deranged. Additionally, it has been known for some time that a variety of animal and human tumours follow a Gompertzian rather than exponential growth curve, which implies that the growth rate declines as tumour size increases (42,43). Whilst a variety of explanations of this phenomenon have been proposed, it is at least compatible with the partial retention of growth control, as illustrated by the model population in the present study.

More broadly, the 'hormone dependence' and 'conditional persistence' of some human tumours has been cited as evidence against their being 'autonomous' (i.e. unresponsive

to normal control mechanisms) (32,44). The property here - partial retention of mitotic autoregulation in non-self-limiting populations - could be considered as a specific case of the more general class of phenomena of non-autonomous proliferation of tumour cells.

Should these arguments prove to have any validity, quite important consequences may follow. All else apart, if mitotic rate is a function of cell population size, assessment of this rate at some point in time provides little guide either to the past or future development of the tumour, unless this information is supplemented by knowledge of a matheamtical law of growth. Moreover, continued (if modified) responsiveness to growth control in tumours raises the possibility of the manipulation of the kinetic state of tumour cells, through normal physiological control mechanisms, to establish optimal therapeutic conditions (33,45).

Additional therapeutic possibilities arise from more general considerations. Self-limitation of the model population is dependent on the existence of certain quantitative relationships between the parameters of the model. Suppose a cellular event occurs (e.g. a somatic mutation or an epigenetic switch) whose macroscopic effect is the alteration of one or other parameter to such an extent that a transition occurs from self-limiting to non-self-limiting population growth.

Now, even if the cellular event which underlies this change were to prove irreversible in practice, should any of the processes which determine the values of other relevant

parameters be physiologically manipulable, it is possible that the self-limiting growth mode could be restored by altering parameters other than those affected by the malignant change. (e.g. if, in 7.27, α were irreversibly increased, reduction of, say μ or ω could restore satisfaction of the condition).

This provides a concrete, although hypothetical, illustration of the practical importance of studying the global properties of populations and tissues as well as the molecular organization of the constituent cells. The excessive concentration on individual cellular properties is, at least potentially, a methodological vice which has been termed 'cytologism' by Smithers (32,45). The point is that if one does not know how the parameters are connected together by the self-limitation conditions, only intuition is available as a guide to research or experimental therapy.

That the present model is simplistic to the point of naïveté has been emphasized several times. Even here, however, the self-limitation condition was not intuitively obvious nor susceptible to derivation by purely verbal reasoning. For realistic models, the task will be correspondingly greater.

Finally, it may be worth noting that the acceptance of a 'cytologistic' paradigm can lead to pessimistic conclusions as to the therapeutic utility of fundamental research. Thus McFarlane Burnet, accepting the malignant event as an irreversible somatic mutation has declared the total impotence of all laboratory research in the field of human cancer (46) (and many other fields, besides).

Such pessimism is unwarranted. Even in the (unlikely) event of the reversal of mutation proving eternally impossible, radically new methods of cancer therapy should be made available by the elucidation of the control mechanisms at the physiological level.

Appendix 7 I

The differential equation for the behaviour of the model (equation 7.14) is

$$\frac{1}{N}\frac{dN}{dt} = \frac{\phi V + \psi N}{kV + \lambda N} \qquad (7.I.1)$$

$$\therefore \int \frac{(kV + \lambda N)}{N(\phi V + \psi N)}\, dN = \int dt \qquad (7.I.2)$$

Now, the integrand on the L.H.S. can be expressed as a sum of directly integrable partial fractions:

$$\frac{kV + \lambda N}{N(\phi V + \psi N)} = \frac{k}{\phi N} + \frac{\lambda - \left(\frac{k}{\phi}\right)\psi}{\phi V + \psi N} \qquad (7.I.3)$$

Hence,

$$\frac{k}{\phi}\int \frac{dN}{N} + \left(\frac{\lambda}{\psi} - \frac{k}{\phi}\right)\int \frac{\psi\, dN}{\phi V + \psi N} = \int dt \qquad (7.I.4)$$

$$\therefore \frac{k}{\phi} \ln N + \left(\frac{\lambda}{\psi} - \frac{k}{\phi}\right) \ln\left|\phi V + \psi N\right| = t + A \qquad (7.I.5)$$

where A is a constant of integration. The constant A may be evaluated from initial conditions, since when t = 0,

$$A = \frac{k}{\phi} \ln\left\{N(0)\right\} + \left(\frac{\lambda}{\psi} - \frac{k}{\phi}\right) \ln\left|\phi V + \psi N(0)\right| \qquad (7.I.6)$$

from which A is evidently a real number for real N (0)

The three inequality conditions for the existence of a finite population limit (conditions (7.24), (7.25) and (7.26)) are

$$-\frac{\phi}{\psi} > 0 \tag{7.II.1}$$

$$-\frac{\phi}{\psi} < \frac{1}{\nu} \tag{7.II.2}$$

and

$$\frac{\lambda}{\psi} < \frac{k}{\phi} \tag{7.II.3}$$

The first of these, (7.II.1), expressed in terms of the original parameters becomes

$$\frac{(\alpha - \omega)\, k}{(\alpha-\omega)\lambda(1-k) - \frac{\omega\beta\gamma}{\mu}} < 0 \tag{7.II.4}$$

Since for the case under consideration $\alpha > \omega$, this requires

$$\frac{\omega\beta\gamma}{\mu} > \nu\,(\alpha-\omega)\,(1-k) \tag{7.II.5}$$

Condition (7.II.2) is, in terms of the original parameters.

$$\frac{(\alpha - \omega)\, k}{\frac{\omega\beta\gamma}{\mu} - (\alpha-\omega)\nu(1-k)} < \frac{1}{\nu} \tag{7.II.6}$$

Now, if condition (7.II.5) is satisfied, the denominator of the L.H.S. of (7.II.6) is positive and the inequality can be rearranged to give

$$\frac{\omega\beta\gamma}{\mu} > \gamma\,(\alpha - \omega) \tag{7.II.7}$$

Now, since $k > 0$, $1 - k < 0$, hence,

$$\gamma(\alpha - \omega)\,(1 - k) < \nu(\alpha - \omega) \tag{7.II.8}$$

Satisfaction of condition (7.II.7) therefore automatically ensures satisfaction of condition (7.II.5) also.

Condition (7.II.3) may be similarily elaborated as

$$\frac{\frac{\beta\gamma}{\mu} + \nu\ (1-k)}{(\alpha-\omega)\ \nu\ (1-k) - \frac{\omega\beta\gamma}{\mu}} < \frac{1}{\alpha-\omega} \qquad (7.II.9)$$

If condition (7.II.7) [and therefore (7.II.5)] is satisfied, the denominator on the L.H.S. of (7.II.9) is negative and the inequality can be rearranged to give

$$\frac{\alpha\beta\gamma}{\mu} > 0 \qquad (7.II.10)$$

Since all the parameters involved are positive, this condition is always satisfied.

Hence, the three inequalities (7.II.1, 7.II.2, 7.II.3) are satisfied if and only if condition (7.II.7) holds.

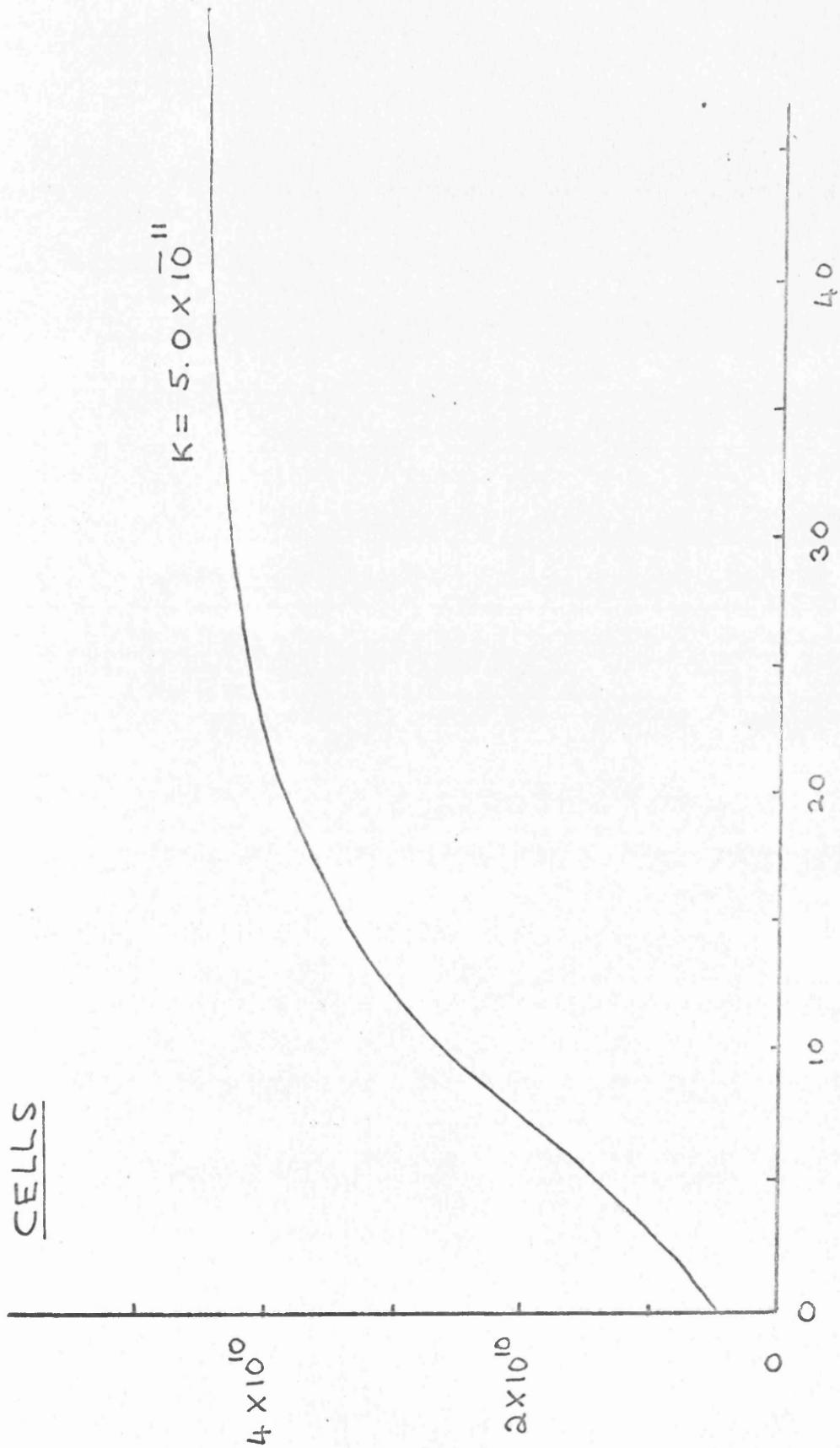
CELLS
$K = 5.0 \times 10^{-11}$
4×10^{10}
2×10^{10}
0
0
10
20
30
40
DAYS

FIG. 7.1

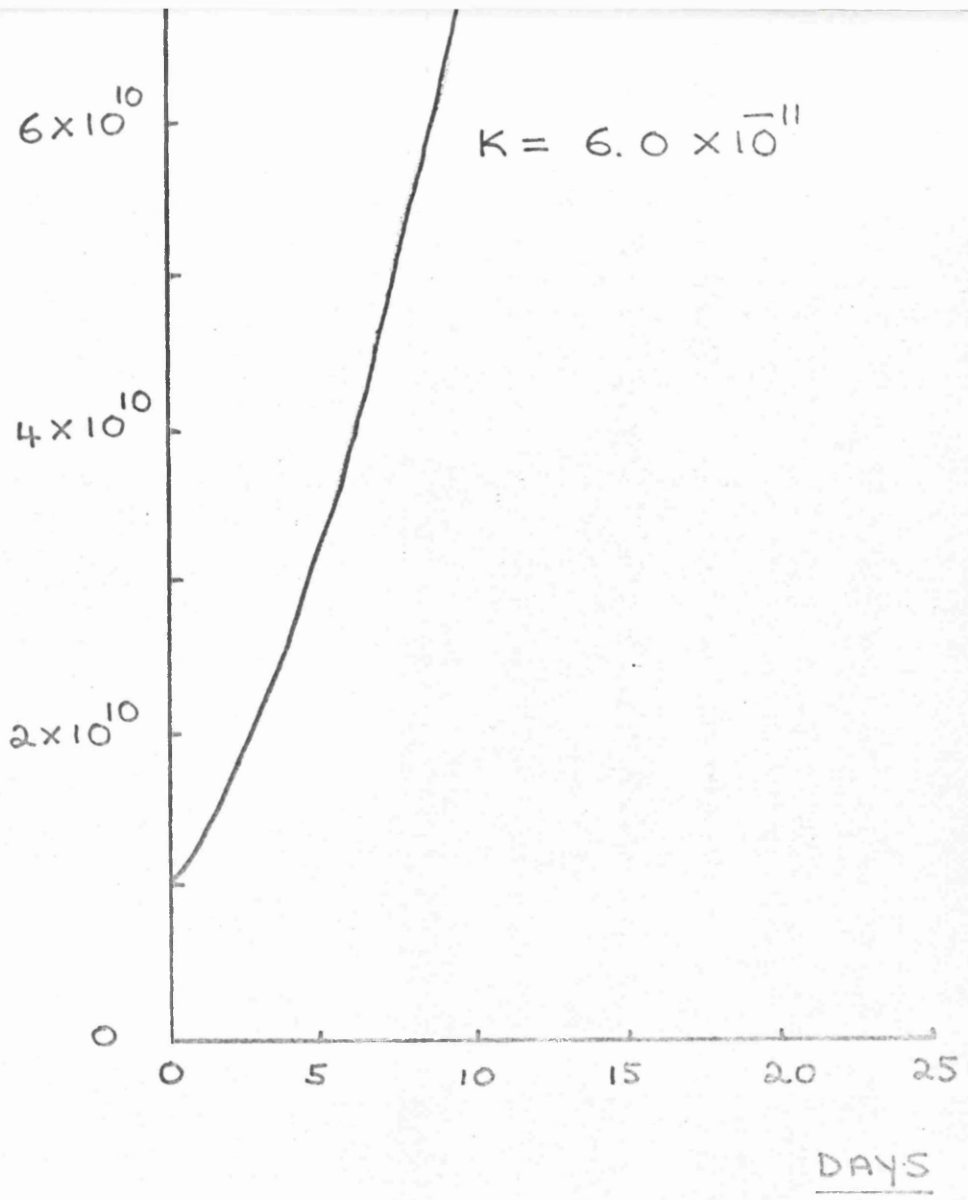
$K = 6.0 \times 10^{-11}$
6×10^{10}
4×10^{10}
2×10^{10}
0
0
5
10
15
20
25
DAYS

FIG. 7.2

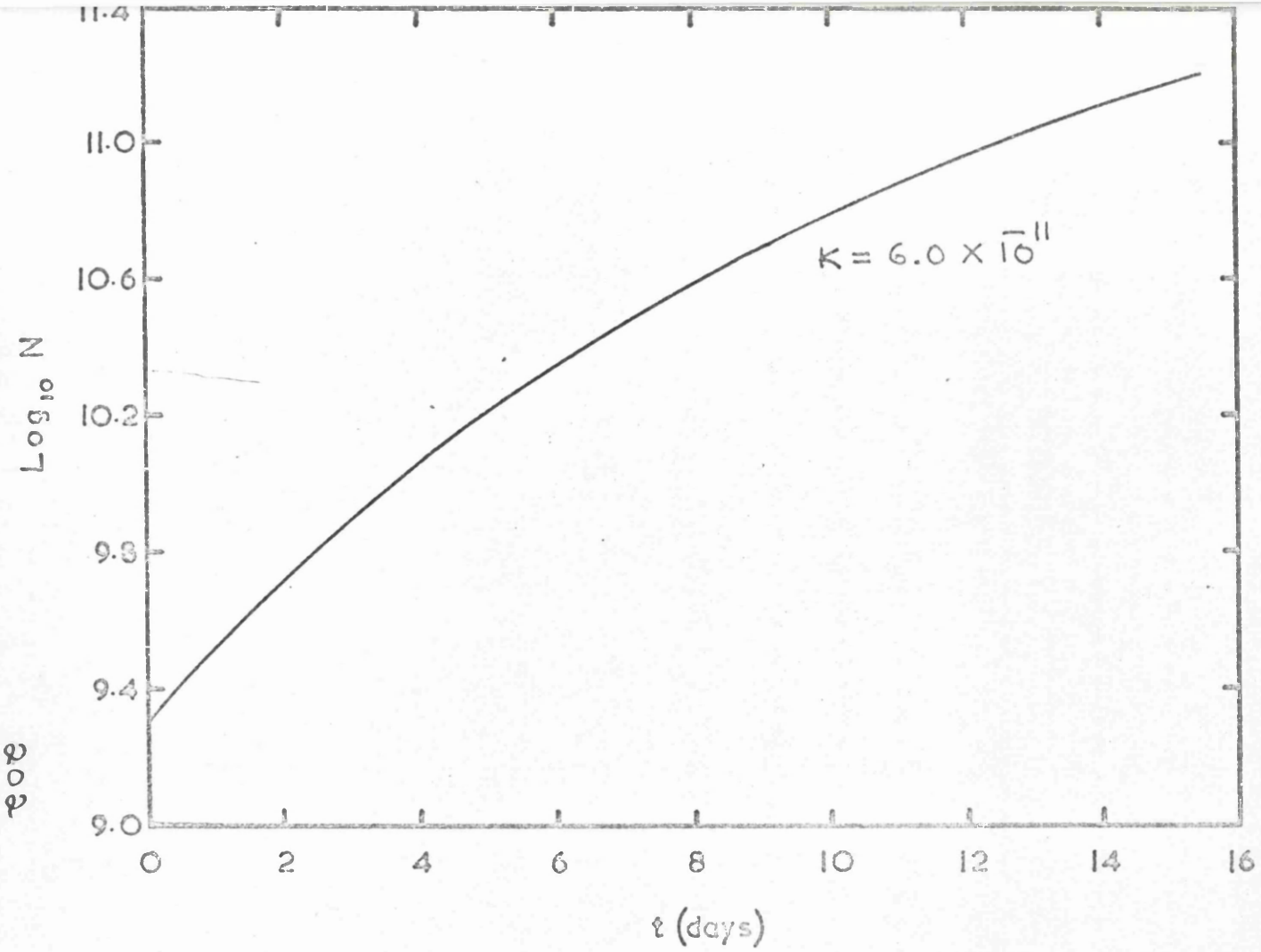

FIGURE 7.3

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8. A THREE-LOOP MODEL FOR THE CONTROL OF GRANULOPOIESIS.

8. A THREE-LOOP MODEL OF GRANULOPOIESIS.

Introduction.

Accepting the model of stem cell mitotic autoregulation proposed in chapter 7, and the tentative conclusions as to control loops and parameter values reached in chapter 6, a three-loop model of the control of granulopoiesis was constructed and examined.

Unlike the isolated stem cell loop of chapter 7, the composite model here considered is described by a third-order system of non-linear delay-differential equations, which are analytically intractable. Model behaviour was therefore assessed entirely using analogue simulation techniques.

However, the analytical theorems derived in chapter 7, though inapplicable to the more complicated equations of this chapter, provide a rough guide to the selection of stem cell loop parameters and an indication of the possible modes of behaviour of the stem cell loop, despite the complications caused by the presence of additional loops.

Derivation of Equations Describing the Three-Loop Model.

The equations describing the three-loop model may be derived by, essentially, combining the equation describing the stem loop of chapter 7 (equation 7.14) with the equations describing the two loops of chapter 6 (equations 6.5 and 6.6)

In so doing, account must be taken of the stem cell loss due to induction of differentiation, and of the amplification effect produced by mitosis of granulocytic precursors during the proliferative phase of maturation.

These considerations lead to equations of the form,

$$\frac{1}{S}\frac{dS}{dt} = \frac{\phi + \psi S}{\Omega + \Theta S} - \frac{\alpha}{1+\beta\,(G_m)^z} \qquad (8.1)$$

$$\frac{dG_m}{dt} = \frac{(\alpha e^{k\tau_1})S}{1+\beta\,[G_m]^{\alpha}_{t-(\tau_1+\tau_2)}} - \frac{\lambda G_m}{1+\mu\,(G_B)^{\gamma}} \qquad (8.2)$$

$$\frac{dG_B}{dt} = \frac{\lambda G_m}{1+\mu\,(G_B)^{\gamma}} - \omega G_B \qquad (8.3)$$

where τ_1 is the time spent in proliferative phase & and τ_2 the rest of maturation (excluding residence in marrow as a mature granulocyte), $k = \frac{\ln 2}{t_c}$ the mean proliferation rate of granulocytic dividing precursors and αS the maximum rate of induction of stem cells to differentiate along the granulocytic pathway. The parameters ϕ, ψ, Ω and Θ are defined on page 184 of chapter 7. The remaining quantities have the meanings of chapter 6. The model structure is shown in fig. 8.1. The parameters were assigned the values of table

8.1. By setting the derivatives of the left-hand sides of equations (8.1), (8.2) and (8.3) to zero, it was calculated that the steady-state (or mean) values of the variable quantities of the model were as given in table 8.2. Additional features of kinetic interest are given in table 8.3.

Simulation Studies of Model Behaviour.

As with the studies described in chapter 6, the behaviour of the model was assessed by subjecting each of the model variables (in this case S, G_m and G_B) to a 'mild' perturbation reducing it to about 50% - 75% of the calculated steady-state (mean) value.

Examination of the response of the present model to perturbations of this magnitude quickly showed that, in its present form, the model was unacceptable as an approximation to a physiologically realistic control system.

Highly unstable behaviour was observed, with all variables either increasing indefinitely (until analogue overload) or decreasing rapidly to zero with extinction of all populations. Moreover, the model was 'capricious' in that very small alterations of the value of critical parameters induced a seemingly sharp transition between unlimited growth and extinction or vice-versa. In some cases of unlimited growth, oscillatory behaviour was seen on the cathode ray oscilloscope, but this was uncommon and may be attributed to artefacts produced by the overloading of the electronic amplifiers of the analogue computer, as can occur when unlimited growth is being simulated.

Additionally, with a given set of parameters, small changes in the value of initial conditions induced 'flip-flop' between extinction and unstable growth. This behaviour is illustrated in figs. 8.2,8.3 and 8.4. The parameter values were those of table 8.2 and the initial conditions those indicated in the figures.

Further study of this system revealed that the erratic behaviour of the model was a direct consequence of its composite nature and could not be attributed to any one of its constituent loops. This was demonstrated as follows :

(a) The equation

$$\frac{1}{S}\frac{dS}{dt} = \frac{\phi + \psi S}{\Omega + \Theta S} - \frac{\alpha}{1+\beta\left[G_m^{(0)}\right]}\,x \qquad (8.4)$$

was simulated and found to be stable for a wide range of initial conditions. Instabilities were generated only when G_m was allowed to vary.

(b) The equations

$$\frac{dG_m}{dt} = \frac{\alpha e^{k\tau_1} S^{(0)}}{1+\beta\left[G_m\right]_{t-\tau}}\,x - \frac{\lambda G_m}{1+\mu (G_B)^y} \qquad (8.5)$$

$$\frac{dG_B}{dt} = \frac{\lambda G_m}{1+\mu (G_B)^y} - \omega G_B \qquad (8.6)$$

where $\tau = \tau_1 + \tau_2$,

were also simulated and also shown to be stable within all ranges of interest. Instabilities immediately resulted when S was allowed to vary.

This provides a concrete illustration of a salient principle of cybernetics : the dependence of a particular feature (here instability or extinction) on the interaction of system components, being absent from the components taken in isolation.

Reformulation of the Model.

Once the source of the instability had been recognized, modifications of the model, to ensure stable behaviour, could be envisaged. Of course, the solution of the problem of restoration of stability need not be unique. In the present case, a particular modification, which seemed physiologically reasonable, was invoked, but the existence of alternative modifications is quite possible.

Since the difficulty results from the dynamic coupling between S on the one hand, and the pair G_m, G_β on the other, a reduction of the 'draw' on the stem cell pool due to granulocytic differentiation should reduce the likelihood of unstable behaviour being provoked.

This produced a difficulty : a stem cell population of $\approx 1.0 \times 10^8$ cells / kg is already large and a substantial increase in stem cell number seemed unreasonable. An alternative is to suppose that additional mitoses occur between the stem cell and the granulocyte, giving increased amplification and reduced 'draw' on the stem cell population itself. In the present case this was done by rewriting equation (7.1) as

$$\frac{1}{S}\frac{dS}{dt} = \frac{\phi + \psi S}{\Omega + \Theta S} - \left(A + \frac{\alpha'}{1 + \beta (G_m)^x} \right) \quad (8$$

where A and α' are each constants chosen such that

$$A + \frac{\alpha'}{1 + \beta (G_m)^x} = \frac{\alpha}{1 + \beta (G_m)^x} \quad (8$$

Modifying A and α' over a suitable range, while ensuring the satisfaction of equation (8.8), it was found that the values

$$\alpha' = 0.13$$
$$A = 0.52 \quad .$$

conferred stability on the model. Of course, to maintain $G_m^{(o)}$ and $G_B^{(o)}$ at their previous values, it was necessary that the 'amplification factor' $e^{k\tau_1}$ be increased. Keeping τ_1 at 6 days as before, the proliferation constant k was increased from 0.59 to 0.95, to just balance the reduced stem cell input. This corresponds to a mean cell cycle time of 17 hours and a total 'amplification factor' of about 300.

This 'amplification factor' is considerably larger than that estimated by various authors for the granulocytic pathway of development.

It is tempting to speculate on the existence of a category of dividing cells - 'committed stem cells' ? - between the true stem cells and myeloblastic cells, capable of making up the necessary amplification. In such a case, the model structure of figure 8.1 should properly be replaced by something like that of fig. 8.5. Although the ' secondary stem cells', S_2 , could be physically the target cells for a supposed 'granulopoietin', a total time constant for the system would ensue if there existed a feedback between S_1 and S_2 , as depicted.

The model here described could be considered a first approximation to such a system, with all stem cells lumped together as S .

Simulation Studies of the Reformulated Model.

With the equations now written as

$$\frac{1}{S}\frac{dS}{dt} = \frac{\phi + \psi S}{\Omega + \Theta S} - \left(A + \frac{\alpha'}{1+\beta(Gm)^{x}}\right) \qquad (8.9)$$

$$\frac{dGm}{dt} = \frac{(\alpha' e^{k'\tau_1})S}{1+\beta[Gm]^{x}_{t-\tau}} - \frac{\lambda Gm}{1+\mu(G_B)^{y}} \qquad (8.10)$$

$$\frac{dG_B}{dt} = \frac{\lambda Gm}{1+\mu(G_B)^{y}} - \omega G_B \qquad (8.11)$$

the behaviour of the model was reconsidered. In this case stable behaviour was observed and is illustrated in fig. 8.6.

A point of interest was that, in this case, the model appeared rather less sensitive to the value of x than had been the prototype model of chapter 6.
Although quantitative comparisons were deemed inappropriate, the model now behaved stably in response to perturbations, and in something the same way as the granulopoietic control system in man and experimental animals (see for example Host 1,2; also Lawrence and co-workers 3).
Unfortunately the modified equations (8.9 - 8.11) cannot really be considered a satisfactory mathematical model of the granulopoietic control system. The arbitrary introduction of the parameter A, which proved necessary for the restoration of system stability, is not consistent with the previously assumed homogeneity of the stem cell pool. Similarly, the amplification factor $e^{k'\tau_1}$ of 300 is now around ten times that estimated experimentally.
The principal result of the studies described in the present

chapter is to cast doubt on the adequacy of a homogeneously - responsive stem cell population. Some kind of heterogeneity would seem essential, either of the population itself, as in the 'unipotential stem cell models'; or in the responsiveness of a stem cell to an inductive signal, depending, perhaps on the phase of the cell cycle. Representation of such heterogeneities lies beyond the scope of the formulations considered here. In conclusion, models of the chapter 6 type appear reasonable for the control of granulopoiesis beyond the myeloblast stage; likewise, the chapter 7 model adequately represents control of pluripotential stem cell number. Between these, the existence is indicated of a further, regulated, cell population about which little is known.

TABLE 8.1 : PARAMETERS INITIALLY CHOSEN FOR THREE LOOP MODEL

PARAMETER	VALUE
ϕ	1.50
ψ	1.00×10^{-8}
Ω	1.40
Θ	3.00×10^{-8}
α	1.32
β	1.00×10^{-12}
x	1.25
k	0.59
τ_1	6
τ_2	2
λ	10.00
μ	4.00×10^{-8}
γ	1.00
ω	2.43

TABLE 8.2 : STEADY-STATE VALUES FOR THREE-LOOP MODEL WITH INITIAL PARAMETERS

QUANTITY	VALUE
$S^{(0)}$	1.00×10^{8} cells/kg
$G_m^{(0)}$	5.00×10^{9} cells/kg
$G_B^{(0)}$	7.00×10^{8} cells/kg

TABLE 8.3 : KINETIC PROPERTIES OF THREE-LOOP MODEL WITH INITIAL PARAMETERS

QUANTITY	REPRESENTATION	VALUE
Minimum Stem Cell Cycle Time	$\ln 2 \left(\frac{\Omega}{\Phi}\right)$	0.65 days
Steady-State Stem Cell Cycle Time	$\ln \frac{\Omega + \Theta S^{(0)}}{\Phi + \Psi S^{(0)}}$	1.22 days
Maximum Rate of Granulopoiesis	$e^{k\tau_1} \alpha S^{(0)}$	4.4×10^9 cells/kg
Steady-State Rate of Granulopoiesis	$\frac{e^{k\tau_1} \alpha S^{(0)}}{1 + \beta (G_m^{(0)})^x}$	2.8×10^9 cells/k

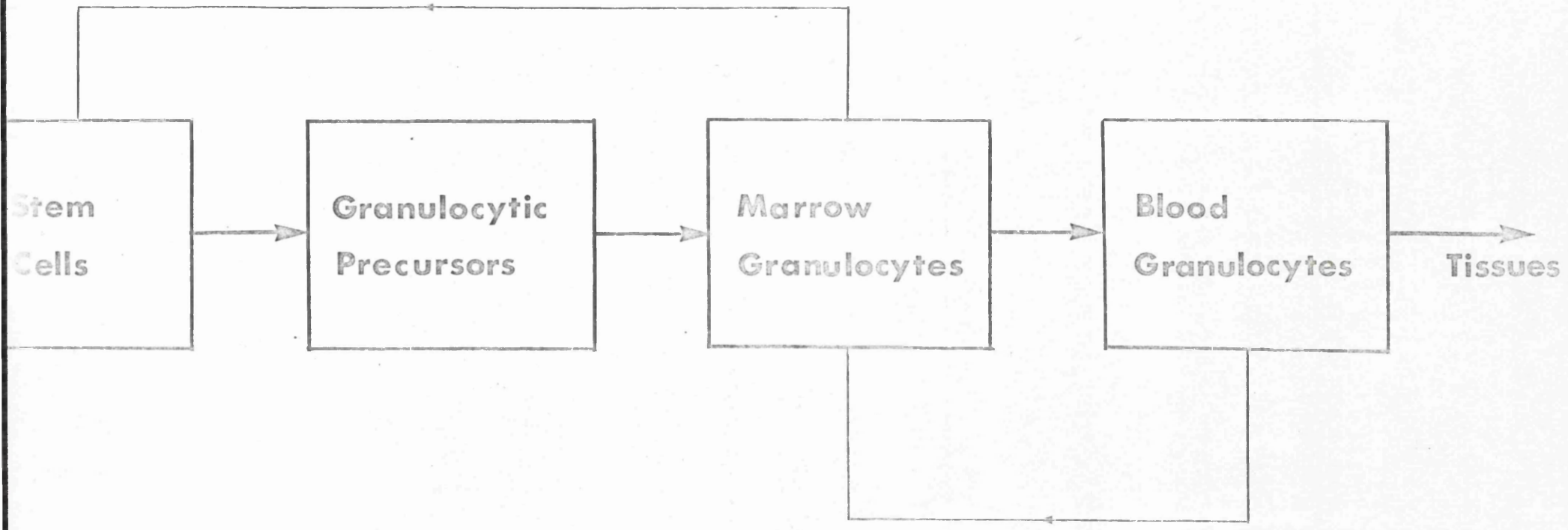

FIG. 8.1 : THREE-LOOP MODEL

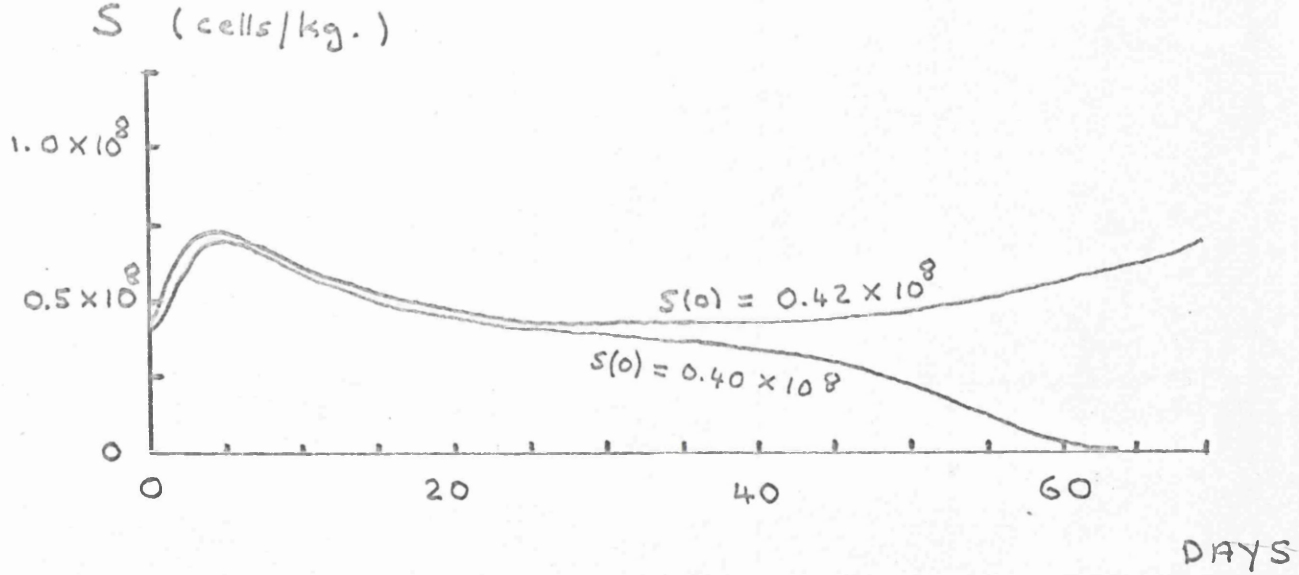
S (cells/kg.)
1.0 x 10^8
0.5 x 10^8
0
S(0) = 0.42 x 10^8
S(0) = 0.40 x 10^8
0
20
40
60
DAYS

FIG. 8.2

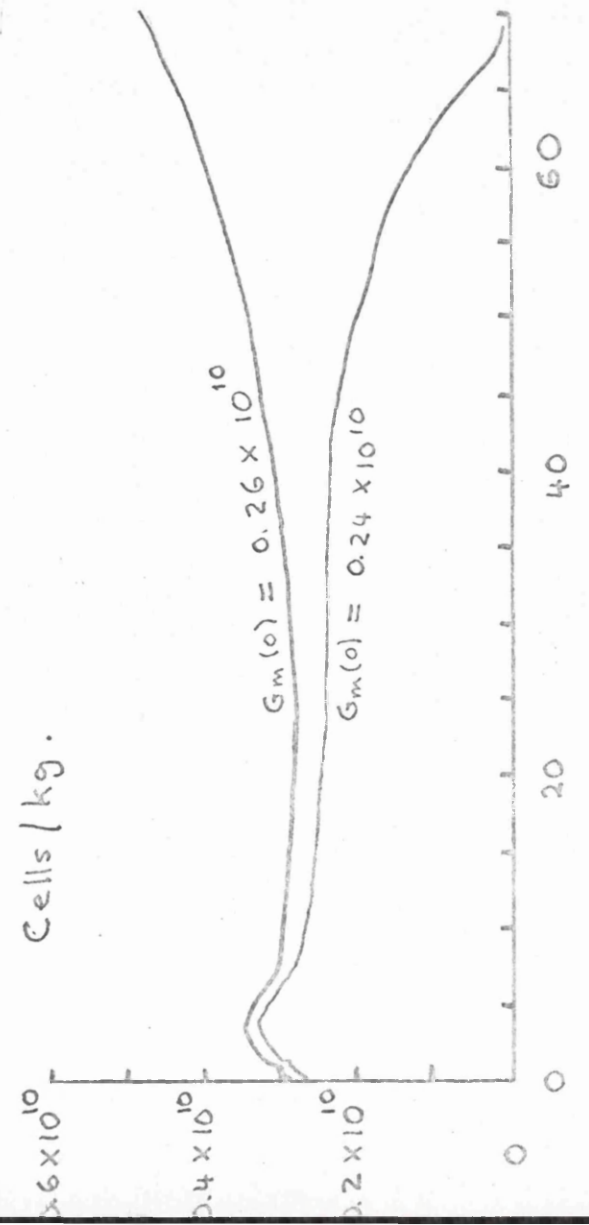
MARROW GRANULOCYTES (Gm)
Cells / kg.
$G_m(0) = 0.26 \times 10^{10}$
$G_m(0) = 0.24 \times 10^{10}$
0
20
40
60
DAYS

FIG. 8.3

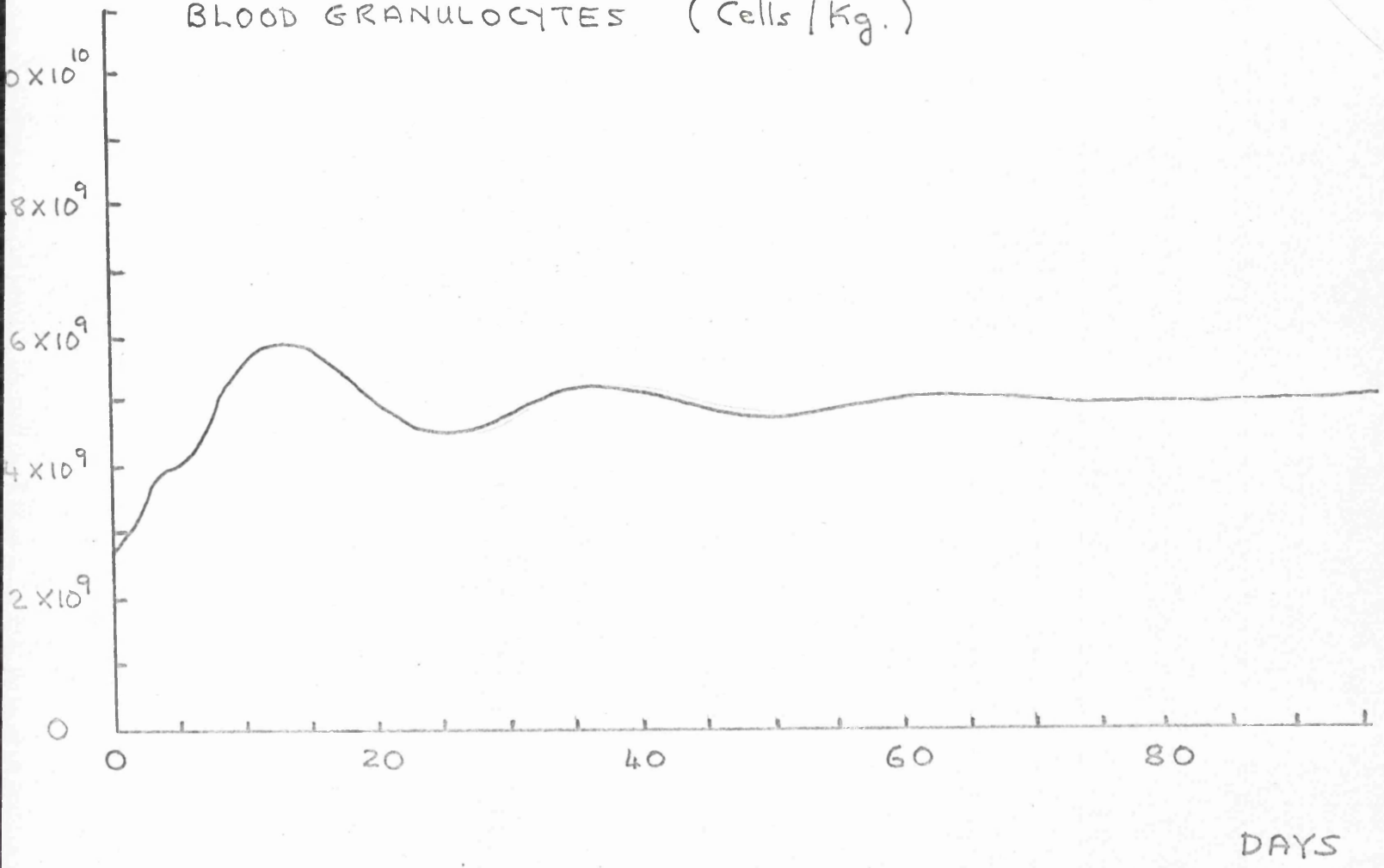
BLOOD GRANULOCYTES (Cells / Kg.)
0 X 10^10
8 X 10^9
6 X 10^9
4 X 10^9
2 X 10^9
0
0
20
40
60
80
DAYS

FIG. 8.6

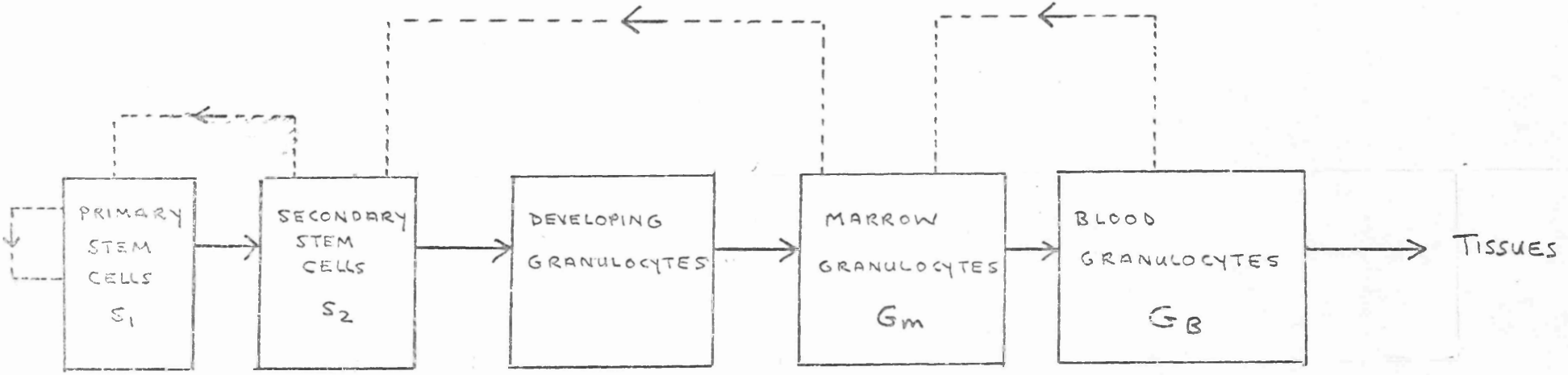
PRIMARY
STEM
CELLS
S_1
SECONDARY
STEM
CELLS
S_2
DEVELOPING
GRANULOCYTES
MARROW
GRANULOCYTES
G_m
BLOOD
GRANULOCYTES
G_B
TISSUES

FIG. 8.5

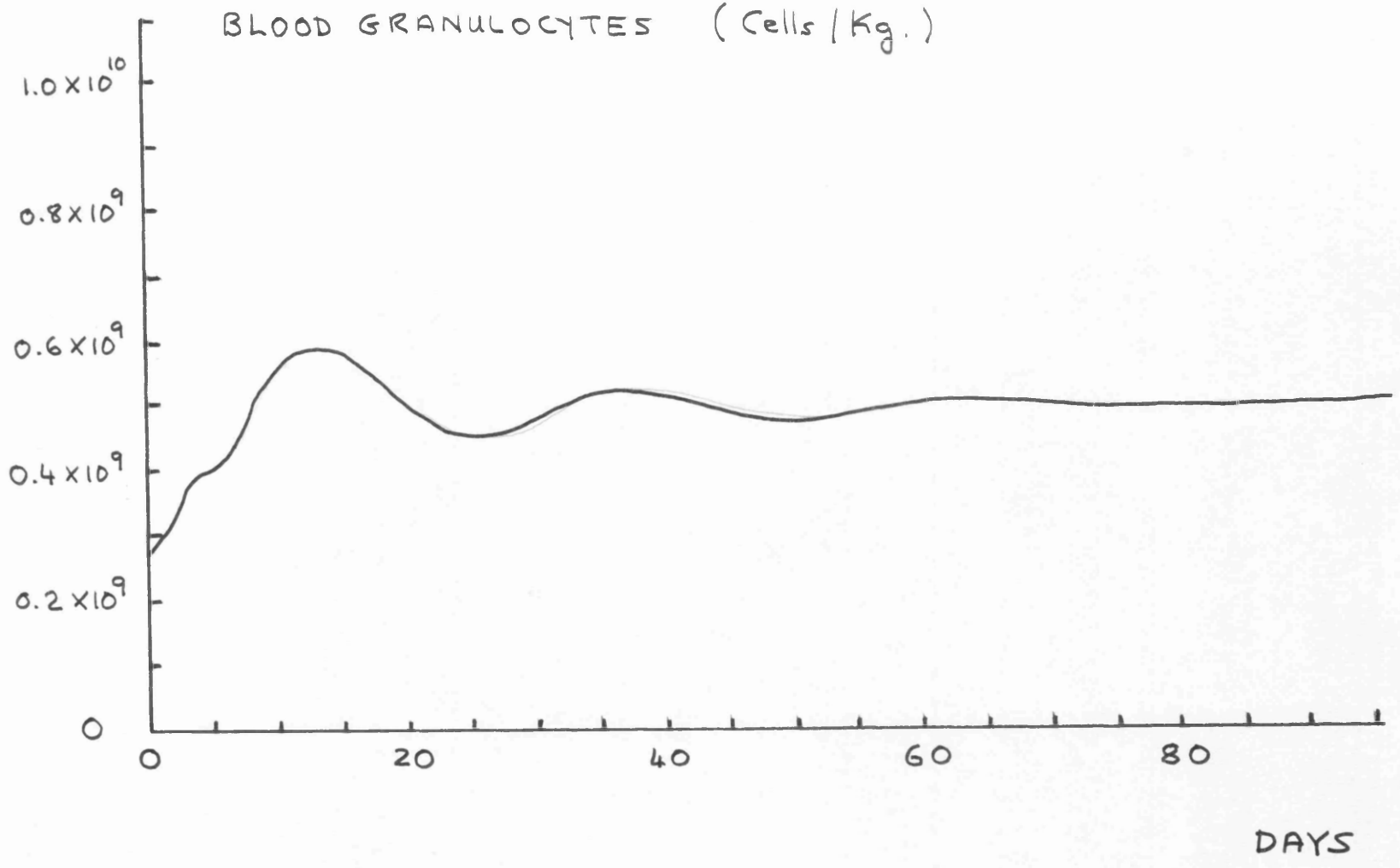
BLOOD GRANULOCYTES (Cells / Kg.)
1.0 X 10^10
0.8 X 10^9
0.6 X 10^9
0.4 X 10^9
0.2 X 10^9
0
0
20
40
60
80
DAYS

FIG. 8.6

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9. AUTOSTIMULATORY MECHANISMS IN ADAPTIVE REGULATION.

9. AUTOSTIMULATORY MECHANISMS IN ADAPTIVE REGULATION.

Introduction.

In all preceding analysis, the existence of a control loop regulating 'de novo' granulopoiesis at the stem cell level was assumed. Evidence for such a loop is discussed in chapter 2.

In this chapter, the physical nature of the presumed loop is considered, with particular reference to the nature of the cells involved in the production of stimulatory or inhibitory signals. At first sight, this problem may appear inappropriate for theoretical analysis and, indeed, theory alone cannot solve it.

Nevertheless, some progress is possible. In particular, cybernetic theory may be used to eliminate certain models which, on experimental grounds, appear reasonably plausible. More significantly perhaps, it may be possible to suggest the minimal changes necessary to render an eliminated model acceptable and to propose experiments discriminating between different types of possibility.

Evidence on the Physical Nature of Control.

Two lines of evidence are especially relevant to the question of the physical basis of granulopoietic stimulation or inhibition. Firstly, a very significant feature of the regulation of granulopoiesis (neglected so far) is the granulocytosis which normally results from infection with bacteria or bacterial endotoxin (1,2). Such an effect is more than a homeostatic recovery from a transient perturbation ; it is an adaptive response to changed conditions, like the erythropoietic response to lowered atmospheric pressure.

Secondly, mention has been made in chapter 2 of 'in vitro' studies on 'colony stimulating factor' (C.S.F.); a significant point in connection with 'in vitro' studies on human cells is the necessity of providing 'feeder layers' of other cells, in order to encourage granulocytic colony growth (3,4). These feeder layers, which evidently produce C.S.F. (5), are usually composed of mixtures of cells, whose nature may provide clues as to the source of C.S.F. under physiological conditions.

At the time of writing, experimental opinion is divided over this question, although the search has evidently been narrowed down to three principal candidates : the blood monocyte (6), the tissue macrophage (7,8) and the granulocyte itself (8,9).

Despite the residual uncertainties, the present evidence is sufficient to raise the possibility that a seeming logical paradox is about to be encountered. The nature of this

apparent paradox may be explained as follows. Although attention has been focussed in the preceding discussion on the granulopoietic properties of C.S.F., it was noted by early workers on the substance that not only granulocyte, but macrophage production, was promoted by this factor (5). Moreover, it now seems likely that the macrophage is actually derived from the granulocyte, with the blood monocyte a probable intermediate class of cell (5). Hence, if C.S.F. production is a property of any one or combination of the monocyte, the macrophage or the granulocyte, an autostimulatory, positive feedback loop would appear to exist.

The stability of such a system, and its utility in bringing about a granulocytosis in response to infection, has recently been asserted by Robinson and Mangalik (9). In fact, of course, a pure autostimulatory system, without any damping components whatsoever, is an untenable proposition (10,11). Pure positive feedback is the 'bête noir' of cybernetics.

However, the experimental evidence noted above is sufficient to generate interest in systems in which an autostimulatory loop exists as one component. Of course, the present evidence is inconclusive ; either C.S.F. or its apparent target cell may prove heterogeneous. Nevertheless, proceeding from 'Occam's Razor' once more, it may be of interest to investigate the minimum additions necessary to a pure autostimulatory loop, in order to render the composite system stable. In addition, the possible implication of an autostimulatory mechanism in infection-induced granulocytosis seems worthy of serious consideration.

Passive Stabilization of an Autostimulatory System by Cellular Death Rate.

An immediate possibility is the stabilization of an autostimulatory system by the damping effect of cellular death or loss. This corresponds to passive rather than active stabilization (e.g. by an inhibitory loop) since no information transfer is involved in the damping side of the process.

Consider a simple model of such a system. Let $N(t)$ be the size of the mature cell population at time t. If the mature cells stimulate further cell production, cell death or loss from the system occurs randomly, and a maturation time-delay is implicated in production, a descriptive equation is

$$\frac{dN}{dt} = \lambda (N)^{x}_{t-\tau} - \omega N \qquad (9.1)$$

In this chapter - unlike those preceding - we are not interested in the presence or absence of oscillatory behaviour. Hence the effect of the time-delay is of lesser importance than previously, and we may gain insight into the mechanisms of primary interest by focussing attention on the simplified equation

$$\frac{dN}{dt} = \lambda N^{x} - \omega N \qquad (9.2)$$

which is obtained by neglecting the time-delay in equation (9.1). This is an example of a Bernoulli equation with constant co-efficients. In appendix 9.I, equation (9.2) is shown to possess the analytic solution

$$N(t) = \left\{ \frac{\lambda}{\omega} + \left[N(0)^{1-\varkappa} - \frac{\lambda}{\omega} \right] . e^{-\omega(1-\varkappa)t} \right\}^{\left(\frac{1}{1-\varkappa}\right)} \qquad (9.3)$$

where $N(0)$ is the initial value of the cell population. The behaviour of such a system is critically dependent on the indicial parameter $\varkappa$ and, in certain circumstances, on the initial value $N(0)$. The following properties of the differential equation (9.2) and its solution (9.3) are established in appendix 9.I :

(a) For $0 < \varkappa < 1$, the system is stable and recovers from perturbations of either sign. This property may be summarized by the limit condition $N(t) \to \left(\frac{\lambda}{\omega}\right)^{\frac{1}{1-\varkappa}}$ as $t \to \infty$. The steady-state value $\left(\frac{\lambda}{\omega}\right)^{\frac{1}{1-\varkappa}}$ is approached regardless of the initial value $N(0)$.

(b) For $\varkappa > 1$ the system is inherently unstable. Granting this, it is not difficult to predict from the differential equation (9.2) that the direction of the instability ($\to \infty$ or $\to 0$) will depend on the value of $N(0)$; for large values of $N(0)$, since $N(0)^{\varkappa}$ will be larger than $N(0)$, $\frac{dN}{dt}\Big|_0 \geq 0$ and the positive feedback will keep N growing indefinitely. Conversely, for small $N(0)$, $\frac{dN}{dt}\Big|_0 \leq 0$ and will remain so for subsequent t.

This argument is borne out, and may be quantitated by examination of the solution (9.3). In summary - for $\varkappa > 1$ we have :

(ii) $N(0) < \left(\frac{\lambda}{\omega}\right)^{\left|\frac{1}{1-x}\right|}$: $N(t) \to 0$ as $t \to \infty$

(iii) For $N(0) = \left(\frac{\lambda}{\omega}\right)^{\left|\frac{1}{1-x}\right|}$, $\left.\frac{dN}{dt}\right|_0 = 0$.

In view of (i) and (ii) however, this constitutes 'neutral stability', which remains vulnerable to the destabilizing effect of any perturbation at any time.

In illustration of this behaviour, solutions of equation (9.2) were computed for $x = 0.5$ and $x = 1.5$ (with $\lambda = \omega = 1$) for initial conditions both above and below the critical value $\left(\frac{\lambda}{\omega}\right)^{\left|\frac{1}{1-x}\right|}$ which, granted stability, corresponds to the steady-state value of N . As fig. 9.1 shows, the system conforms to theoretical expectation.

Qualitative Criteria for Stability in a Passively-Damped Autostimulatory System.

Intuitively, it is reasonable to expect that cell death or loss can stabilize an autostimulatory system only if the cellular production rate initially rises faster with mature cell number than does the death rate, but this relationship reverses for sufficiently large values of cell number. In the present instance, the assumption of random cell death means that the production rate must initially rise faster than, but subsequently slower than, a linear dependence on cell number (fig. 9.1).

This criteria may assist in determining whether cell death is alone sufficient to stabilize an autostimulatory system or whether additional damping mechanisms (e.g. active, inhibitory, loops) are logically necessary. In the case of granulopoiesis and macrophage production, present techniques may not be too far from permitting an experimental approach to this question.

Of course, time-delays (neglected here) are likely to augment any tendency to instability which, in a non-delayed system, may be contained. The above conjecture therefore provides a criterion for rejecting a suggested mechanism, but satisfaction of the criterion does not guarantee that the mechanism will actually work.

Capacity of a Passively-Damped Autostimulatory System for Adaptive Regulation.

Robinson and Mangalik (9) have recently proposed that a form of autostimulation of granulopoiesis could provide for granulocytosis in infection. Roughly speaking, it is suggested that C.S.F. is released on lysis of dying granulocytes, so that the increased death rate which occurs in in bacterial infection could, paradoxically, cause a granulocytosis rather than a granulocytopenia.

This means that the autostimulatory effect should be taken as a function of cell death rate ωN instead of cell number. Similar considerations apply if the macrophage or monocyte is the major source of C.S.F. since autostimulation still occurs.

These considerations suggest a modification of equation (9.2). viz.

$$\frac{dN}{dt} = \lambda (\omega N)^{x} - \omega N \qquad (9.4)$$

Equation (9.4) is identical to equation (9.2) with λ replaced by $\lambda \omega^{x}$. The criterion for stability remains $x < 1$, but the steady-state value (granted stability), N_s becomes

$$N_s = \left(\frac{\lambda \omega^{x}}{\omega} \right)^{\frac{1}{1-x}} \qquad (9.5)$$

$$= \left(\lambda \omega^{x-1} \right)^{\frac{1}{1-x}} \qquad (9.6)$$

$$= \lambda^{\frac{1}{1-x}} \; \omega^{\frac{x-1}{1-x}} \qquad (9.7)$$

For $x < 1$ (stability), $\omega^{\frac{x-1}{1-x}}$ is a monotonic decreasing function of ω. Hence N_S must decrease as ω increases - increased cell death leads to reduced cell number. The converse is possible only if $x > 1$ - which leads to an unstable system.

It follows that an autostimulatory system whose only damping component is passive and linear cannot both be stable and regulate in the required fashion. The suggestion of Robinson and Mangalik (9) must therefore be rejected. Autostimulation coupled with adaptive autoregulation evidently requires the existence of one or more non-passive damping loops.

Alternative Mechanisms for Stabilization of an Autostimulatory Control System Regulating Cell Production.

Other than passive damping through cell loss or death, active control loops (involving information transfer) must be invoked to stabilize an autostimulatory system. It is simplest in the first instance to consider only one such damping loop and to assume that only one maturational series is involved in it.

The requirements of such a loop are basically that it prevent the action of the autostimulatory loop becoming excessively violent which requires that it be capable of inducing and suppressing production of the autostimulating cell series. If only the one maturational series is assumed to be involved in both loops, it is necessary to suppose that the autostimulating and the damping loop sense or act on cells at different stages of maturation in a given series.

This kind of mechanism - differential control action with maturity - is a natural supplement to mechanisms regulating very primitive populations (stem cell mitotic autoregulation), mature populations (marrow release of granulocytes) and those operating to connect the very primitive and the fully matured (control of granulocytic differentiation at the stem cell level).

Two simple types of mechanism which involve different points of control with different levels of maturity are described below.

Mechanisms Involving Differential Signalling with Maturity.

One intuitively attractive scheme results from supposing that the cells of a given series can synthesize both stimulators and inhibitors of cell production, the ratio between the two being a function of cellular age or maturity.

Simple-mindedly, one may suppose that the inhibitor and the stimulator are capable of intracellular neutralization of each other so that a given cell has a net export of either inhibitor or stimulator.

On such a scheme, inhibitory cells could be either more mature (fig. 9.) or less mature (fig. 9.) than the inhibitory cells. Preliminary studies of models of the two possibilities (Wheldon, unpublished) indicate that the latter possibility seems the more likely, on stability grounds. However, at the time of writing, these studies are incomplete and further work is required.

Experimentally, an approach seems possible if the stimulator is identified with C.S.F. and the inhibitor with a molecular species capable of 'masking' C.S.F. (See Metcalf and Moore). In a sudden leukapheresis, C.S.F. should promptly increase, while in an acute radiation experiment C.S.F.-mask should increase, the presently favoured model (fig. 9.4). If the alternative model (fig. 9.) is correct, exactly the opposite would be expected.

Improving assays of C.S.F. may soon allow experimental discrimination between the two types of model.

CONCLUSIONS.

Positive feedback control deserves more attention than it has received. In some cases, a given equation admits either a negative or a positive feedback interpretation. Riley, for example, has argued that the kinetics of the autostimulatory model presented in this chapter are consistent with negative rather than positive feedback (13). In appendix 9.II therefore, the way in which the model could exemplify purely positive feedback is made explicit.

In order that the trees do not conceal the wood, however, the argument that purely positive feedback systems may be stable is presented in appendix 9.III, in a way which is less dependent on particulars.

So far as the granulopoietic system is concerned, the issue remains unresolved. In attempting to resolve it, it is worth keeping in mind the fact that positive autostimulation is not logically eliminated by stability arguments, although other considerations do suggest the existence of inhibitory loops as well.

Appendix 9.1: Solution of Bernoulli's Equation with Constant Coefficients

Equation (9.2) states

$$\frac{dN}{dt} = \lambda N^{x} - \omega N \tag{9.A.1}$$

It may be linearized by the following device:

$$\text{Set} \qquad N = Z^{\left(\frac{1}{1-x}\right)}$$

$$\text{Hence} \qquad \frac{dN}{dt} = \left(\frac{1}{1-x}\right) Z^{\left(\frac{x}{1-x}\right)} \frac{dZ}{dt}$$

and equation (10.A.1) becomes

$$\frac{dZ}{dt} + \omega(1-x)Z = \lambda(1-x) \tag{9.A.2}$$

This is a linear equation with constant coefficients. Multiply both sides by the integrating factor

$$p = e^{\int \omega(1-x)\,dt} = e^{\omega(1-x)t}$$

to give

$$\frac{d}{dt}\left[e^{\omega(1-x)t} \cdot z \right] = e^{\omega(1-x)t} \lambda(1-x) \tag{9.A.3}$$

$$\therefore \quad e^{\omega(1-x)t} z(t) - z(0) = \lambda(1-x) \int_0^t e^{\omega(1-x)t}\,dt \tag{9.A.4}$$

$$e^{\omega(1-x)t} z(t) - z(0) = \frac{\lambda}{\omega}\left[e^{\omega(1-x)t} - 1 \right] \tag{9.A.5}$$

$$\therefore \quad z(t) = z(0)e^{-\omega(1-x)t} + \frac{\lambda}{\omega}\left[1 - e^{-\omega(1-x)t}\right] \tag{9.A.6}$$

Hence,

$$N(t) = \left\{ N(0)^{1-x} e^{-\omega(1-x)t} + \frac{\lambda}{\omega}\left[1 - e^{-\omega(1-x)t}\right] \right\}^{\frac{1}{1-x}} \tag{9.A.7}$$

$$N(t) = \left\{ \frac{\lambda}{\omega} + \left[N(0)^{1-x} - \frac{\lambda}{\omega} \right] e^{-\omega(1-x)t} \right\}^{\frac{1}{1-x}} \qquad (9.A.8)$$

The behaviour of this equation is rather complicated; it is critically dependent on the values of index parameter x and the starting condition $N(0)$.

Case I : $x < 1$

We here assume $0 < x < 1$ (for $x < 0$, feedback is negative). Then, if $x < 1$, the exponent $-\omega(1-x)t$ is negative and the exponential dies away.

Hence $N(t) \to \left(\frac{\lambda}{\omega}\right)^{\frac{1}{1-x}}$ with $t \to \infty$, which is independent of the initial value $N(0)$.

Case II : $x > 1$

Unstable behaviour occurs in this case, but its direction does depend on the initial value.

Case II(a) : $N(0)^{1-x} > \frac{\lambda}{\omega}$

When $N(0)^{1-x} > \frac{\lambda}{\omega}$, $\left[N(0)^{1-x} - \frac{\lambda}{\omega} \right] > 0$, then, since $x > 1$, $N \to 0$ as $t \to \infty$. Since $1-x < 0$, $N(0)^{1-x}$ may be written in the form $\frac{1}{N(0)^{|1-x|}}$ so that $N(0)^{1-x}$ is a monotonic decreasing function of $N(0)$. The condition $N(0)^{1-x} > \frac{\lambda}{\omega}$ may therefore be written in the alternative form $N(0) < \left(\frac{\lambda}{\omega}\right)^{\left|\frac{1}{1-x}\right|}$.

Case II(b) : $N(0)^{1-x} < \frac{\lambda}{\omega}$

In this case, $\left[N(0)^{1-x} - \frac{\lambda}{\omega} \right] < 0$ hence $\left\{ \frac{\lambda}{\omega} + \left[N(0)^{1-x} - \frac{\lambda}{\omega} \right] e^{-\omega(1-x)t} \right\}$ begins positive and gradually decreases towards zero. Since $1-x < 0$, This means that $\left\{ \frac{\lambda}{\omega} + \left[N(0)^{1-x} - \frac{\lambda}{\omega} \right] e^{-\omega(1-x)t} \right\}^{\frac{1}{1-x}}$ approaches $+\infty$ as t increases.

The "post-infinity" situation, with $\left\{ \frac{\lambda}{\omega} + \left[N(0)^{1-x} - \frac{\lambda}{\omega} \right] e^{-\omega(1-x)t} \right\}$ having passed through ∞ to become negative is not of physical significance. As pointed out above, the condition $N(0)^{1-x} < \frac{\lambda}{\omega}$ may, for $1-x < 0$ be alternatively written $N(0) > \left(\frac{\lambda}{\omega}\right) \left| \frac{1}{1-x} \right|$.

Summary

For $x < 1$, $N(t) \to \left(\frac{\lambda}{\omega}\right)^{\frac{1}{1-x}}$ whatever $N(0)$.

For $x > 1$, $N(t) \to 0$ for $N(0) < \left(\frac{\lambda}{\omega}\right) \uparrow \left| \frac{1}{1-x} \right|$

$N(t) \to +\infty$ for $N(0) > \left(\frac{\lambda}{\omega}\right) \uparrow \left| \frac{1}{1-x} \right|$

Appendix 9.2.

It has been asserted that the equation

$$\frac{dN}{dt} = \lambda N^{x} - wN \quad , \quad 0 < x < 1 \tag{9.2.1}$$

is consistent with the existence of positive rather than negative feedback.

Let m represent the strength of a stimulatory signal and S the size of the (stem cell) population receptive to this signal.

If mature cells, N in number, produce this molecular signal at a uniform rate, then, approximately,

$$m \approx \alpha N \tag{9.2.2}$$

Cellular production rate $\xi(t)$ may be assumed to be given by

$$\xi(t) = f(m,S) \tag{9.2.3}$$

where f is a function in two variables.

A simple form for f is

$$\xi(t) = \beta\, m.S(t) \tag{2.2.4}$$

In general, the stem cell population will be depleted by the recruitment of stem cells in receipt of the signal.

viz $$S(t) = F(m) \tag{9.2.5}$$

where F(m) is a decreasing function of m, and, since

$$m \approx \alpha N, \tag{9.2.6}$$

$$F(m) \approx F^{1}(N) \tag{9.2.7}$$

where $F^{1}(N)$ is a decreasing function of N.

Thus, if

$$\frac{dN}{dt} = \xi(t) - wN \tag{9.2.8}$$

we have

$$\frac{dN}{dt} \approx \beta m F^{1}(N) - wN \tag{9.2.9}$$

$$= (\beta\alpha) NF^{1}(N) - wN \tag{9.2.10}$$

Since $F^{1}(N)$ is decreasing with N, in any approximation of the form

$$N F^{1}(N) \approx N^{x}, \tag{9.2.11}$$

it is evident that $x < 1.$ (9.2.12)

Equation (9.2.1) is therefore consistent with the kinetics of an autostimulatory system in which the number of stem cells is finite and subject to depletion by the signal.

More General Statement Of The Conditions For Stability In Cell Population Kinetics

The equation

$$\frac{dN}{dt} = \lambda N^{x} - wN \qquad (9.3.1)$$

admits of more than one kinetic interpretation (12 – 14)

To avoid ambiguities, consider the autonomous regulation of a cell population. The kinetics of cell number depend on a simple balance of cellular production rate $f_1(N)$ and loss rate $f_2(N)$.

viz $$\frac{dN}{dt} = f_1(N) - f_2(N) \qquad (9.3.2)$$

While it is usual in models of mitotic homeostasis ~~the~~ to postulate functions f_1 and f_2 such that

$$\frac{\partial f_1}{\partial N} < 0 \quad \text{and} \quad \frac{\partial f_2}{\partial N} > 0,$$

this is strictly unnecessary.

The minimum conditions for the existance of a non-zero steady-state for equation (9.3.2) are that

$$\frac{dN}{dt} > 0 \text{ for small N but } \frac{dN}{dt} < 0 \text{ for}$$

large N.

Considerable latitude exists in the choice of f_1 and f_2 consistant with steady-state kinetics. In particular, functions having the properties $\frac{\partial f_1}{\partial N} > 0$ and $\frac{\partial f_2}{\partial N} > 0$ are perfectly consistent with stability, provided that $\frac{f_1}{f_2} \to 0$ as $N \to \infty$, and that $\frac{f_1}{f_2} \to K$ (where $K > 0$) as $N \to 0$. The quantity K need not be finite, but physical constraints usually require that it is.

A function f_1 such that $\frac{\partial f_1}{\partial N} > 0$ i.e. cellular production increases with cell number is consistent with positive feedback (autostimulation), although it is equally consistent with other mechanisms. The essential point is that a system whose only feedback is autostimulatory is not necessarily unstable.

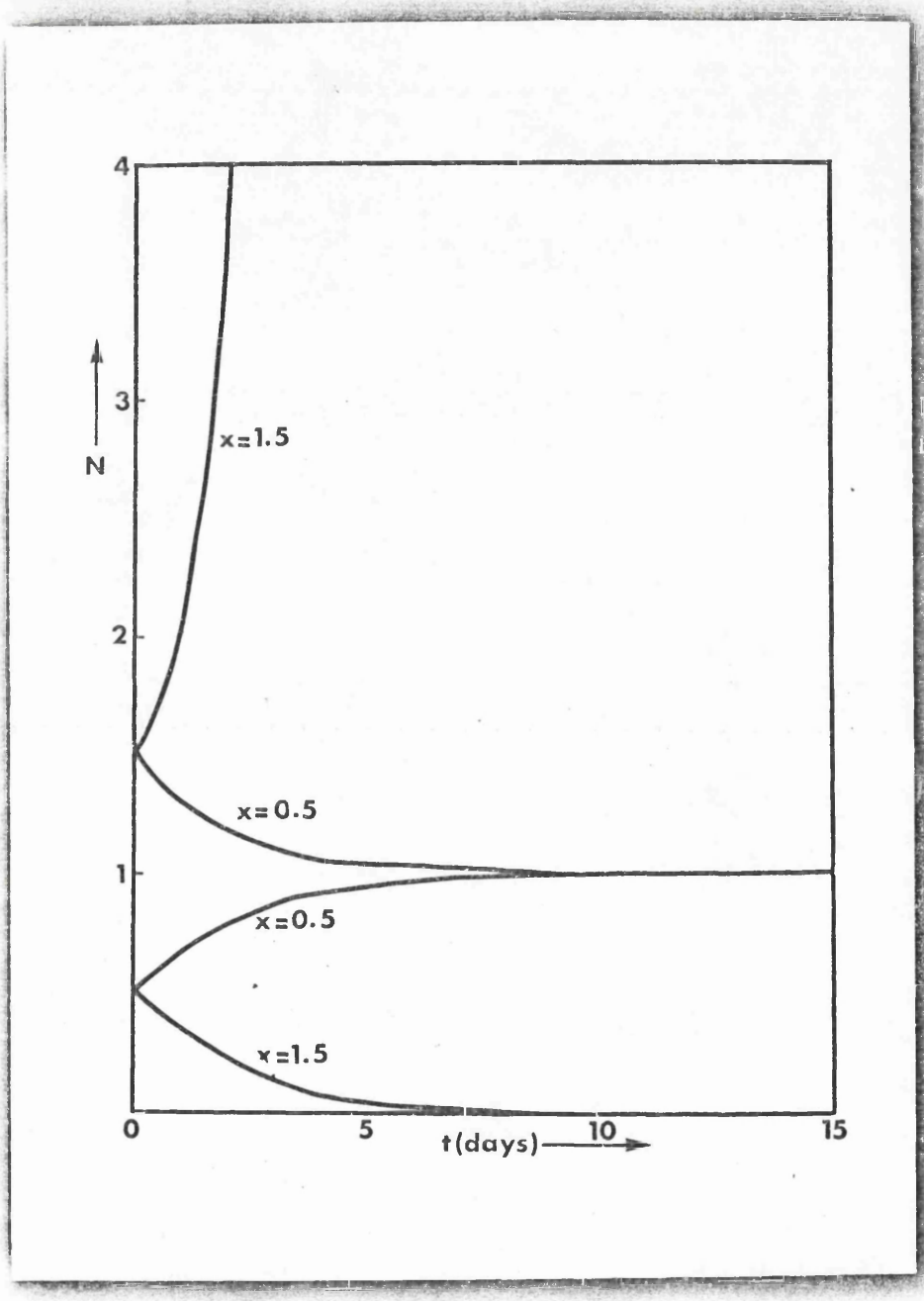

FIG. 9.1

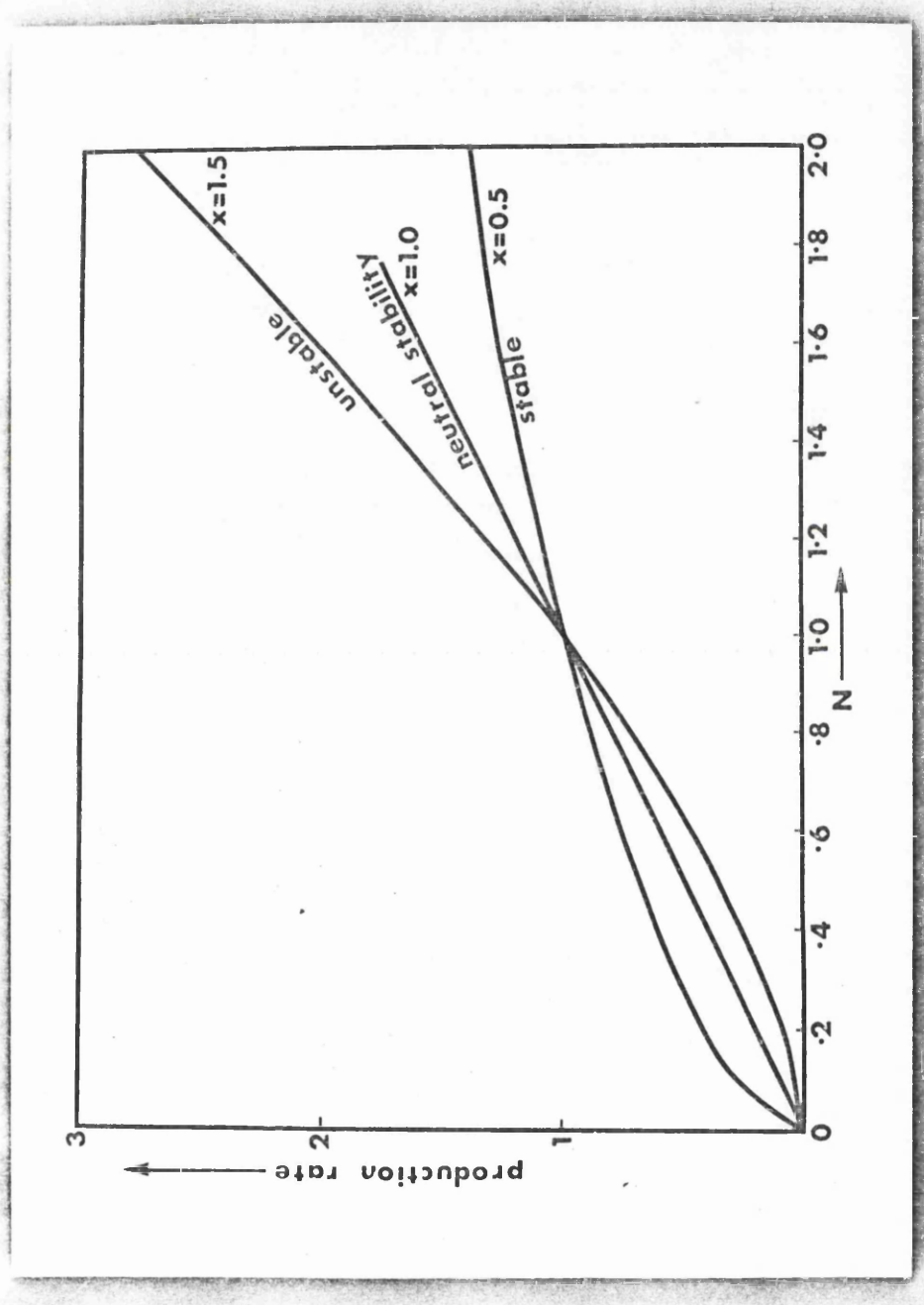
production rate
N
0
·2
·4
·6
·8
1·0
1·2
1·4
1·6
1·8
2·0
1
2
3
x=1.5
unstable
neutral stability
x=1.0
x=0.5
stable

FIG. 9.2.

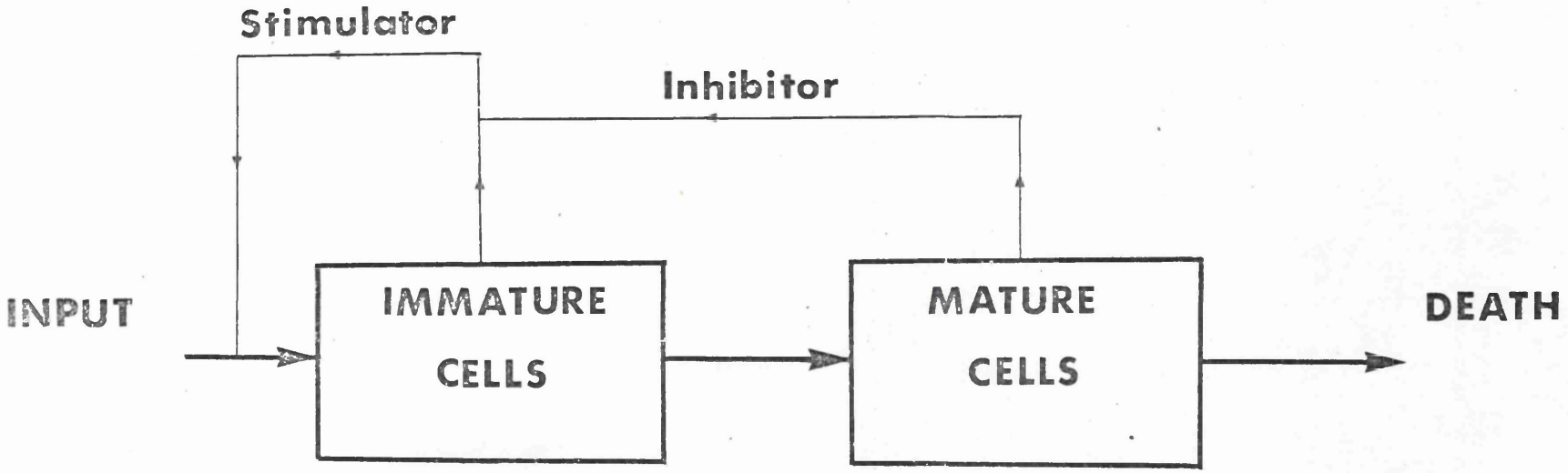
Stimulator
Inhibitor
INPUT
IMMATURE CELLS
MATURE CELLS
DEATH

FIG. 9.3

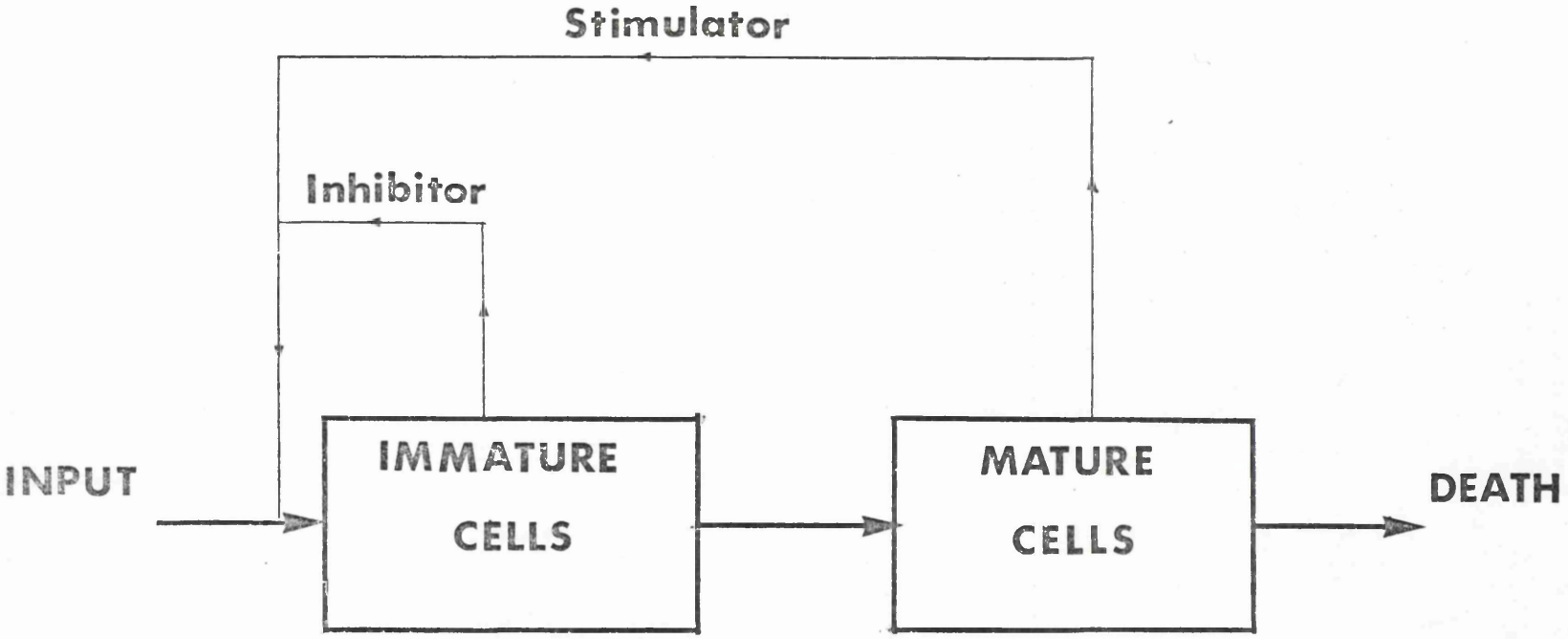
Stimulator
Inhibitor
INPUT
IMMATURE CELLS
MATURE CELLS
DEATH

FIG. 9.4

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10. SIMULATION OF PATHOLOGICAL GRANULOPOIESIS.

10. SIMULATION OF PATHOLOGICAL GRANULOPOIESIS.

Introduction.

The studies described in previous chapters permit tentative conclusions regarding the organization of granulopoiesis in normal subjects. It seems probable that the regulation of granulopoiesis involves the integrated action of a number of control loops, at least some of which are prone to oscillations and instability. The possibility therefore arises that some recognized pathologies of the control of granulopoiesis are best understood in terms of the altered operation of individual control loops, or the deranged integration of several.

Alternatively, primary pathological disturbances unrelated to the dynamic control of granulopoiesis may, as a secondary effect, so alter the operation or integration of control loops as to give rise to overt derangement of control. In either case, it is possible that simulation studies may illuminate some aspects of the pathology of granulopoiesis.

The studies previously described have left a number of problems unresolved. In particular, the studies described in chapters 8 and 9 provide indirect evidence for heterogeneity of the stem cell population and for the need for inhibitory as well as stimulatory controls to enable granulopoiesis to be adaptively regulated with functional demand. Both the nature of presumed heterogeneity and the nature of presumed loops responsible for adaptive regulation remain uncertain.

In chapter 6, however, preference was established for a two-loop model which seems a not unreasonable 'first-order'

representation of the homeostatic control of granulocyte numbers. It may be appropriate therefore to consider the minimal changes to this model necessary for the representation of certain pathological modes of granulopoiesis, bearing in mind that the model can hardly fail to be a gross simplification of the real system.

Pathologies of the control of granulopoiesis can take many forms. In this chapter attention is focussed on disorders of granulopoiesis in which cyclical behaviour is apparent.

Cyclicity, whether spontaneous or induced experimentally is a phenomenon which may provide information pertinent to the temporal characteristics and mode of organization of the governing control system (1). In the case of granulopoiesis, cyclical neutropenia, and the cyclical form of chronic granulocytic leukaemia, are disorders which may be amenable to study from this point of view.

A Two-Loop Model for the Control of Granulopoiesis.

The model to be considered here is essentially that of chapter 6. This model incorporates two control loops (fig. 10,1) and is described by the delay-differential equations

$$\frac{dG_m}{dt} = \frac{\alpha}{1+\beta\,[G_m]^{x}_{t-\tau}} - \frac{\lambda G_m}{1+\mu\,[G_B]^{y}} \qquad (10.1)$$

$$\frac{dG_B}{dt} = \frac{\lambda G_m}{1+\mu\,[G_B]^{y}} - \omega G_B \qquad (10.2)$$

where G_m, G_B are the marrow and blood granulocyte populations respectively and α, β, λ, μ, x, y, ω, τ are all parameters.

In chapter 6, interest was focussed on stability, with little interest in a model once it was known to be unstable. In this chapter, it is necessary to consider particular modes of instability, so that unstable models are interesting as well as stable ones. This means that analogue simulation techniques are not suitable in the present case because, once instability occurs, 'analogue overload' is induced and the analogue representation of the form of the instability is likely to be distorted.

For present purposes, equations (10.1) and (10.2) were numerically integrated by digital means using a FORTRAN procedure adapted to solve time-delayed equations by Mr. A. Griffiths of Y-Ard (Glasgow). A 0.1 day integration step was employed and the equations solved on the Y-ARD PDP-15 digital computer.

In initially choosing a set of parameters, it was possible

to draw on the experience gained from the analogue simulation studies. This offset one disadvantage of digital computation - the relative slowness of extensive parametric searching - by enabling the desired parametric changes to be specified inadvance (i.e. on-line parametric variation was necessary).

After a few preliminary computer runs, a set of parameters was chosen to represent normal granulopoiesis (table 10.1). For technical reasons connected with scaling of display facilities, it was found convenient to slightly alter the parameters from the values used in the studies described in chapter 6. The changes are much too small to be of physiological significance. Some derived quantities of physiological interest are given in table 10.2 and the response of the model to a mild perturbation is shown in fig. 10.2. With the parameter values specified in table 10.1, a 70 kg. man with blood volume of 5 litres and one half of all blood granulocytes marginated rather than circulating, a blood granulocyte concentration of 7.7×10^3 cells / m.m. is the steady-state value. This is well within the norman physiological range, and quite close to the mean (2).

Dependence of System Behaviour on Value of Time-Delay.

With all other parameters as in table 10.1, the obligatory maturation delay τ was altered ans its effect on model response to a mild perturbation examined. The results of this study proved to be unambiguous : increasing increased the overshoot, reduced damping, increased the period of the oscillation and - with sufficient increase - induced divergent oscillations and model instability. Reducing the time-delay did the opposite in all cases.

The results of increasing the delay from 7 days, as in fig. 10.2, to 10 days, 14 days and 20 days are shown in figs. 10.3, 10.4 and 10.5 respectively. With increasing τ, the oscillation period increases monotonically, but not necessarily uniformly, as the period also depends on marrow transit time which is different with different phases of the oscillation.

With an abnormally large value of τ (10 days) the qualitative effect on the oscillation of other parameters was examined over a range larger than had been convenient with the analogue simulation. With one exception, all noted effects were exactly as given in chapter 6. The exception was the parameter α ; increasing this parameter over a wide range produced improving stability (as expected), but decreasing it showed a qualitative reversal of effect at a critical threshold.

As α was reduced from 1.0×10^{10} to around 2.5×10^{9}, the stability worsened, with increasing divergence of oscillation - as indicated by table 6.11. Thereafter, the

effect reversed and stability improved with further decreases in α . Although such behaviour was not observed with any other parameter (in the ranges examined) it emphasizes the danger of drawing far-reaching conclusions from a restricted number of computer runs in restricted parametric ranges.

The de-stabilizing effect of increasing τ , and stabilizing effect of reducing it, appeared however, to be consistent over a sufficiently wide range to include all physiologically important conditions.

Pathogenesis of Cyclical Neutropenia.

From the studies described in chapter 6 and in the present chapter it is possible to identify several possible causes of oscillatory granulopoiesis.

Since the primary loop controlling stem cell differentiation is prone to oscillation and is stabilized by the action of the loop regulating marrow transit, increasing the gain of the primary loop (increasing α), increasing the gain of the secondary loop (increasing γ) or 'decoupling' the one loop from the other (decreasing μ) can induce sustained oscillations, or even instability. Likewise, increasing the time-delay τ or reducing the intrinsic damping of the primary loop (reducing β) will tend to induce oscillatory or unstable behaviour.

In assessing the probability of any one of the above factors being causally implicated in the pathogenesis of cyclical neutropenia, two features of this disorder seem particularly noteworthy :

(a) In cyclical neutropenia, neutropenia is particularly evident at the nadir of each cycle, but is usually low at the peak as well, giving a reduced mean level overall.

(b) The period of the cycle is usually normal.

These features suggest that granulopoiesis is reduced overall in cyclical neutropenia and that maturation is basically normal. Attention is therefore directed to reduced granulopoiesis, either through low production or high death.

Reduced production (reduced α) has already been mentioned above ; its effect seems to depend on the amount

of reduction which occurs. However, with the parameters chosen to represent normal granulopoiesis on the present model, up to a four-fold reduction in granulopoiesis ($1.0 \times 10^{10} < \alpha < 2.5 \times 10^{9}$) consistently decreased stability. Thereafter, oscillations tended to damp down, as the level of granulopoiesis fell still lower. This suggests that a mild neutropenia resulting from reduced production is likely to be oscillatory, but a severe neutropenia is unlikely to be oscillatory.

Increasing cell death rate (increasing ω) consistently reduced stability over the range considered. This is illustrated in figs. 10.6 and 10.7 which show the model behaviour with two-fold and (about) eight-fold increases in the cell death rate ($\omega = 5.00$ and $\omega = 20.00$ respectively). (It should be noted however that this model does not incorporate any of the adaptive mechanisms discussed in chapter 9, which might complicate this conclusion).

On the evidence of the present studies, cyclical neutropenia is likely to be a variant of any mild chronic neutropenia resulting from decreased production (shortage of stem cells, C.S.F., death in maturation?) or increased cell death (autoimmune neutropenia, production of defective cells?).

This conclusion is in broad agreement with that reached by King-Smith and Morley (3) in similar studies to those described here. While much remains uncertain, cyclical neutropenia need no longer be considered a mysterious syndrome but rather a not unlikely accompaniment of neutropenias arising in a variety of ways.

Simulation of Cyclical Granulopoiesis in Myeloid Leukaemia.

As described in chapter 5, cyclical granulopoiesis has been reported to occur in some cases of chronic granulocytic leukaemia, an observation which may bear on the temporal organization of granulopoiesis in this disease.
Interpretation of such phenomena depends on the acceptance of a particular model. For example, a linear analysis leads to the suggestion that some biological equivalent of 'negative friction' (i.e. a forcing term) should be sought (4). However, the almost certainly non-linear character of the granulopoietic control system, and the presence of the obligatory time-delay, make linear analysis inappropriate for this problem (5).
Instead, the matter is best considered using computer simulation of a mathematical model.
The oscillation in chronic granulocytic leukaemia differs from that in normal granulopoiesis or cyclical neutropenia in two major respects :
(a) The period of the oscillation is considerably greater than 20 days in all known cases - 40-50 days being common.
(b) The mean level of the blood neutrophil count rises with time unless therapy is instituted.
The first of these observations strongly suggests that the granulocytic maturation time, and hence the time-delay, is substantially increased in C.G.L. (3,5). To investigate this possibility, all parameters of the present model were set to the values given in table 10.1 and the time-delay increased until instability occurred, at about a value of 17 days

for τ .

With τ = 20 days, overt instability is seen, and takes the form of a divergent oscillation with a period of rather more than 40 days (fig. 10).

However, several other features of C.G.L. are not accounted for by delayed maturation alone.

(a) The divergent oscillation seen in cyclical C.G.L. has a distinct tendency to 'climb away' from the x-axis, rather as though a divergent oscillation were superimposed on a rising base-line.

(b) The clonal domination of Ph' positive cells in C.G.L. is unexplained. This anomaly, which occurs in erythroid as well as granulocytic cells is usually considered evidence for the selective advantage of a leukaemic pluripotential stem cell.

(c) Increased stem cell → mature cell amplification, due to delayed maturation, could account for the granulocytosis of C.G.L. Evidence suggests however that the amplification factor is decreased rather than increased (6).

The simplest explanation of each of these features is that the C.G.L. involves an overgrowth of a stem cell population.

To simulate this, τ was restored to its previous value (7 days) and the parameter α was replaced by the quantity $\alpha . (1 + k e^{mt})$ to represent exponential growth of the stem cell population. With $\alpha = 1.0$, $k = 0.1$, and $m = 0.03$, the model behaves as shown in fig. 10.8 .

A mild oscillatory tendency is seen, but this is of normal period and not divergent.

Seemingly, both a growing stem cell population and a delayed maturation in the granulocytic pathway are indicated.

With τ = 20 days, $\alpha \to \alpha(1 + k e^{mt})$, $\alpha = 1.0$, $k = 0.1$ and $m = 0.03$ as before, the model exhibits the rising divergent oscillation seen in fig. 10.9 . At least ~~quantitatively~~ qualitatively, this does now resemble the oscillation seen in the cyclical form of C.G.L.

Discussion.

Cyclical C.G.L. is a recently discovered syndrome, and it is not yet possible to assess its frequency of occurrence amongst all cases of C.G.L. Even if rare, however, a syndrome which may illuminate the pathogenesis of any form of leukaemia deserves consideration.

On the basis of the simulations described above, two separate changes appear necessary to the model for normal granulopoiesis in order that the cyclical form of C.G.L. be represented. Apparently, continuous growth of the stem cell population, and delayed maturation of leukaemic granulocytes, must be introduced. If this conclusion is accepted, it seems natural to enquire whether the association of stem cell overgrowth with delayed maturation of granulocytes is causal or fortuitous.

If the extended period of cyclical C.G.L. is an incidental rather than essential accompaniment of leukaemogenesis, at least some cases of cyclical C.G.L. should exhibit normal periodicity. Conversely, some cases of cyclical neutropenia should result from an extended time-delay and exhibit an extended period. Neither of these possibilities has been reported to occur.

A non-fortuitous association might therefore be expected, and could arise in a variety of ways, not necessarily causal. For example, the Ph' deletion presumably involves a number of different genes. Association of functionally independent events could result from the spatial proximity on a chromosome of deleted genes. Such a mechanism shifts

attention to the regulation of chromosome conservation and duplication.

An alternative mechanism, directly arising from the preceding considerations on C.G.L., leads to a theory of the pathogenesis of C.G.L. which may (in principle) be applicable to other varieties of neoplasia. Although speculative, this theory does not appear to have been proposed previously and it is suggested that it deserves consideration.

The basic hypothesis is that the initial stages of cellular maturation following induction of differentiation are comparatively labile, stability being acquired during maturation. This permits the reversion of early cells to the stem cell pool and can provide a two-way link between defects affecting a given channel of development and the pluripotential pool.

Stability of Differentiation in Developing Cells.

As reviewed in chapter 2, there exists a body of evidence that pluripotential stem cells differentiate in a particular developmental pathway via an intermediate unipotential or 'committed' stem cell. Stem cell differentiation appears to be akin to embryonic induction and it is appropriate to take account of evidence in this field.

From a review of embryonic induction in amphibia Jacobson (7) concluded that "Any group of cells may respond simultaneously to several specific inductors, the organ that emerges depending on which sort of inductor first has sufficient cummulative effects.....Late in the sequence, the responding cells become more firmly committed to a particular course of differentiation". In some cases, it is possible to demonstrate experimentally that progress toward formation of one organ need not interfere with progress toward formation of another organ from the same cells, until a critical stage is reached (7-9).

This evidence, together with that previously quoted, gives rise to the possibility that stem cell commitment is preceded by a phase of potentially reversible maturation during which reversion to the original (pluripotential) condition may still occur.

This possibility is consistent with recent 'multi-hit' models of the induction of cytodifferentiation. In particular, Okunewick's model (10) of erythroid development postulates two separate inductive events, each mediated by erythropoietin. The possibility of reversion to the

pluripotential pool if the second inductive event does not follow sufficiently rapidly on the first is a feature of the model which can explain the ability of 'pure' erythroid nodules to generate granulocytic progeny and vice-versa.

A multi-hit model is also favoured by theoretical considerations of the advantage of having a single molecular species activating or repressing separate genes or groups of genes (11). Reversion of cells which have received inadequate induction for commitment again arises as a possibility.

Finally, recent radiobiological evidence suggests that the transformation of a pluripotential to a committed stem cell is a temporally protracted process which involves maturation and cell division (12). Taken in total, the cited evidence is consistent with the conceptual model of induction of granulocyte development shown in fig. 10.10.

Kinetics of Stem Cell Induction and Reversion.

Assuming the stem cell induction of fig. 10.10, the mathematical formulation depends on explicit assumptions regarding the mechanism of reversion of maturing stem cells. We now consider two simple models of this mechanism.

(a) Random Mechanism.

Suppose that maturing stem cells have constant probability of reversion per unit time until a critical point in their maturation (commitment) beyond which this probability is zero.

Let $\xi(t)$ be the instantaneous induction rate of stem cells to differentiate in some given pathway, $N(t)$ the number of maturing stem cells in the reversible phase of maturation, τ the transit time of the reversible phase, αN the proliferation rate and βN the reversion rate, with α and β positive constants.

Then,

$$\frac{dN}{dt} = \xi(t) + (\alpha-\beta)N(t) - e^{(\alpha-\beta)\tau}\xi(t-\tau) \qquad (10.3)$$

or

$$N(t) = \int_0^{\tau} e^{(\alpha-\beta)\psi}\xi(t-\psi)\,d\psi \qquad (10.4)$$

If $R(t)$ is the instantaneous reversion rate and if a random reversion mechanism is postulated,

$$R(t) = \beta\int_0^{\tau} e^{(\alpha-\beta)\psi}\xi(t-\psi)\,d\psi \qquad (10.5)$$

For a constant induction rate ξ_0 we have the steady-state solutions :

$$N_0 = \frac{\xi_0}{\alpha-\beta} \cdot \left[e^{(\alpha-\beta)\tau} - 1 \right] \qquad (10.6)$$

$$R_0 = \frac{\beta \xi_0}{\alpha-\beta} \left[e^{(\alpha-\beta)\tau} - 1 \right] \qquad (10.7)$$

For each stem cell induction therefore,

No. of committed cells = $e^{(\alpha-\beta)\tau}$ (10.8)

No. of reverted cells = $\frac{\beta}{\alpha-\beta} \cdot \left[e^{(\alpha-\beta)\tau} - 1 \right]$ (10.9)

(b) Non-Random Model.

An alternative model results from supposing that maturing stem cells all complete the earliest phase of maturation until a critical commitment step is scheduled, which only a fraction f of the cells take successfully.

The number of maturing stem cells preceding the critical step is therefore unaltered by the existence of reversion.

Thus,

$$N(t) = \int_0^\tau e^{\alpha\psi} \xi(t-\psi)\, d\psi \qquad (10.10)$$

while

$$R(t) = (1-f)\, e^{\alpha\tau} \xi(t-\tau) \qquad (10.11)$$

Then, for each stem cell induction,

No. of committed cells = $f\, e^{\alpha\tau}$ (10.12)

No. of reverted cells = $(1-f)\, e^{\alpha\tau}$ (10.13)

A Theory of the Pathogenesis of Myeloid Leukaemia.

A theory of the pathogenesis (at least the cyclic form of) of C.G.L. may now stated. Suppose that the single intrinsic defect of a leukaemic cell is a quantitative reduction in its rate of maturation between the pluripotential and granulocytic unipotential stem cell stages of development. Hence the maturation time is increased, with extended period of any observed granulopoietic cycling. However, the reversion rate and number of reverted cells per induced cells also depend positively on τ .

Let us consider how increased τ could result in the number of reverted cells per unit cell exceeding unity.

On the random model, equation (10.9) gives the necessary condition to be

$$\frac{\beta}{\alpha-\beta}\left[e^{(\alpha-\beta)\tau}-1\right] > 1 \qquad (10.14)$$

or

$$\tau > \frac{1}{\alpha-\beta}\ln\left(1+\frac{\alpha}{\beta}\right) \qquad (10.15)$$

while, on the non-random model, the condition is given by (10.13) as

$$(1-f)\,e^{\alpha\tau} > 1 \qquad (10.16)$$

or

$$\tau > \frac{1}{\alpha}\ln\left(\frac{1}{1-f}\right) \qquad (10.17)$$

The relationships (10.15) and (10.17) show that with both models, a critical value of τ exists beyond which induction of differentiation constitutes a source of gain rather than of loss of pluripotential stem cells.

Recalling the parametric sensitivity of the model for stem

cell homeostasis which was developed in chapter 7, it is not difficult to see that increasing τ could have the effect of de-stabilizing the stem cell population control. Of course, total de-stabilization would be a relatively late development. Initially, the clone arising from the original deviant would gradually accumulate over normal clones, due to the increased reversion from induction. Eventually, however, continuous stem cell growth, of a pluripotential population consisting mainly of daughters of the primary deviant, might come about.

With such a theory, a causal association between a protracted duration of maturation and the accumulation and steady growth of deviant stem cells, becomes comprehensible. Moreover, since responsiveness of the deviant cells to mitotic homeostatic control is presumed normal, the 'cybernetic' responsiveness of leukaemic cells to population depletion is explicable (see chapter 3, refs. 18 - 22).

Much of the above rests on the proposition that the cycle length seen in cyclic C.G.L. reflects a delayed maturation of leukaemic granulocytes. This is by no means the only possible explanation. For example, in linear oscillation theory, two superimposed oscillations can generate a 'beat' oscillation whose frequency is that of the difference between the primary oscillations. Non-linear oscillations are more complex, but similar phenomena occur.

If oscillatory processes occur in adjacent channels (e.g. granulopoiesis and erythropoiesis) which share a pluripotential stem cell pool, a small difference between the

periods of the two processes could be manifested as a 'beat' oscillation of large period. Assessment of such a possibility would require a complex model representing several channels of cell production and their mutual coupling.

At present, the interpretations presented above seem to provide the simplest and most direct explanations of a class of kinetic phenomena in myeloid leukaemia for which no explanations at all have previously been available.

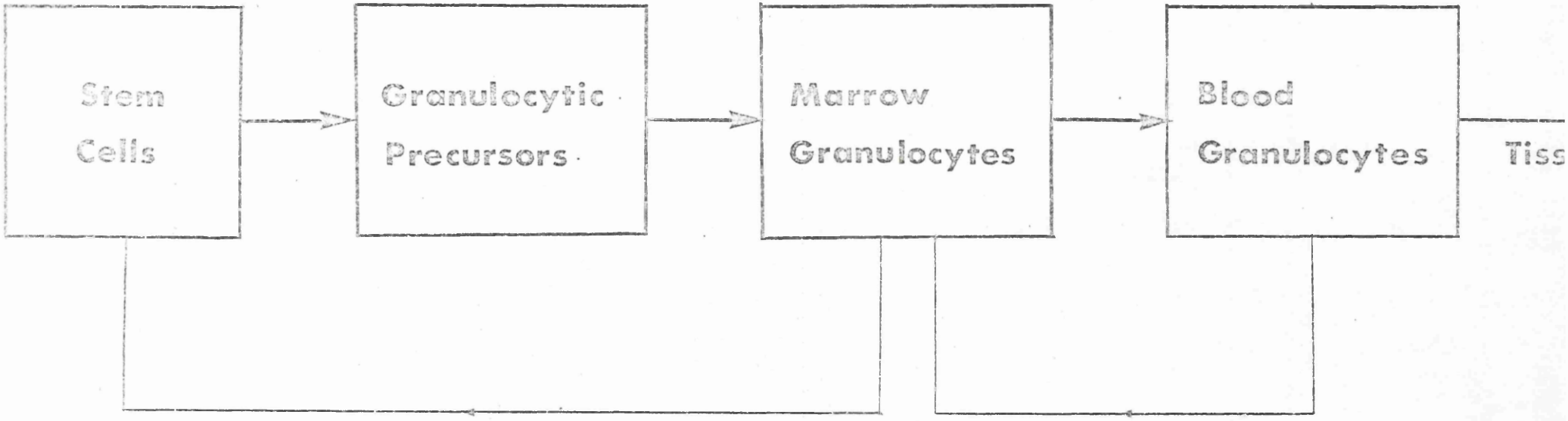

FIG. 10.1 TWO-LOOP MODEL

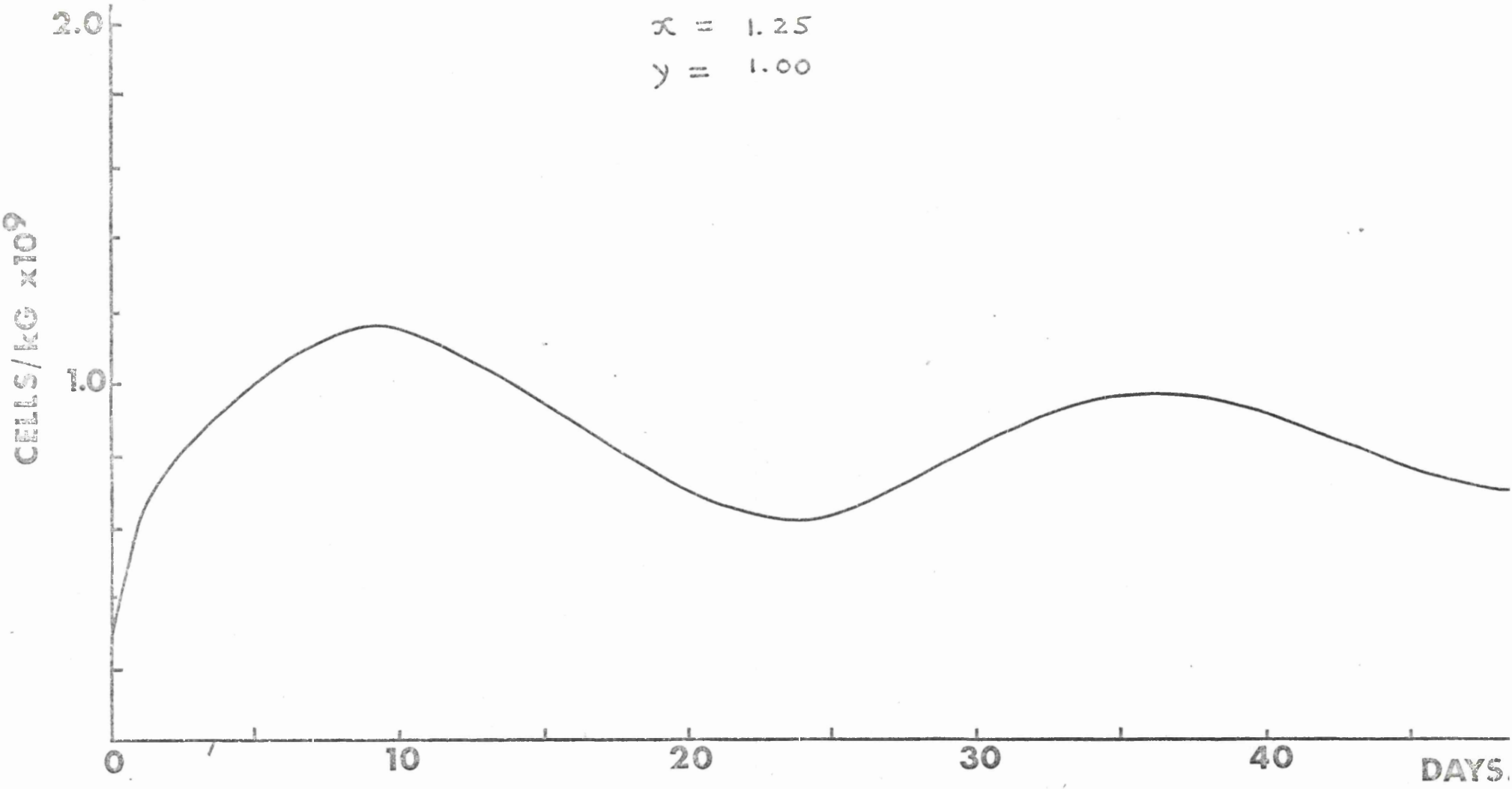

FIG. 10.2

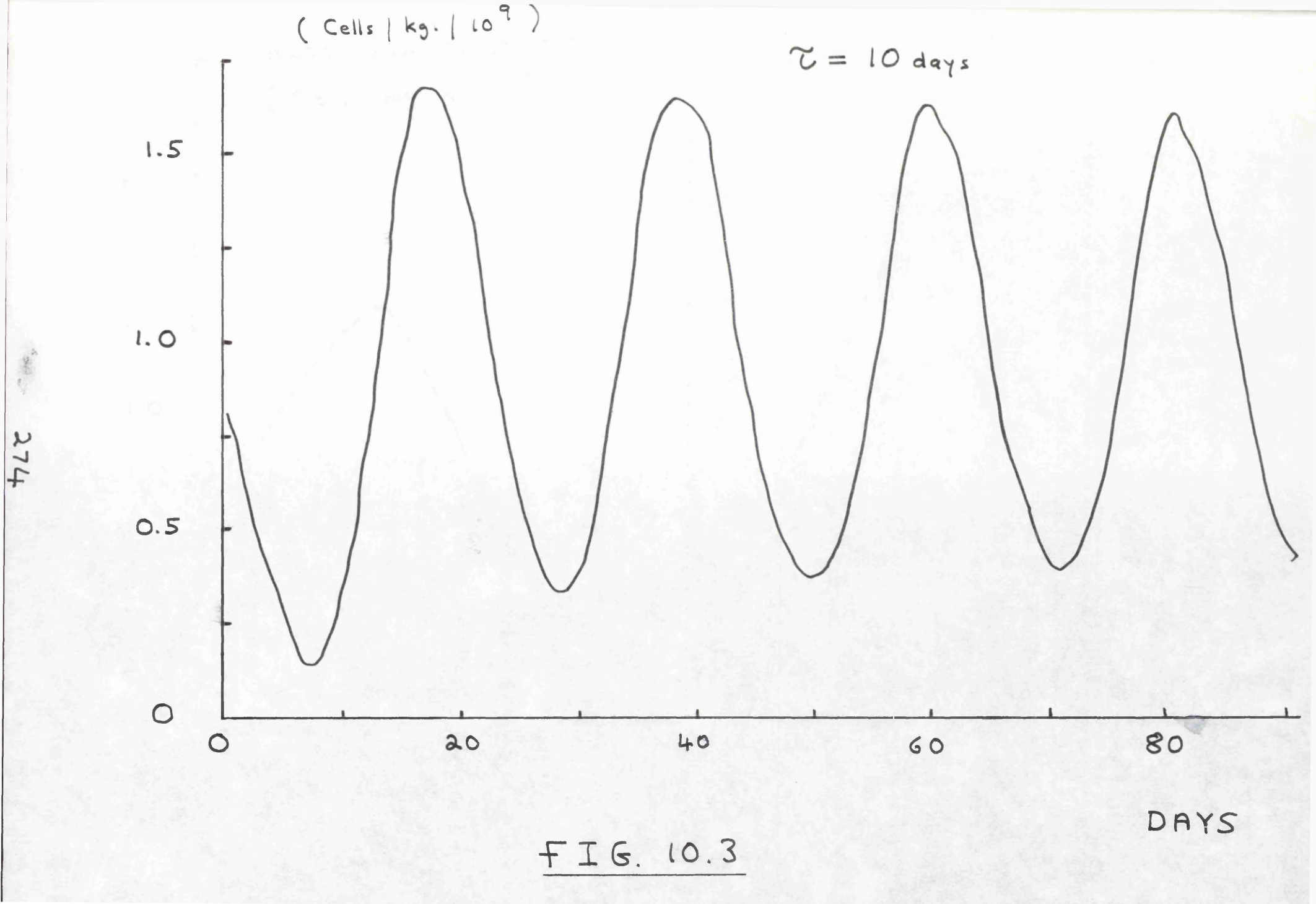

FIG. 10.3

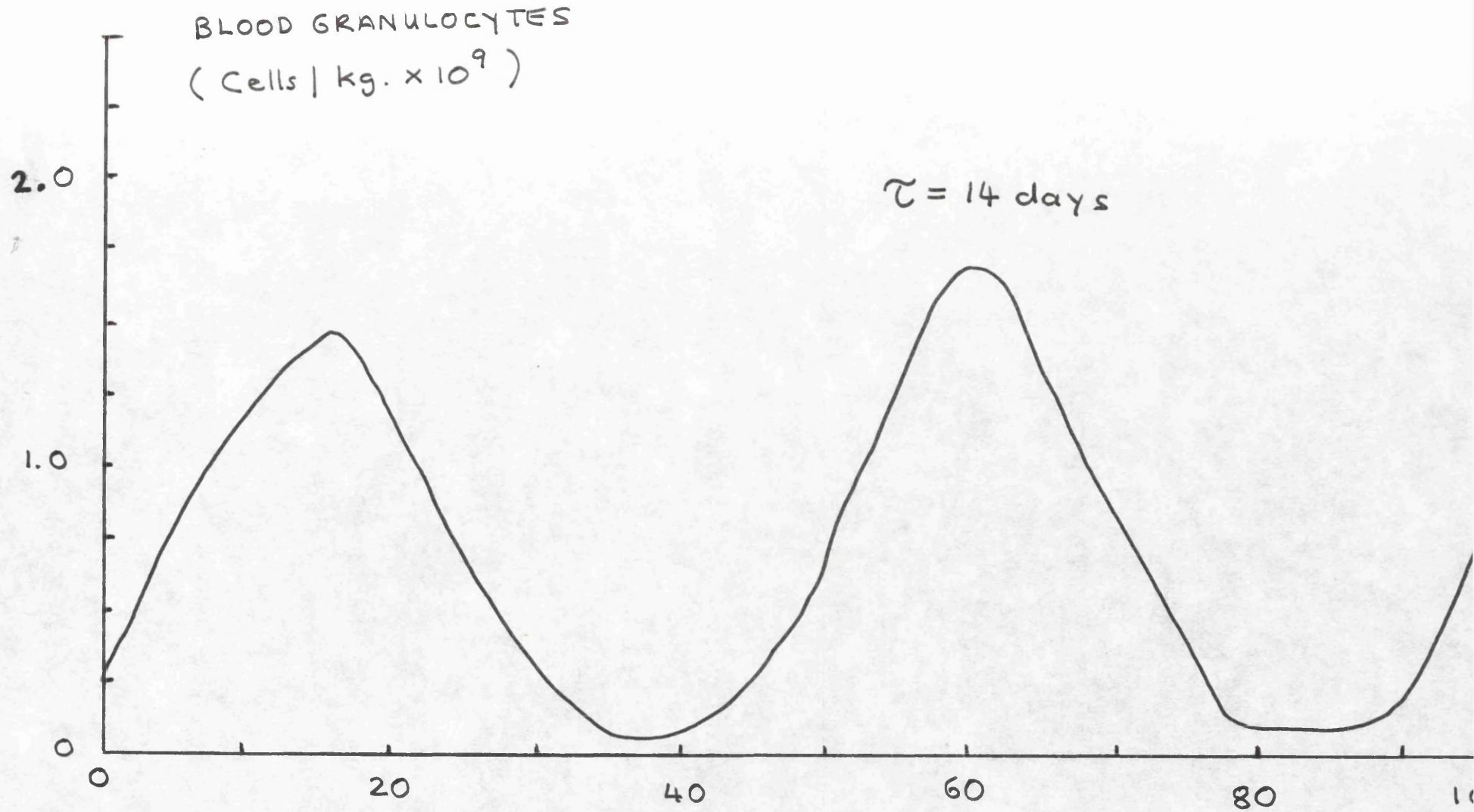
BLOOD GRANULOCYTES
(Cells | kg. x 10^9)
2.0
1.0
0
τ = 14 days
0
20
40
60
80
DAYS

FIG. 10.4

$\tau = 20$ days

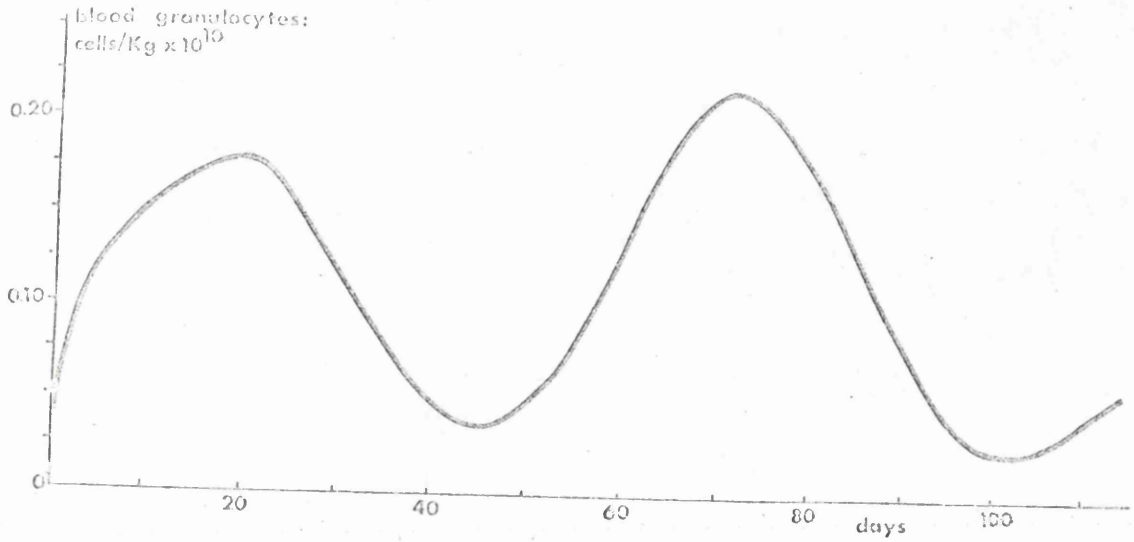

FIG. 10.5

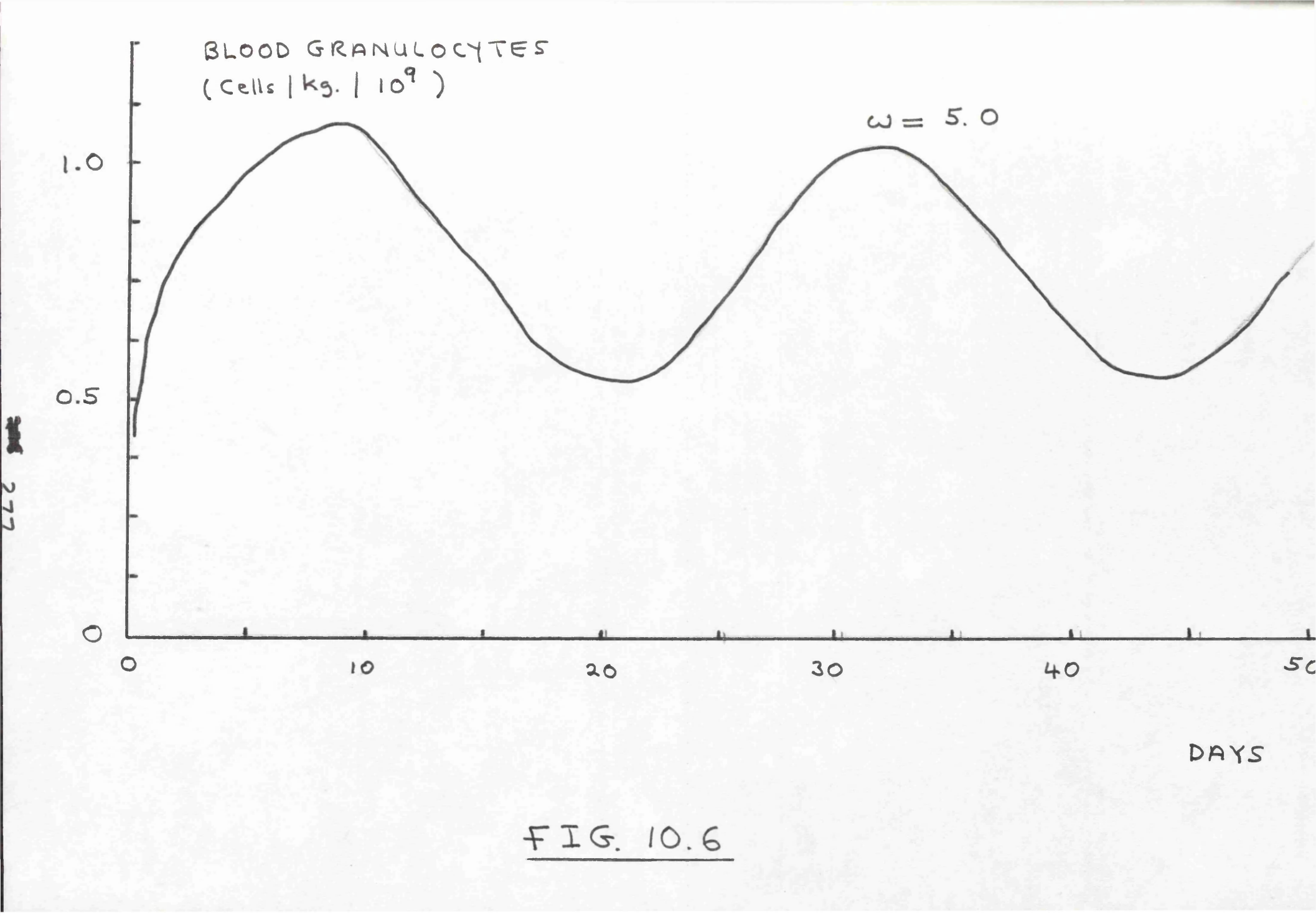
BLOOD GRANULOCYTES
(Cells | kg. | 10^9)
$\omega = 5.0$
1.0
0.5
0
0
10
20
30
40
50
DAYS

FIG. 10.6

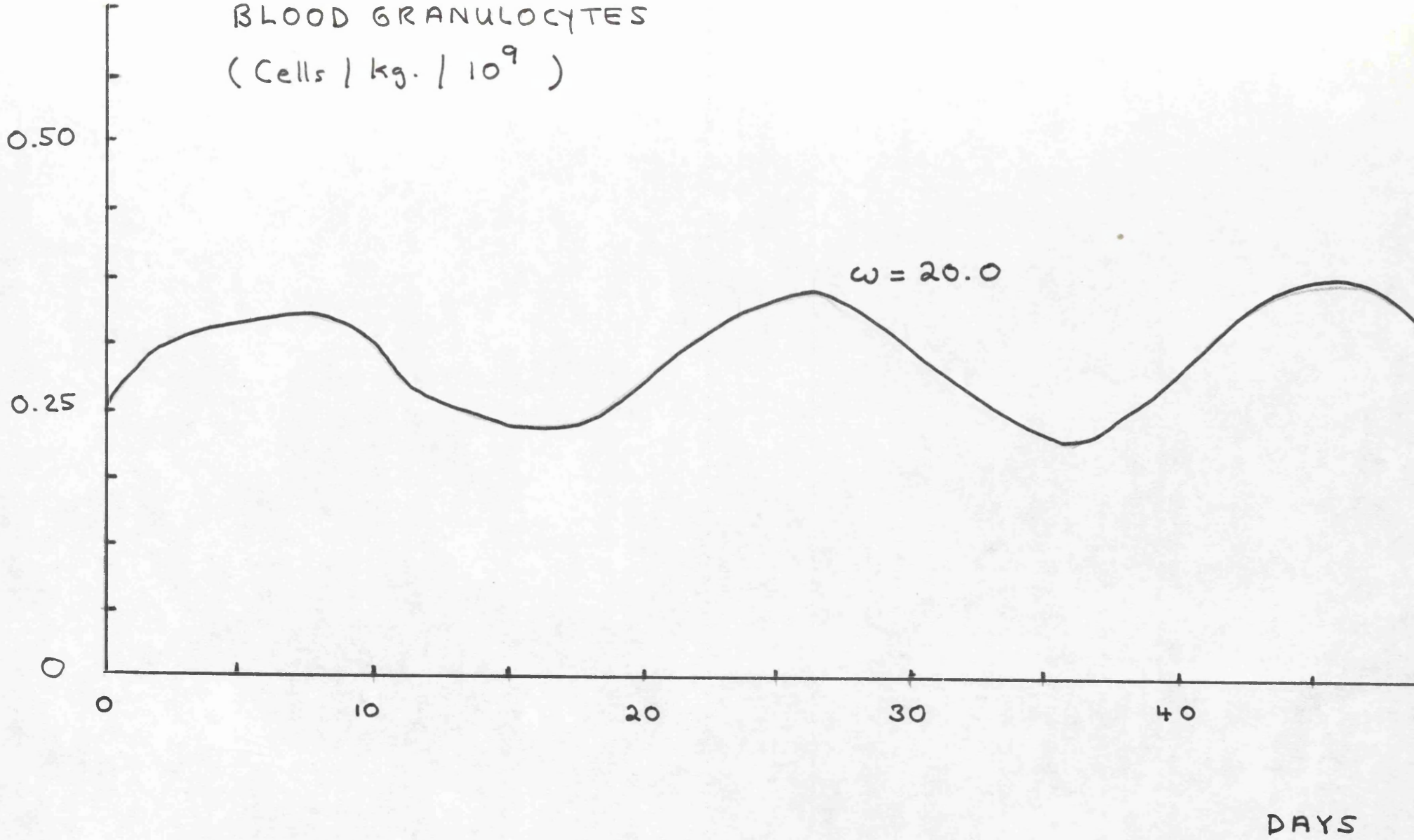
BLOOD GRANULOCYTES
(Cells / kg. / 10^9)
0.50
0.25
0
ω = 20.0
0
10
20
30
40
DAYS

FIG. 10.7

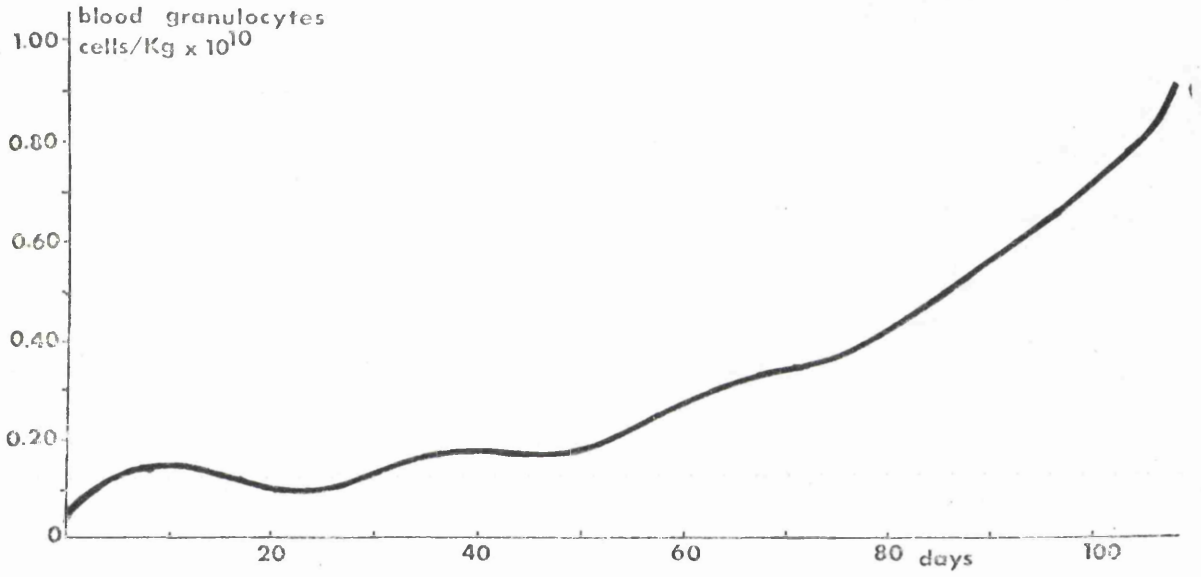
blood granulocytes
cells/Kg x 10^{10}
1.00
0.80
0.60
0.40
0.20
0
20
40
60
80
days
100

FIG. 10.8

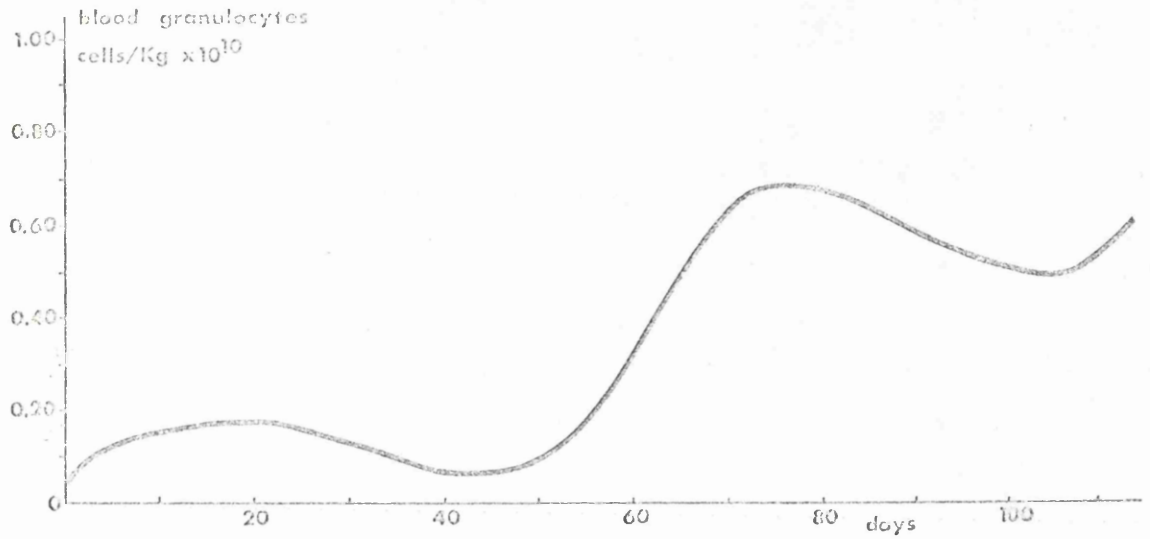
blood granulocytes
cells/Kg x10^10
1.00
0.80
0.60
0.40
0.20
0
20
40
60
80
days
100

FIG. 10.9

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CONCLUSIONS.

The studies described constitute a preliminary attempt towards the conceptual unification of the major known facets of granulocyte production. This unification remains to be accomplished.

Nonetheless, recognition of small 'islands' of unification may precede the emergence of a coherent picture of the whole. The attempt to discern some such 'islands' has proved informative.

Granulopoiesis appears to be regulated by mechanisms belonging to a class of systems which are potentially unstable. The main source of instability is the time-delay representing obligatory maturation of granulocytic precursors. Relatively low gains for the main feedback loops therefore seem to be indicated (chapter 6).

There seems little doubt that at least one class of bone marrow stem cells displays mitotic homeostasis. Consideration of this form of control cannot in general be divorced from consideration of pattern formation and organ topology. In the particular case of stem cells, where a partial separation of these concepts may be possible, a simple theory of mitotic homeostasis leads to consideration of a further class of instabilities which may apply to mitotic control systems of more general kinds (chapter 7).

In reality, mechanisms regulating granulocyte production must co-exist harmoniously with those responsible for mitotic homeostasis of bone marrow stem cells. The model studies, on the other hand, suggest the existence of

incongruity between these two classes of mechanism.
Resolution of this (apparent) paradox probably depends on a clearer understanding of stem cell heterogeneity, or possibly on a cell-cycle dependence of responsiveness to signals for differentiation (chapter 8).

A striking feature of granulopoiesis is its adaptive response to infections and endotoxins. A previously suggested explanation (Robinson-Mangalik) invoked the concept of positive-feedback (autostimulatory) regulation. Somewhat surprisingly, pure positive feedback systems are not necessarily unstable. The conditions for stability, however, appear to be such as to eliminate adaptive regulation when stability is present, and vice-versa. A more complicated system seems to be indicated (chapter 9).

Neutropenia and leukaemia represent the two sides of deranged control of granulopoiesis. Phenomenological theories of the nature of these conditions can be proposed, using the model studies to provide conceptual guidance. It seems unlikely that these theories (whether true or false) would have been conceived without the use of models of the control of granulopoiesis (chapter 10).

The inadequacies of the work presented fall into two main groups.

In the first place, the models considered are conceptually simple - perhaps naïve, contain numerous parameters of uncertain value and cannot yet provide a comprehensive representation of all known features of granulocyte production.

More seriously, however, it is illusory to suppose that any

form of controlled cellular production will prove amenable to understanding at the supracellular level.

The gravity of this asymmetry may be appreciated when it is considered that in the several discussions of neoplasia and leukaemia, no explanation was advanced for the occurrence of tumour progression. It seems unlikely that this enigmatic phenomenon will prove comprehensible at any level which takes cells or their multiples as its units.

Equally, however, the step from the knowledge of a cell to its behaviour as a unit of a tissue is not a small one. A number of difficulties arise, some of them mathematical rather than chemical or anatomical. Studies of the behaviour of interacting feedback loops using mathematical models may assist in the resolution of these difficulties.

Finally, the 'spin-off' from an investigation may sometimes turn out to be its principal justification. In the present instance, it may be hoped that the methods of calculation developed in chapter 4 will permit analysis of cellular development in a wider class of situations than hitherto considered possible.

One important application might be the analysis of patterns of maturation of human granulocytes growing in culture. The experimental techniques have been available since 1970; the mathematical techniques are presented here.

By comparing the development in culture of normal and leukaemic cells, it should be possible to determine whether leukaemic development exhibits any of the anomalies suggested in chapter 10. If so, the other questions raised will deserve consideration. If not, new information will have been obtained, on which to base new theories.

GLOSSARY.

The definitions given below are not comprehensive. They are intended only to explain the meaning of a term as it is specifically used in the foregoing dissertation.

adaptive : teleologically responsive to changed conditions.

analytic : expressible in terms of known mathematical

cybernetics : study of control processes involving information transfer.

deterministic : obeying strictly causal laws.

differentiation : conversion of cells from (relatively) unspecialized to specialized.

erythrocyte : red blood cell; involved in gas transfer.

erythropoiesis : erythrocyte production.

eukaryocyte : having a nuclear membrane (i.e. a higher cell).

genotype : genetic complement of a cell.

granulocyte : a white blood cell; involved in defence against infection.

granulopoiesis : granulocyte production.

haemopoiesis : (generic term for) blood cell production.

homeostatic : tending to preserve equilibrium against disturbances.

induction : commencement of differentiation.

'in vivo' : outside the living system (e.g. tissue culture).

'in vitro' : inside the living system (e.g. animal experiments).

limit cycle : 'steady-state' oscillation of fixed period.

macrophage : scavenger cell found in tissues.

maturation : cellular development between induction and completion of differentiation.

mitosis : cell division (in eukaryotic cells).

monocyte : blood cell, possibly macrophage precursor.

myeloid : (strictly) of marrow origin; (commonly) granulocytic.

neoplastic : 'new tissue'; used in reference to malignant tumours.

phenotype : cellular characteristics other than the genetic complement.

platelet : blood cell; involved in coagulation processes.

pluripotential : able to generate diverse progeny.

prokaryctic : lacking a nuclear membrane (i.e. bacterial cell).

stable : having a finite, non-zero, well-defined equilibrium.

stochastic : obeying probabilistic laws.

teleological : goal-directed.

thrombopoiesis : platelet production.

transcription : synthesis of RNA from a DNA template.

translation : synthesis of protein from an RNA template.