Cellular growth, injury and death

Mathematical formulation and analog computer analysis

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KURZFASSUNG: **Zellwachstum, Schädigung und Tod. Mathematische Formulierung und Analog-Computer-Analyse.** Vom Gesichtspunkt molekularer Mechanismen sind biologische Vorgänge außerordentlich komplex. In der vorliegenden Studie wird der Versuch unternommen, auf der Basis eines Modellsystems das Zusammenwirken funktioneller Grundeinheiten der Zelle zu einem System aufzuhellen. Die Flußgleichungen des Modellsystems werden aufgestellt und in die entsprechenden Differentialgleichungen transformiert. Für den Wachstumsvorgang wird unter verschiedenen Parameterbedingungen eine Analog-Computer-Analyse durchgeführt; ferner werden die Phänomene Zellschädigung und Tod analysiert. Experimentelle Studien am Computer gewähren wichtige Einblicke in die grundlegenden Wachstumsvorgänge und in die Wachstumsdesorganisation durch äußere Einwirkungen.

INTRODUCTION

Growth is a basic property of biological species, and growth coupled with the division process leads to the increase of population. Cellular growth can be considered from various points of view, for example, as it exists at the present time, thus ignoring evolutionary aspects, since evolution represents the development of new biological systems and new biological properties. The complexity of biological systems is obvious. It is not so obvious how to gain more insight into detailed mechanisms inherent in cell growth and integrated process. Collecting data from isolated experiments yields important raw material for the synthesis of more complex systems. However, detailed information obtained from experiments performed in conditions which are not equivalent to cellular environment represents only a limited type information, and such information has to be integrated into a larger framework of cellular operations. Current analysis of cellular growth processes in the literature is essentially carried out by a descriptive process. However, verbal nonquantitative analysis of nonlinear systems is not adequate. The usual intellectual reasoning process involves only a few steps of linear logic. Biological processes are essentially non-linear. Furthermore, they represent a multiple network of interactions and, consequently, application of linearity into such framework is leading to an illusion of understanding rather than a real understanding of the basic process. Since biological processes when considered at molecular level involve a large number of functional entities, the descriptive analysis of such a system has only a limited value.

In order to comprehend the basic cellular processes, it is essential that relationships between individual functional units should be analyzed as a function of time, since the growth process represents the change of absolute concentration of those entities. Therefore, it is essential that formalized relationship be established between the operational elements of the system and that the problem be solved in a quantitative manner. In order to do this it is imperative that a model-system be established. In cellular metabolic systems there are a number of similar-type elementary units which are organized into complex patterns. It is considered that an analysis of a model-system which contains the principal functional entities, such as DNA, RNA, and enzymes, may lead to an understanding of growth processes and their regulation.

The development and construction of a m o d e l - s y s t e m is not at all an easy process. It would be desirable to have a very complete model, which would help interpret many complex cellular processes. However, quantitative formulation is limited by many prohibitive factors. Consequently, the complexity of the model is limited to that level where the mathematical and computer techniques permit it to be carried out. Our model-system contains all basic functional entities which are operational in the cell, and they are organized in a framework of mutual interaction as known from the data in the literature.

In general, the basic elements of the system are: genes, messengers (RNA), templates, enzymes, repressors and activators. The model-system contains four basic functional genes which represent group-properties. This approach is justified if one considers that group-property is based on the fact that all basic functional entities are built up with a certain number of integral building blocks, and a complete number of blocks have to be there in order to build a basic functional entity. If one building block is missing, a functional unit cannot be built. Consequently, group-property will give a cross-behavior of the system. In the initial condition it is assumed that all functional entities have a relative value of unity, and as a function of time all entities will start to grow. They reach approximately double value at the end of the generation time, which has been selected as a convenient time interval in the framework of computer observation time. The growth of the model-system is initiated from initial conditions by activating the external pool. This is equivalent to taking cells and placing them in a nutrient medium and measuring their growth.

FORMULATION AND DESCRIPTION OF MODEL-SYSTEM

The terminology used in model building is represented in Table 1 and the flow schemes in Table 2. A schematic diagram of the model is presented in Figure 1.

Let us review briefly the operational characteristics of a model-system. The first functional process can be considered the complexing of messenger M with ribosome B, yielding the template N. Messenger M is produced by interaction of complex $[E_p P_n]$ with gene G_E. Enzyme synthesis results when templates interact with transport RNA and the amino acid pool complex $[C P_a]$. The total protein E contains three fractions: E_n – enzymes which convert internal pool P_i into RNA percursors; E_a – enzymes which convert internal pool P_i into amino acids; E_t – enzymes which convert external

pool P_e into internal pool P_i. Rate constants (k_n, k_a, and k_t) determine what fraction of the total protein is converted to respective enzymes. The formation of polymerase E_p follows generally the same line represented by total protein E synthesis. External

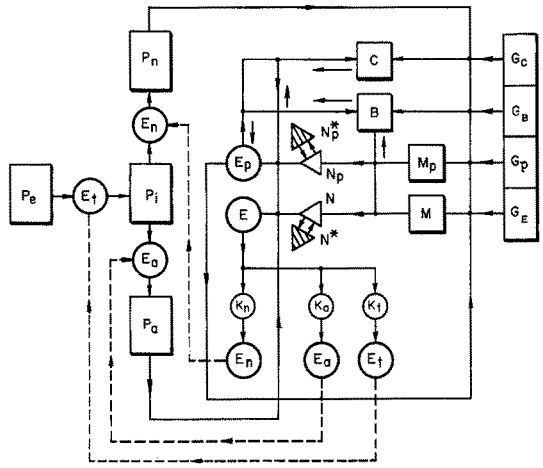

Fig. 1: A model-system for cell growth analysis. (Compare with Table 1)

Table 1

Terminology and symbols

POOLS

P_e – Extracellular nutrient pool
P_i – General intracellular metabolic pool
P_a – Amino acid pool for protein synthesis
P_n – Nucleotide pool for RNA synthesis

ENZYMES

E – Total protein
E_n – Enzymes which convert internal pool (P_i) into RNA precursors
E_a – Enzymes which convert internal pool (P_i) into amino acids
E_p – RNA polymerase for messenger RNA (M) synthesis
E_t – Enzymes which convert external pool (P_e) into internal pool (P_i)

Rate constant k_n, k_a, and k_t determine what fraction of total protein represents respective enzymes.

GENES

G_E – Genes for messenger RNA (M) synthesis
G_P – Genes for messenger RNA (M_p) synthesis
G_B – Genes for the synthesis of RNA fraction of ribosome
G_C – Genes for transport RNA (C) synthesis

MESSENGERS

M – Messenger (RNA) for protein (E) synthesis
M_p – Messenger (RNA) for E_p synthesis
B' – RNA fraction of ribosome
B – Ribosome
C – Transport RNA
N – Ribosome and messenger complex for protein (E) synthesis (template)
N_p – Ribosome and messenger complex for E_p synthesis (template)
N^* – Inactive state of N
N^*p – Inactive state of N_p
s_i – Metabolic which converts templates N and N_p into inactive state
s'_i – Metabolic which converts inactive template N^* and N^*_p into active state
$k_1 \ldots k_n$ – Various rate constants

nutrient pool P_e is converted into an internal pool P_i. In addition, part of the pool P_i leaks out from the cell via k_{30}, but the product X will not be associated with the external pool. The principal regulatory element in the system is the complexing of total protein with the internal pool (Eq. 22, Table 2). This complexing is reversible and the ratio between the two rate constants, k_{22}, k_{-22}, will determine the degree of regulation at that level. Additional regulatory features are the complexing of polymerase with

Table 2

General scheme

1. $E_p + P_n \xrightarrow{k_1} [E_p\, P_n]$
2. $B' + E \xrightarrow{k_2} B$
3. $G_B + P_n \xrightarrow{k_3} G_B + B'$
4. $G_C + P_n \xrightarrow{k_4} G_C + C$
5. $G_P + P_n \xrightarrow{k_5} G_P + M_p$
6. $G_E + [E_p\, P_n] \xrightarrow{k_6} G_E + E_p + M$
7. $B + M \xrightarrow{k_7} N$
8. $B + M_p \xrightarrow{k_8} N_p$
9. $C + P_a \xrightarrow{k_9} [C\, P_a]$
10. $N + [C\, P_a] \xrightarrow{k_{10}} B + C + M + E$
11. $N_p + [C\, P_a] \xrightarrow{k_{11}} B + M_p + C + E_p$
12. $M \xrightarrow{k_{12}} P_n$
13. $M_p \xrightarrow{k_{13}} P_n$
14. $B \xrightarrow{k_{14}} P_i$
15. $C \xrightarrow{k_{15}} P_i$
16. $E \xrightarrow{k_{16}} P_i$
17. $E_p \xrightarrow{k_{17}} P_i$
18. $P_e + E_t \xrightarrow{k'_{18}} P_i + E_t$
19. $P_i + E_n \xrightarrow{k'_{19}} P_n + E_n$
20. $P_i + E_a \xrightarrow{k'_{20}} P_a + E_a$
21. $E_p + C \underset{k_{-21}}{\overset{k_{21}}{\rightleftarrows}} [E_p\, C]$
22. $E + P_i \underset{k_{-22}}{\overset{k_{22}}{\rightleftarrows}} [E\, P_i]$
23. $E_p + B \underset{k_{-23}}{\overset{k_{23}}{\rightleftarrows}} [E_p\, B]$
24. $N_p + s_i \xrightarrow{k_{24}} N'_p$
25. $N'_p + s'_i \xrightarrow{k_{25}} N_p$
26. $N + s_i \xrightarrow{k_{26}} N'$
27. $N' + s'_i \xrightarrow{k_{27}} N$
28. $N \xrightarrow{k_{28}} P_i$
29. $N_p \xrightarrow{k_{29}} P_i$
30. $P_i \xrightarrow{k_{30}} X$

where:

$E_n = k_n E$ $\qquad$ $k_{18} = k'_{18}\, k_t$

$E_t = k_t E$ $\qquad$ $k_{19} = k'_{19}\, k_n$

$E_a = k_a E$ $\qquad$ $k_{20} = k'_{20}\, k_a$

transport RNA (Eq. 21) and the complexing of polymerase with ribosomes (Eq. 23). All functional entities have different degrees of stability, and they decay into their respective pools. The system is growing because there is an input via transport enzyme E_t from the external pool. In order to analyze cellular dormancy, two additional entities are introduced into the system. It is visualized that template N can be converted into inactive form N* and template N_p into N^*_p. This conversion can result from the action of metabolites or hormones and is considered to be reversible by activation process.

In order to analyze this type of model-system in a quantitative manner, it is essential that a systematic formulation be made. Consequently, the flow scheme in Table 2 is organized into a set of simultaneous differential equations, and these are programmed for the analog computer. This procedure is not further discussed here, since this has been analyzed in previous publications (HEINMETS 1964a, 1964b, 1966). When the program has been assembled on the analog computer, it is essential to establish a f u n c t i o n a l s y s t e m. The principal characteristics of a functional system for the cell growth can be considered to be the requirement that during the generation time all cell components have to double their initial value. Organization of this requires a great deal of patience and experience in computer technology since the model contains many entities (31 rate constants). Only after tedious and prolonged trials is it possible to develop a functional system. The principal cause of the difficulty is the fact that the system is highly nonlinear. As a matter of fact, it is a network of nonlinearities. This is of course the principal feature of the biological system, where many degrees of interdependence exist among functional entities.

Once the functional system has been established on the computer, then it is possible to carry out experiments on the model-system and observe kinetic behavior of the system on an oscilloscope screen. It is instructive to observe the results when certain rate constants of the system are changed or the initial conditions altered. Varieties of experiments of this nature can be carried out and subsequently recorded for permanent records. Thus laboratory experiments can be carried out on the computer. For example, many biological experiments are using techniques in which a certain compound is introduced into the system and perhaps removed or neutralized later. In order to carry out experiments of this nature, various electronic switches were incorporated in the program of the model-system. They were synchronized with computer solutions so that operations could be carried out at any desired time. Also, experiments of a more complex nature were designed so that two compounds could be simultaneously introduced into the system and simultaneously removed from the system. The transient introduction of various elements into the model-system enables one not only to follow the events at the site where the interaction takes place, but also to follow a sequence of events which take place throughout the system. This is in contrast to experimental procedures in biology where very few entities can be simultaneously studied because of limitations of experimental techniques. Consequently, the amount of information obtained from laboratory experiments can be more limited so far as the total system is concerned. This is especially true for the kinetic aspects of the process. On all recordings the initial value for the functional entity is indicated by 1, and 0 indicates

that the functional entity has disappeared from the system. Value 2 indicates that the particular entity has doubled.

ANALYSIS OF GROWTH PROCESS AS A FUNCTION OF TIME

A computer solution is obtained for the cell growth when the model-system is made operational in the presence of external pool P_e. Since all basic functional entities have the initial value of unity, then the growth will be initiated from that level. In order for the system to grow, it is essential that there should be an in-flow of pool P_e

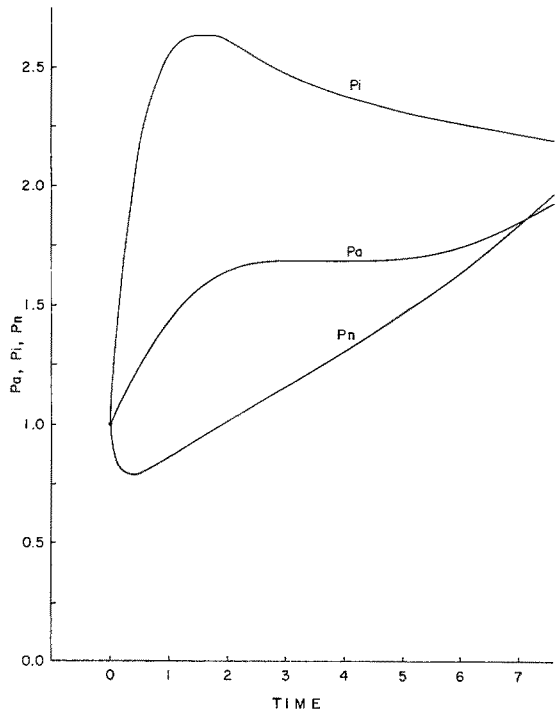

Fig. 2: Concentration of pools P_i, P_a and P_n during growth

via transport enzymes E_t. Rate constant k_{18} value has been so selected that during the generation time all cell components approximately double the initial value. The growth of the model-system when it starts from the initial values is in an arbitrary state. In the initial phase there are formations of various complexes and adjustment of equilibrium at various steps. Only after the initial transient phase has passed can we consider the normal growth conditions established and the system to be suitable for kinetic analysis. In order to minimize the initial transients, one has to assign initial values for complexes (see Eqs. 1, 9, 20, 21, 22, Table 2). These were determined empirically for

conditions where there are only small initial transients present. Figure 2 shows the growth of three pools, P_i, P_a, and P_n. Pool P_i increases rapidly up to a maximum value and then decreases gradually. When observation time is extended beyond generation time, this curve will flatten out and gradually start to rise again. Pool P_a after the initial transient will also start to grow, finally reaching a plateau. It is obvious that the amino acid pool and general internal pool have quite different growth characteristics. In contrast, the nucleotide pool P_n will pass through a steep reduction phase before it will start to grow. This of course can be expected since the first step of synthesis will be utilization of P_n by genes, and this causes a rapid initial loss of this pool. In general, processes are so complex that no attempt is to be made here to analyze the curve forms specifically. The formation of messenger M, template N, enzyme E, and ribosome B all have different kinetic characteristics (not presented here). However, finally all entities start to grow and reach approximately the same value at the same generation time. Obviously this system would grow and explode if cellular division did not take place. Since we are considering at the present only growth, no attempt is to be made to analyze the growth beyond generation time.

THE EFFECTS OF INTERNAL POOLS ON THE GROWTH CHARACTERISTICS OF THE FUNCTIONAL ENTITIES

At first we consider the rate effects arising from the conversion of pool P_i into amino acid pool P_a, which is accomplished via rate constant k_{20}. Figure 3 shows the enzyme E_p growth at various k_{20} values. It is evident that higher k_{20} values produce a more rapid growth and low values terminate the growth. Other entities (not shown here) exhibit in general the same growth trends.

In order to study the effect of nucleotide pool formation, the rate constant k_{19} is varied, while the growth of functional entities is recorded. This effect is demonstrated in Figure 4, where transport RNA C growth is presented at various k_{19} values. It is evident that at a low k_{19} value ("3") there is after small initial rise a rapid reduction of C concentration. This is followed by a leveling off phase, and finally an extremely slow growth is established. At higher k_{19} values, the final growth becomes progressively larger, but growth of C will be suppressed at very high k_{19} values ("15"). This experiment shows clearly that it is difficult to predict by casual reasoning how a complex system behaves at various parametric values. Other entities have been recorded in similar conditions, but are not presented here. The general conclusion which is drawn on the basis of experimental data reveals that excessive values of k_{19}, either too large or too small, will produce abnormal concentration relations between functional entities. As a consequence, abnormal growth characteristics develop in the model-system.

The effect of a sudden reduction of pool P_a on the growth of various functional entities is shown in Figure 5. It is evident that all entities exhibit different kinetic characteristics following such a transient event. Enzymes E and E_p start to decline while ribosome B, after the initial decline, starts to grow rapidly. All other entities

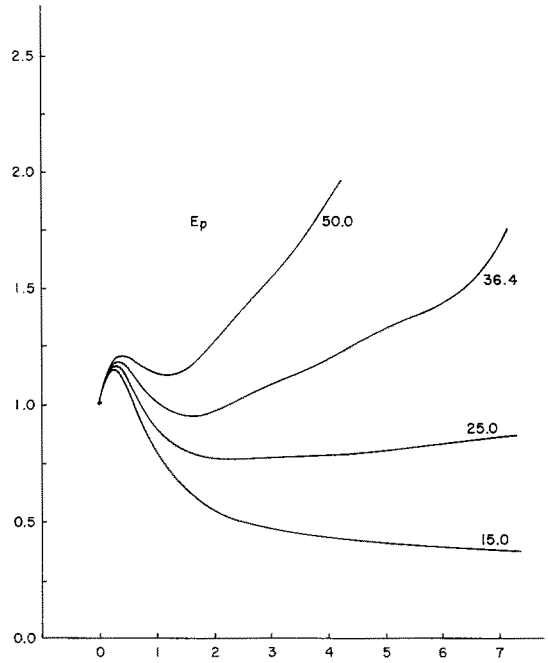

Fig. 3: The effect of k_{20} on enzyme (polymerase) E_p. Numbers on curves indicate the relative k_{20} values

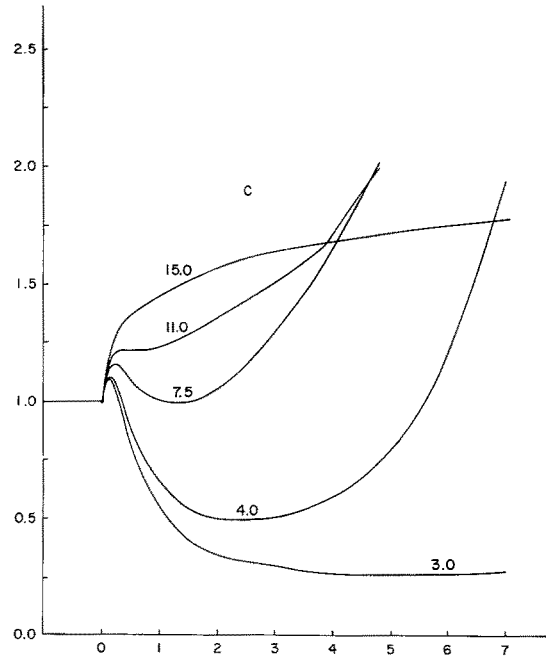

Fig. 4: The effect of k_{19} on transport RNA C. Numbers on curves indicate the relative k_{19} values

suffer concentration changes in various degrees. However, if computer observation time is extended to the range of several "generation times", it is evident that finally all functional entities start to decline, and system becomes extinct. It appears that the

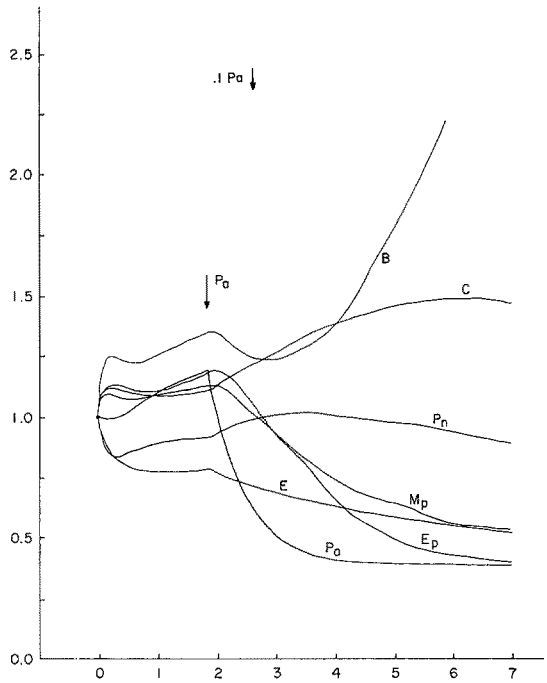

Fig. 5: The effect of reduction of pool P_a (90 % at time indicated by the arrow) on various functional entities

model-system becomes non-operational when excessive changes are made in single parameter values. The significance of this event in terms of biological phenomenon will be evident when cellular injury mechanisms are studied.

CELLULAR INJURY AND DEATH

Introduction

The phenomenon of cellular injury and death is a very perplexing problem in nature and has many ramifications. It has been a subject of many speculations and many theories have been proposed to interpret the mechanism of cellular death. Before we analyze this problem in more detail, it is important to consider the viability problem in general and explore its practical aspects. It is essential to put the concept of cellular viability on a rational basis, and one should attempt to interpret cellular injury and death via basic metabolic and synthetic processes. Furthermore, one cannot

consider cellular death only from the point of view of complete and absolute killing. There are degrees of injury, and consequently there may be cellular recovery. In principal, there is no absolute concept of cell life or death. There are states of functional disorganization, and those states can be altered by internal or external factors.

In general, a cell can be killed or injured in a multitude of ways. It can be killed by radiation, by excessive temperatures, by mechanical injury, by chemical agents, etc. This raises the question of whether the process of death is basically a singular process or is a diverse and complex set of disintegrative phenomena. It would be of benefit to review briefly the basic views existing at the present time on this subject in the literature. Many theories have been proposed to interpret the cellular killing process, but of foremost importance from the view of popularity is the so-called target theory. It is based essentially on population statistics where cellular inactivation or killing rate will provide information in regard to the killing process which may be a single hit or multihit type (DAVIS & FEINGOLD 1962, LEA 1947, RAHN 1945). Despite its obvious shortcomings, the target theory is still widely accepted as a means of interpreting and elucidating the mechanisms of cellular death (GRAY 1961, BARENDSEN 1961, MARCOVICH 1961, HAYNES 1964). Since target theory is based on the curve form analysis, which itself is affected by a multitude of experimental parameters (WEBB 1964, POWERS 1962), one cannot expect that the "death kinetics" of a population will yield information with regard to the cellular killing process and mechanism of cellular recovery on a single cell basis. In the literature numerous curves have been published for such analysis. No significant results have been obtained. One could ask whether the theory is founded on the premise which potentially could give information for cellular death mechanism, or are we dealing here with an obscure illusion? The primary source of information for target theory is the relationship between the rate of killing and the exposure dose. In our opinion, this type of relationship does not contain any basic information which is necessary for interpreting cellular killing mechanisms.

A few decades ago it became obvious to some that target theory was not capable of explaining cellular injury nor cellular death, but no substitute for the theory appeared for a while. Then some new proposals were made by us for the interpretation of cellular injury and death phenomena (HEINMETS 1954). Later, a wider and more comprehensive treatment of the subject was made (HEINMETS 1960). The basic premise proposed was based on the concept that there is a definite relationship between cellular injury and functional disorganization of the metabolic system. One could say that the factors that interfere with or excessively modify cellular organization and cellular processes can be considered in a biological sense to injure or kill the cell. Such interference could result from the interaction of cellular functional entities with a multitude of agents, for example, absorption photones, collision with elemental particles, interaction of its various molecules, etc.

As a result of these interactions, in terms of functional sub-units of the cell, the following changes could take place: inactivation or alteration of enzymes; modification of messenger and transport RNA; inactivation of genes; alteration of metabolites, intermediates, and co-factors. Since various agents and molecules can interact differently with cellular functional entities and molecules, a multitude of modes of

functional disturbances may develop, depending on the character of the agent involved. It can also be considered from a chemical point of view that the molecular alterations produced within the cell may be reversible or irreversible. Furthermore, metabolic damage produced in the cell can be repaired by the cell, or if this is not possible, then the cell will die. However, at certain levels of injury the cell may survive when it can compensate for the deficiencies itself, or when external agents are applied which help cellular recovery. Consequently, we can generally define cellular injury as a phenomenon in which normal functional processes of the cell are altered by interfering agents. Since various operational entities of the cell have specific molecular composition and organization, the chemical interaction specificity determines which of the operational units are altered by a particular agent. The specificity of the agents may determine their character of functional injury. This can be a singular injury in a single locus, or it can be a multiple injury in many loci. For example, injury on a single gene level would be sufficient to kill the cell if this gene were irreversibly inactivated. By contrast, when several of the enzymes are inactivated, we have widespread functional damage, and it is not at all obvious how the cell would be affected by such injury. As a matter of fact, cellular processes are so complex and so interwoven that it is very difficult to perform a cleancut analysis of the system unless the system is analyzed quantitatively. It is of interest that the cellular model-system which has been developed can serve as useful purpose as a means of analyzing the problem of cellular viability as well as an aid to studies on growth regulation and growth in general.

In order to gain more insight into cellular disorganization, it is essential that a systematic study be made on the effects which can be produced by interfering with the activity of important functional entities in the cells. Furthermore, it would be important not only to view the problem of cellular disorganization from a phenomenological point of view but also to study the level and degree of disorganization.

Basic premises and procedures

Quantitative studies on the cell growth model reveal that the system is sensitive to alterations at the level of any functional entity and that the change which occurs is followed by adjustment at all levels of functional entities in the model-system. Therefore, in order to understand the events that are taking place after the interaction at a particular site, it is essential that all changes occurring in other entities be recorded simultaneously. Such a computer study is equivalent to the study of cell physiology under conditions in which, simultaneously with the cellular injury, physiological and biochemical measurements are made with all possible entities which enter into the scheme. This is essential since the measurements of a single entity are not sufficient to characterize the kinetic events in the cellular metabolism after a certain type of injury. However, in many practical conditions in cellular biology, injury occurs simultaneously at multiple sites. This is especially true in cases when a heterogeneous agent is introduced into the system, for example, radiation, heat, etc. No attempt is made to explore the multiple injury pattern, not that it would be too

complex to be analyzed, but rather it is too involved and needs a special programming for that purpose. Here only a limited set of experimental data can be presented. A more complete set of treatment of this subject will be presented elsewhere (HEINMETS 1966).

Inactivation of functional templates

Since genes represent only a small fraction of total cellular elements, it is evident that non-genetic injury plays a much larger role than genetic injury. Due to limited space we cannot analyze non-genetic injury at many sites, and therefore we limit ourselves in this study only to template type injury.

In our previous studies, it was shown that the template is a suitable site for exercising growth control (HEINMETS 1966). One can pose a question: What would happen

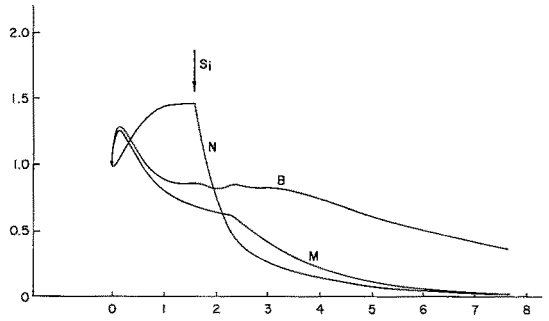

Fig. 6: Continuous repression of templates N and N_p. The effect on: template N, messenger M and ribosome B

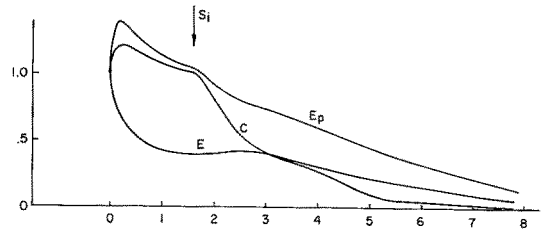

Fig. 7: Continuous repression of templates N and N_p. The effect on: enzymes E and E_p and transport RNA C

to the functional system if regulatory compounds such as hormones or chemical agents were applied on the templates in excessive amounts for long periods of time? Furthermore, are the effects on the system only temporary and always reversible, or would there possibly be permanent damage produced by the regulatory mechanisms when they are operational at an extreme level? Template inactivation results when substrate s_i (eqs. 24, 26, Table 2) interacts with N and N_p. Then substrate s'_i can restore template activity (Table 2, eqs. 25, 27). In the normal system where there is limited growth for

the model-system, these rate constants have zero value. Only when it is desired to control growth at the template level are these rate constants introduced. This mechanism is equally applicable to an external agent(s) introduced into the system which causes specific template inactivation or activation.

Figures 6 and 7 show an experiment where an external agent s_i is introduced into the system (at time indicated by the arrow). This compound interacts with templates N and N_p and converts these into inactive state. It is evident that there is an immediate reduction of template N followed by a slower reduction of other entities. The system will become finally nonfunctional since all entities will disappear gradually.

The experiment presented in Figure 8 shows that a system which is growing normally can be disorganized when an external agent, s_i, is introduced into the system. Observation time on the computer is about 7 generation times. Agent s_i is introduced about at $^3/_4$ of generation time. The first effect is rapid loss of template N. This is

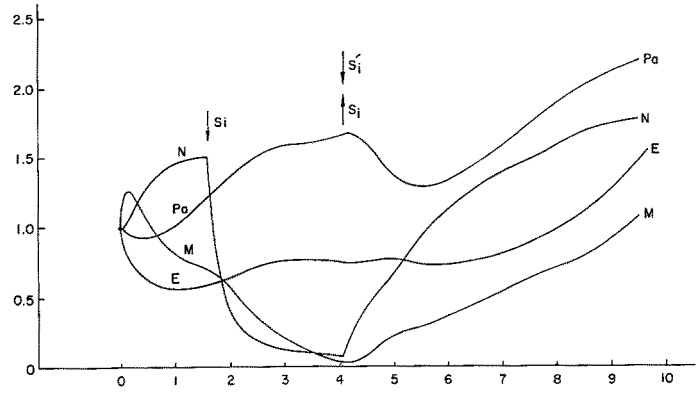

Fig. 8: Inactivation and reactivation of templates N and N_p. The effect on: template N, messenger M, enzyme E and pool P_a

followed by reduction of template M synthesis. The rate of enzyme E synthesis is reduced, while pool P_a continues to grow at a lower rate. Other entities which have been recorded (not shown here) all reveal a reduction of concentration in varying degrees. If the system is left indefinitely in such a condition, the model-system becomes extinct since all entities finally cease to exist. It was of interest to study the effect of an activating agents s'_i when this is introduced into the system. Figure 8 shows the effect in conditions where s_i is removed and s'_i is simultaneously introduced into the system. It is evident that there is immediately a rapid increase of template N concentration, while other entities start to grow subsequently. This experiment shows that a decaying model-system may be restored into the normal functional state when a template activating compound is introduced into the system. However, other experiments reveal that the functional system may be restored only in conditions where activating agent is introduced before a "critical time limit", otherwise system cannot recover. This experiment shows that system's survival is conditional, depending on the duration of injury.

Genetic injury

Basically, genetic injury can be reversible or irreversible. In irreversible injury, gene activity is partially or completely destroyed, and this effect is permanent. Reversible injury can be corrected either by removal of the agent which interacts with the gene or by the action of repair mechanisms. For example, if there is a complexing process between an agent and the gene, then the [gene-agent]-complex can be maintained as long as the agent is present in the system. However, when the agent is removed, the complex dissociates and the gene is free to operate again. Another aspect of genetic injury is that it can be either partial or complete. In the latter case, the gene is completely blocked and is not functionally operative. Partial injury means

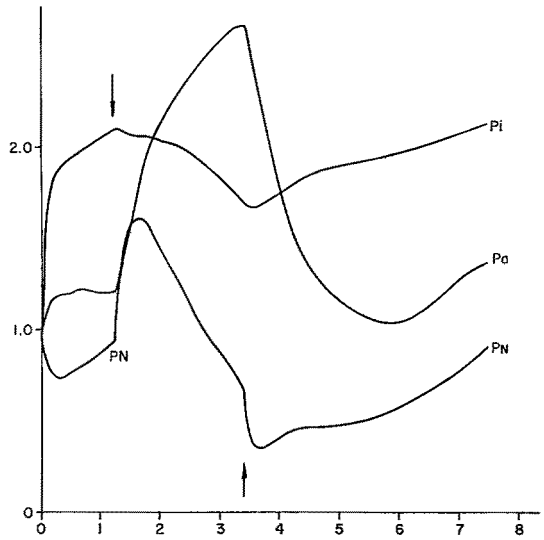

Fig. 9: The effect of complete genetic block on gene G_0 level. At the time indicated by first arrow, k_4 is made zero. At time indicated by second arrow, the genetic block is removed. Duration of genetic block is such that cell is capable of recovery. Pools P_i, P_a, and P_n are recorded

that the gene is operational, but it does not function at the normal rate. This condition can arise when the special configuration of the genetic structure suffers some minor modifications. In partial genetic injury, the average genetic activity is reduced. Furthermore, we assume that when the gene is blocked, it is not operational and does not produce RNA. We do not analyze the case where the gene is producing a defective, non-functional RNA. This can be done, but it requires some special programming and model-system changes.

Genetic injury can be produced at any gene level presented in the model-system. However, due to limited space we can treat the problem only at one gene group level. Further details are available in another publication (HEINMETS 1966). Here we consider genetic injury at gene G_0 level, whose function is to synthesize transport RNA C.

The rate constant k_4 determines the activity of the gene. Here only complete gene activation is considered. When a complete genetic block is introduced at $^3/_4$ of generation time, the components of the system start to decline and all the system becomes extinct. However, when the genetic block is removed at a certain interval, system may recover. Figure 9 shows experiments where various pools are recorded during 8 generation times. Other entities have been recorded, but only their behavior is discussed here. The first event taking place when the gene is reactivated is the increase of transport RNA C concentration. This is associated with the decrease of pool P_n and pool P_a while pool P_i starts gradually to increase (Fig. 9). Practically at the same time an increase of enzyme E and ribosome B occurs. In contrast, M_p and E_p both continue to decrease. Both templates N and N_p were near the maximum concentration when k_4 was restored to the original value, and both started immediately to decline. Template N declines initially very rapidly, but after a while reduction proceeds at a slower rate. Messenger M shows initially practically no change, but subsequently there is a decrease of M concentration. After passing a minimum, M gradually starts to increase again. The recovery of the system takes place rather unevenly and various functional entities which were reduced start to increase at different times. The first to increase is enzyme E_p, then messengers M_p and M, followed by pool P_a. Finally, at the end of generation time, N_p starts to increase while N reaches a constant level. N finally also starts to increase, but this can be seen only when the observation time

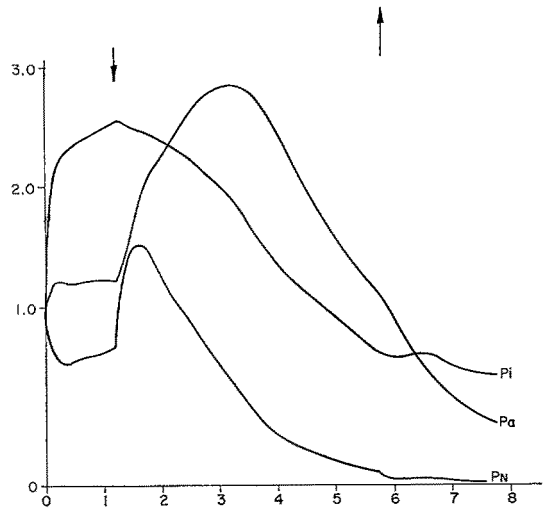

Fig. 10: This is the same experiment as in Figure 9 except the duration of block is longer. System does not recover from such injury. Pools P_i, P_a, and P_n are recorded

is further extended on the computer. This experiment reveals that introduction of the genetic block really disorganized the system. However, removal of the block intiated a sequence of events which permitted the system to recover. The recovery is not uniform, however, and it will take numerous generations to stabilize the system. An

important conclusion which can be drawn from this simulation experiment is that a cell may be capable of self-recovery from a prolonged genetic injury. In these experiments duration of the genetic block was slightly longer than a generation time. This raises the question of what the minimum duration of genetic block is which would prohibit recovery of the system. In order to explore this, the experiment was performed with the same model-system, but the growth rate was slightly reduced by increasing k_{30} and k_{17} and by decreasing k_{18}. This was necessary in order to avoid computer overloading in transient phases of the experiments. In the absence of the genetic block, this system starts to grow slowly and continues to do so indefinitely. Rate constant k_4 was reduced to zero at the same time as previously, but duration of the zero period was markedly increased. Only pools are shown here in Figure 10. In these conditions, after removal of the genetic block, the system was not able to recover and all entities continued to decline. There is a small increase of ribosome B, but the rise is very small compared with the previous experiments which recovered from the injury. Also, transport RNA C has a very small increase in concentration, but it is trivial and C does not continue to grow. Enzyme E has practically no increase, and the concentration is maintained at a low level. Pool P_i remains constant for a while, then starts to decline. The kinetic behavior of the system indicates that synthesis initiated after the removal of the genetic block is extremely small, and this synthesis is not sufficient to make the system again operational. Some further experiments were carried out on the computer to determine critical duration of the genetic block. Experiments revealed that if the blocking time is slightly reduced, the cell is indeed capable of self-recovery. Consequently, the experiment reported in Figure 10 represents rather the borderline conditions where the system loses its ability to recover. It appears that there is a critical time limit for a given system to recover from a particular type of genetic block.

This simulation experiment represents a very important concept in cellular injury analysis because it clearly reveals the significance of the time factor in the process of recovery. However, if the genetic block is not complete, then different time relationships appear and only systematic study can give useful information in this area. These simulation experiments on the model-system suggest some considerations for cellular systems. By an application of a temporary genetic block, the model-system is terminated, and the question can be asked whether there is a requirement for permanent genetic injury to kill the cells. It appears that perhaps a certain length of the blocking time is sufficient during cellular growth to inactivate the cell. If this were the case, then differential killing of different cell types could be possible simply on a time basis. If we assume that a long-lasting genetic block can kill off the cell, then the question can be raised whether all cells in a population can be killed off with the same critical block lasting a definite time. This, of course, seems possible if one assumes that all cells behave equally. This assumption, of course, is contrary to practical experience, since there are variations in various biological properties among individual cells. This brings up the point of whether the properties of various functional entities will effect the critical blocking time in the model-system and how much variation is necessary. This problem, of course, would need a systematic study which is not done

here, but we shall present an example to demonstrate that the model-system can be used to analyze such a problem. Furthermore, we shall show that stability variation in the values of individual functional entities is the determining factor in determining viability of the system.

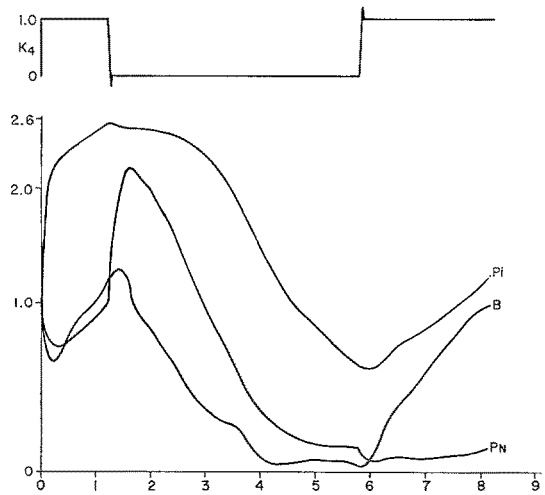

Fig. 11: Same experiment as in Figure 10 except protein stability is increased. Rate constant k_{16} is reduced from .01702 to .0107. This increased enzyme E stability permits recovery of the system. Pools P_i, P_n, and ribosome B are recorded

Figure 11 represents a similar experiment as in Figure 10 except that protein stability has been increased by reducing rate constant k_{16}. It is evident that all pools start to grow again after the removal of the genetic block. All other functional entities (not shown here) also start to increase, and system becomes again functional.

GENERAL COMMENTS

On the basis of the few experiments presented here and the extensive studies published elsewhere (HEINMETS 1966), one can conclude that cellular recovery and death are highly conditional phenomena. Many interdependent factors exert effective control on such processes and more insight into basic operational mechanisms can be gained by extensive research on the model-system basis. Model-system studies also indicate that when severe damage occurs on the level of any entity, inactivation of the system results (HEINMETS 1966). Obviously, the living cell is much better regulated than a model-system. However, a basic concept which has been established on model-system studies reveals that disorganization of the system can be produced on the level of practically any functional entity, and extensive disorganization leads to decay of the system. However, when interfering agent is removed, the cell may be capable of initiating growth again, but the cellular fate is conditional. When such an experiment is carried out in practice, the time factor, of course, will play an important role. If

conditions of injury are continued for a long time, cellular destruction will take place because there is no compensatory synthesis. Any biological system will decay by the inherent property that all functional entities are unstable, and in order to maintain the system's maintenance, synthesis should occur. On the other hand, for a limited amount of time, the cell can sustain a certain amount of decay and still be able to recover when the condition for the growth is soon re-established. We should like to make a few comments on cellular viability studies within a mass population on the basis of experience which we have obtained by analyzing the model-system. Model-system studies indicate that a viable system can sustain a certain amount of disturbance. The resulting disorganization depends upon how long the state of disturbance of normal functional processes is maintained as well as on the stability of functional entities of the system. It is a well known experimental observation that all cells do not have exactly equal characteristics. For example, if a group of synchronized cells are placed into growth medium, then within a few generations synchrony is lost, indicating that all cells are not equal in terms of metabolic and synthetic processes. This is, of course, expected since the cell is a highly complex organizational and functional system where statistical events in the terms of molecular interaction and reactions represent the basic process. Consequently, the structure, as well as the function of entities is probabilistic in nature. Furthermore, variations on the single entity level are supplemented by sets of higher order interactions. Thus, systems develop having a large number of probablistic states. Consequently, a population contains species which have a wide variation in survival potential under normal conditions. Subsequently when the cellular population is put under stress, one would expect that there would be among individual cells a wide range of deviations from the average behavior. If it is decided to destroy a cellular population completely, a process, for example, which is essential for sterilization, then we may be able to kill off the majority of population with a relatively small dose, but for the elimination of few individuals which have high survival potential (extremely effective functional organizations) a large dose may be required. We feel that on the basis of general concepts emerged from computer studies on the individual cell model-systems, new theories could be developed which could determine kinetics characteristic of population death.

REQUIREMENTS FOR COMPUTER PERFORMANCE AND LAYOUT

This is a mathematical problem. The model-system (Fig. 1) is represented by nineteen simultaneous differential equations containing thirty rate constants. In order that a functional system be established, the computer must have certain performance characteristics as well as a suitable physical layout. The organization of a functional system on the computer is time-consuming and tedious. It also makes great demands on the man who organizes the system, particularly in terms of memory, as well as evaluation and pertinent intellectual reasoning during the operation. For a solution to be obtained to such a problem, it is essential for the computer to possess certain performance characteristics. We shall therefore outline a number of essential requirements on the basis of our own personal experience:

(1) The computer must contain a sufficient number of operational elements (amplifiers, integrators, multipliers, electronic switches, potentiometers, etc.). These must perform with great dynamic accuracy. (2) The programming patchboard must be removable so that the problem can be taken off the computer. This is imperative for prolonged studies as well as for organizing the system. (3) All operational control knobs and switches should be within easy hand-reach of the operator. (4) Computer performance should have the following principal characteristics: (a) High speed, a compressed time scale, a broad frequency band for accuracy and reproducibility. (b) It must operate with high dynamic accuracy in real time, as well as in repetitive performance. The latter is required for organizing the system by using an oscilloscope for observations. If the repetitive solution on the scope is not accurate, it is not possible to organize the functional system. The real-time solution is used for recording the data with an X-Y recorder. Switching from repetitive to real-time and vice versa should be simple. (c) The computer should be able to maintain stable performance when square-wave potentials are introduced into the system, and it must be able to recover rapidly from overload conditions. (d) The noise level must be low. This is extremely important when characteristics of the system are studied in conditions of decay (for example, in studies of viability).

ADVANCED MODEL-SYSTEM FOR CELL GROWTH

It is desirable to extend model-system analysis to systems which contain more basic growth regulation at genetic level. A more advanced model-system has been developed (Fig. 12). Flow equations and a simultaneous set of differential equations are represented in Tables 4 and 5. Such system can be analyzed at the time when more advanced hybrid computers are available.

Table 3

Supplementary terminology

Gene groups

G_b – Ribosomal gene; G_p – RNA polymerase (E_p)
G_c – Transfer RNA gene; G_t – enzyme E_t
G_n – Enzyme E_n; G_a – enzyme E_a
G_e – For the synthesis of effector compound (e) to increase the "activity-state" of the genes from normal state.
G_r – For the synthesis of depressor compound (r) to decrease the "activity-state" of the genes from normal state.

Genetic states

a) $G^s1 + e \rightarrow \overset{*}{G}{}^s1\,(e)$

b) $G^s1 + r \rightarrow \overset{*}{G}{}^s1\,(r)$

where absolute values for activity are:

$\overset{*}{G}{}^s1\,(e) = e\,G^s1$

$\overset{*}{G}{}^s1\,(r) = \frac{1}{r}\,G^s1$

Factors "e" and "r" are variable modifiers which have maximum limit values.

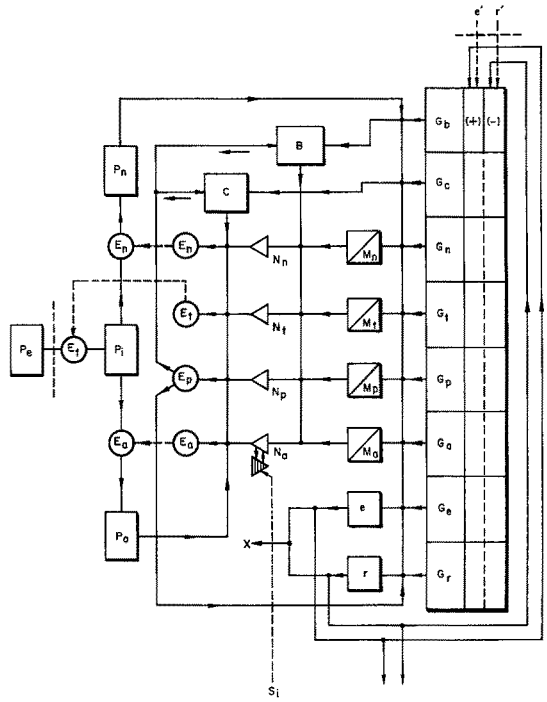

Fig. 12: Advanced model-system for cellular growth

Table 4

General scheme

1. $E_p + P_n \xrightarrow{k_1} [E_p\, P_n]$

2. $\overset{*}{G}_b + [E_p\, P_n] \xrightarrow{k'_2} \overset{*}{G}_b + E_p + B$

3. $G_b + [E_p\, P_n] \xrightarrow{k_2''} G_b + E_p + B$
$\overset{*}{}$ $\overset{*}{}$

4. $G_b + [E_p\, P_n] \xrightarrow{k_2} G_b + E_p + B$

5. $G_c + [E_p\, P_n] \xrightarrow{k_3} G_c + E_p + C$

6a. $G_n + [E_p\, P_n] \xrightarrow[k_4]{} G_n + E_p + M_n$

6b. $G + [E_p\, P_n] \xrightarrow{k'_4} G + E_p + M$
for: M_n, M_t, M_a combined equation

7. $G_t + [E_p\, P_n] \xrightarrow{k_5} G_t + E_p + M_t$

8. $G_a + [E_p\, P_n] \xrightarrow{k_6} G_a + E_p + M_a$

9. $G_p + [E_p\, P_n] \xrightarrow{k_7} G_p + E_p + M_p$

10. $G_e + [E_p\, P_n] \xrightarrow{k_8} G_e + E_p + e$

11. $G_r + [E_p\, P_n] \xrightarrow{k_9} G_r + E_p + r$

11a. $e + r \xrightarrow{k_{60}} X$

12a. $B + M_n \xrightarrow{k_{11}} N_n$

Table 4 (Continuation)

12b. $B + M \xrightarrow{k'_{11}} N$
for N_{n1} N_t and N_a

13. $B + M_t \xrightarrow{k_{12}} N_t$

14. $B + M_a \xrightarrow{k_{13}} N_a$

15. $B + M_p \xrightarrow{k_{14}} N_p$

16. $C + P_a \xrightarrow{k_{15}} [c\,P_a]$

17a. $N_n + [c\,P_a] \xrightarrow{k_{16}} B + M_n + C + E_n$

17b. $N + [c\,P_a] \xrightarrow{k'_{16}} B + C + M + E$
where:
$E_n = k_n E$
$E_t = k_t E$
$E_a = k_a E$

18. $N_t + [c\,P_a] \xrightarrow{k_{17}} B + M_t + C + E_t$

19. $N_a + [c\,P_a] \xrightarrow{k_{18}} B + M_a + C + E_a$

20. $N_p + [c\,P_a] \xrightarrow{k_{19}} B + M_p + C + E_p$

21a. $M_n \xrightarrow{k_{20}} P_n$

21b. $M \xrightarrow{k'_{21}} P_n$

22. $M_t \xrightarrow{k_{21}} P_n$

23. $M_a \xrightarrow{k_{22}} P_n$

24. $M_p \xrightarrow{k_{23}} P_n$

25. $B \xrightarrow{k_{24}} P_i$

26. $C \xrightarrow{k_{25}} P_i$

27a. $E_n \xrightarrow{k_{26}} P_i$

27b. $E \xrightarrow{k'_{26}} P_i$

28. $E_t \xrightarrow{k_{27}} P_i$

29. $E_a \xrightarrow{k_{28}} P_i$

30. $E_p \xrightarrow{k_{29}} P_i$

31. $P_e + E_t \xrightarrow{k_{30}} P_i + E_t$ or
$P_e + k_t E \longrightarrow P_i + k_t E$

32. $P_i + E_n \xrightarrow{k_{31}} P_n + E_n$ or
$P_i + k_n E \longrightarrow P_n + k_n E$

33. $P_i + E_a \xrightarrow{k_{32}} P_a + E_a$ or
$P_i + k_a E \longrightarrow P_a + k_a E$

34a. $E_p + C \underset{k_{-33}}{\overset{k_{33}}{\rightleftarrows}} [E_p\,C]$

34b. $E_p + B \underset{k_{-34}}{\overset{k_{34}}{\rightleftarrows}} [E_p\,B]$

35. $E + P_i \underset{k_{-35}}{\overset{k_{35}}{\rightleftarrows}} [E\,P_i]$

36. $N_p \xrightarrow{k_{36}} \overset{*}{N}_p$

37. $\overset{*}{N}_p + s_i \xrightarrow{k_{37}} N_p$

38. $N \xrightarrow{k_{38}} \overset{*}{N}$

39. $\overset{*}{N} + s_i'' \xrightarrow{k_{39}} N$

40. $G_b + e \xrightarrow{k_{40}} \overset{*}{G}_b$

41. $G_b + r \xrightarrow{k_{41}} \underset{*}{G_b}$

42. $\overset{*}{G}_b \xrightarrow{k_{42}} G_b$

43. $\underset{*}{G_b} \xrightarrow{k_{43}} G_b$

44. $e \xrightarrow{k_{44}} x$

45. $r \xrightarrow{k_{45}} x$

Table 5

Differential equations

1. $\dot{E}_p = k_{19}\, N_p\, [c\, P_a] - k_{29}\, E_p - k_{33}\, E_p C + k_{-33}\, [E_p C] - k_{34}\, E_p B + k_{-34}\, [E B_p] - k_1\, E_p P_n + k'_2\, \overset{*}{G}_b\, [E_p P_n] + k''_2\, \underset{*}{G}_b\, [E_p P_n] + k_2\, G_b\, [E_p P_n] + k_3\, G_c\, [E_p P_n] + k'_4\, G\, [E_p P_n] + k_7\, G_p\, [E_p P_n] + k_8\, G_e\, [E_p P_n] + k_g G_r\, [E_p P_n]$

2. $\dot{P}n = k_{31}\, k_n\, E\, P_i - k_1\, E_p P_n + k'_{21}\, M + k_{23}\, M_p$

3. $[\dot{E_p P_n}] = k_1\, E_p P_n - [E_p P_n]\, (k'_2\, \overset{*}{G}_b + k''_2\, \underset{*}{G}_b + k_2\, G_b + k_3\, G_c + k'_4\, G + k_7\, G_p + k_8\, G_e + k_g\, G_r$

4. $\dot{\overline{G_b}} = k_{40}\, e\, G_b - k_{42}\, \overset{*}{G}_b$

5. $\dot{\underset{*}{G}}_b = k_{41}\, r\, G_b - k_{43}\, \underset{*}{G}_b$

6. $G_b = k_{42}\, \overset{*}{G}_b + k_{43}\, \underset{*}{G}_b - k_{40}\, e\, G_b - k_{41}\, r\, G_b$

7. $\dot{B} = [E_p P_n]\, (k_2\, G_b + k'_2\, \overset{*}{G}_b + k''_2\, \underset{*}{G}_b) - k'_{11}\, BM - k_{14} - BM\, p - k_{24}\, B + k'_{16}\, N\, [c\, P_a] + k_{19}\, N_p\, [c\, P_a] - k_{34}\, E_p B + k_{-34}\, [E_p B]$

8. $\dot{C} = k_3\, G_c\, [E_p P_n] - k_{15}\, c\, P_a + k'_{16}\, N\, [c\, P_a] + k_{19}\, N_p\, [c\, P_a] - k_{25}\, C - k_{33}\, E_p C + k_{-33}\, [E_p C]$

9. $\dot{M} = k'_4\, G\, [E_p P_n] - k'_{11}\, BM - k'_{21}\, M + k'_{16}\, N\, [c\, P_a]$

10. $\dot{M}_p = k_7\, G_p\, [E_p P_n] - k_{14}\, BM_p + k_{19}\, N_p\, [c\, P_a] - k_{23}\, M_p$

11. $\dot{e} = k_8\, G_e\, [E_p P_n] - k_{40}\, G_b e - k_{44} e - k_{10}\, er$

12. $\dot{r} = k_9\, G_r\, [E_p P_n] - k_{41}\, G_b r - k_{45}\, e - k_{10}\, er$

13. $\dot{N} = k'_{11}\, BM - k_{38}\, N + k_{39}\, s''_i\, N^* - k'_{16}\, N\, [c\, P_a]$

14. $\dot{N}_p = k_{14}\, BM_p - k_{36}\, N_p + k_{37}\, s'_i\, \overset{*}{N}_p - k_{19}\, N_p\, [c\, P_a]$

15. $\dot{\overset{*}{N}}_p = k_{36}\, N_p - k_{37}\, s'_i\, \overset{*}{N}_p$

Table 5 (Continuation)

16. $\dot{N}^* = k_{38} N - k_{39} s''_i N^*$

17. $\dot{P}_a = k_a k_{32} \underline{P_i E_a} - k_{15} \underline{c P_a}$

18. $\dot{\overline{[c P_a]}} = k_{15} \underline{c P_a} - k'_{16} \underline{N [c P_a]} - \underline{k_{19} N_p [c P_a]}$

19. $\dot{E} = k'_{16} \underline{N [c P_a]} - k'_{26} E - k_{35} E P_i + k_{-35} [E P_i]$

20. $\dot{P}_i = k_{30} k_t \underline{P_e E} = k_{31} k_n \underline{E P_i} - k_{32} k_a \underline{E P_i} - k_{35} \underline{E P_i} + k_{-35} [E P_i] + k_{24} B + k_{25} C + k'_{26} E + k_{29} E_p$

21. $\dot{\overline{[E_p C]}} = k_{33} \underline{E_p C} - k_{-33} [E_p C]$

22. $\dot{\overline{[E_p B]}} = k_{34} \underline{E_p B} - k_{-34} [E_p B]$

23. $\dot{\overline{[E P_i]}} = \underline{k_{35} E P_i} - k_{-35} [E P_i]$

SUMMARY

1. A functional model-system has been helpful in elucidating the problem of cellular growth kinetics. Computer studies with the model-system have made it possible for us to obtain some information in regard to interrelationships between various functional entities as well as on the effect of various parameters on the growth process.
2. These studies include the effects of the various entities on normal growth. The phenomenon of cellular viability and the recovery of viability has been elucidated, and it has been shown that the organization pattern plays a dominant role in a functional system.
3. While the simulation of the cellular system with the model has produced some highly interesting results, the complexity of the problem, raises many new issues. In order to make a model-system analysis more penetrating for gaining insight into biological processes, the model-system has to be expanded. It should especially contain more regulatory mechanisms.
4. In our experience, it was extremely difficult to obtain a solution on the computer for the differential equations representing the model-system. Sometimes it was thought to be impossible. Consequently, one has to introduce new features into the model-system gradually.
5. Since a functional system has been obtained, however, there is no apparent difficulty

to supplementing within certain limits additional features involving regulatory mechanisms at various levels. This can be done with available computer facilities.

6. More expanded model-systems will also require new computer developments. Here again, there is no apparent limitation from the computer technology point of view. The problem is mainly economic.

ACKNOWLEDGMENTS

In these studies, the GPS Instrument Company (Newton, Mass., U.S.A.), General Purpose Computer, Model GPS 10000, was used.

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Discussion following the paper by HEINMETS

HESS: Which reaction triggers gene activity? How do you get the genetic level going?

HEINMETS: The model system for cell growth is analyzed during one growth cycle. Genetic division is not considered here. All gene groups are active in the beginning of the growth cycle. The problem of initiation of genetic activity by trigger mechanisms has been discussed elsewhere (HEINMETS, Analysis of normal and abnormal cell growth. Plenum pr. New York, 1966).

BRANSON: In the solution of such a complex system, we have found that many sets of parameters lead to the same conclusion. How then do you decide among the various sets?

HEINMETS: Model system is formulated by 19 time dependent differential equations, containing 33 rate constants. Here indeed exist many possible solutions. On the basis of general experimental information, we have assigned certain values to rate constants and the analog computer solution is obtained within the framework of such numerical data.

SUGITA: One of the difficulties in this work may be to get a suitable function generator. Why are you using the ultra-high speed analogue computer?

HEINMETS: In this work we do not need a function generator because all entities in the model system are derived by analog computer from the basic inter-relationship between the functional entities. The computer is used as a mathematical tool to solve the set of differential equations. It has to have high speed and accuracy in order to get solutions rapidly enough for visual observation on oscilloscope (at least 20 solutions per second are required for such a purpose). Visual observation of the complete solution on the scope is essential for obtaining a functional model system.

KRÜGER: Sie haben den Versuch unternommen, ausgehend von Elementarvorgängen, die Stoffumsetzungen im Organismus zu analysieren. Ich selbst versuche zunächst die Veränderungen im Makrobereich möglichst exakt zu beschreiben und von hier aus analytisch zu den Elementarvorgängen zu kommen. Hier scheinen sich einfachere mathematische Lösungen zu ergeben. Diese Bemühungen sind auch für die tägliche Arbeit des Biologen wertvoll.

HESS: I admire Dr. HEINMET's work, which I have followed for a long time; he has greatly advanced the study of group systems. In our work we concentrated more on specific chemical reactions. We started to construct a mathematical model of this type by writing down the chemical equations representing each of the glycolytic steps including the enzyme substrate interactions and product substrate interactions. In this system we write down about 120 equations and let the digital computer solve them.

KIEFER: (1) I do agree that the target theory cannot account for many facts we have found in radiation biology, but it is the only approach we have so far. Can your treatment give an explanation for the survival curves commonly found? (2) You told us that recovery is probably dependent on protein synthesis, but recovery processes after irradiation are not brought about by protein synthesis. This seems to be inconsistent with your findings.

HEINMETS: Our analysis of cellular viability via the model system can provide an explanation for the survival curves of cell populations. However, the problem is mathematically too complex to be presented here. Model system analysis of cellular recovery after a reversible type of injury revealed that protein synthesis is essential. However, from literature it is well known that many injuries are irreversible (especially extensive genetic disorganization) and it is not expected that protein synthesis is effective here. It was also pointed out during my presentation that when cellular injury is extensive, the ability of the cell to synthesize some protein does not restore the viability.

HESS: What is the time scale in your model; is the conservation law fulfilled? The digital computer model, which I have developed with B. CHANCE and D. GARFINKEL, is based on experimental data and well-known biochemical reactions, which are treated in form of common

mass action kinetics. Thus, it is possible to compute data of cell metabolism on the basis of well-known facts. We do not need a complicated analog technique but can rely on appropriate computer programs which handle the complex and otherwise rather unapproachable mathematical equations.

HEINMETS: The basic time scale in our experiments is generation time. During that time all functional entities of the model system have to double their value. It is considered that subsequently cellular division takes place and all entities again acquire the unit value.

LOCKER: Ich möchte gerne die Meinung von Dr. HEINMETS über den für die Computer-Anwendung so wichtigen Begriff der „Simulation" hören. Wenn wir auf der Basis gewisser Kenntnisse über ein System ein theoretisches Modell für dieses formulieren, legen wir bereits fest, welche experimentell prüfbaren Resultate ein solches Modell liefern soll. Durch Vornahme einer indirekten Messung, nämlich durch Untersuchung der Antworten des Modells auf verschiedene inputs prüfen wir seine Brauchbarkeit. Kommt es hierbei zu einer nicht vertretbaren Differenz zwischen den vom Modell vorausgesagten und den tatsächlichen experimentellen Werten, muß die Komplexität des Modells erhöht werden, und zwar solange, bis eine signifikante Verminderung der Abweichungen zwischen vorhergesagten und beobachteten Werten eintritt. Gibt es nun einen prinzipiellen Einwand gegen die Verwendung des Begriffes „Simulation" für dieses Verfahren?

HEINMETS: There is no basic objection to use the word "simulation" in describing models. However, it is essential that it should be made clear what kind of simulation procedures are used and whether the method is adequate to simulate a complex system. For example, in many occasions in the past, complex biological processes were simulated directly via electronic circuitry. In many cases, such simulation is so crude that hardly any useful information can be obtained. This is especially so when kinetics of the system are considered. A complex model system, when represented via time-dependent differential equations, can yield informative data, provided that adequate computing facilities are used. In my opinion there should always be an interplay between experimental work and model system development.

BALAZS: (1) Welche Art genetischer Verletzungen wurde gewählt und was verstehen Sie unter der Behebung eines genetischen Blocks? (2) Bei atmungsgeschädigten Strahlungsmutanten der Hefezellen ist eine Art Selbstregulation durch Rückmutation zu beobachten. Wie wäre es möglich, dies mathematisch zu deuten? (3) Es wurde beobachtet, daß bei atmungsgeschädigten Zellen der genetische Block durch andere enzymatische Wege kompensiert werden kann, wodurch genügend Energie freigesetzt wird. Wie könnte man ein solches System mathematisch deuten?

HEINMETS: (1) Genetic injuries were produced in the model system by converting an active gene group into inactive state. This was accomplished on the analog computer by using special electronic switches which were operated in synchrony with a computer solution. After a desired time interval, the inactive gene was made again functional and the effect was observed on the computer solution. Genetic inactivation was studied on all gene groups presented in the model system. (2) Radiation mutation processes can be simulated on the analog computer, but a proper model system has to be established for such purpose. (3) Respiration damage and its compensation via other enzymatic pathways presents an interesting problem. In principal, such systems are highly suited for analog computer analysis, provided there is enough information to develop a plausible and adequate model system.

HAWKINS: To set up an analog of a car suspension is difficult and requires a specialist. Once the computer is set up, anyone can study the effect of, say, altering the stiffness of one spring. Is it true in regard to your analysis that once the difficult part of setting up the computer is done, another biologist or physiologist could use it to study, for example, enzyme inactivation?

HEINMETS: Once a model system has been developed and a solution obtained on a computer, any competent biologist can carry out further experiments on the computer (perhaps with the aid of a computer technician) and test out properties of the system.

MARMASSE: I would like to return to Dr. BALAZS' second question. Would it be possible to translate mathematically a genetic mutation, in Dr. HEINMETS' scheme, by a change in rate constants?

HEINMETS: Yes, a rate constant change can represent genetic mutation.

ZERBST: Wie die Frage von Herrn LOCKER zeigt, ergeben sich Schwierigkeiten mit dem Begriff der Simulation. Entspricht sie einer homomorphen Wiedergabe eines Vorganges ohne energetische und stoffliche Analogie? Vielleicht würde man besser Analogrechnung an Stelle von Simulation sagen. Bei den mathematischen Modellen besteht die Gefahr, daß wir sie zu einfach oder zu kompliziert ansetzen. Leider verfügen wir heute noch nicht über genügend Informationen hinsichtlich der Lösungsräume. Hier wäre, neben der Zusammenarbeit mit den Physiologen und Biochemikern, eine Zusammenarbeit mit den Morphologen erforderlich.

HEINMETS: It is quite true that the organization of a complex model system, which includes, in addition to symbolic interactions, also geometry and energetics, is indeed difficult. Collaboration between various specialists is required to develop and solve advanced model systems.