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An analysis of the concept of cellular injury and death†

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The concept of cellular viability is extremely complex and has been the subject of much speculation. A new theory is proposed to interpret cellular injury and killing process. Factors that interfere with or extensively modify cellular organization and reactions can be considered, in a biological sense, to injure or kill the cell. Such interference may result from the interaction of cellular molecules with a multitude of agents. In the terms of *functional sub-units* of the cell the following changes may take place: (1) inactivation or alteration of enzymes, (2) modification of templates for synthetic process, and (3) alteration and decomposition of metabolites, intermediates and co-factors.

An analysis is made of how cellular processes may be affected by external agents and how functional disorganizations may occur. It is proposed that there is a relation between the phenomenon of functional disorganization and cellular injury and death.

1. INTRODUCTION

The concept of cellular viability is extremely complex and has been the subject of much speculation. How a cell is 'killed' or 'altered' when exposed to various physical and chemical agents has not only theoretical significance concerning 'living processes' at the cellular level but also has great practical importance. An altered cell may lead to an abnormal growth phenomenon, and this may be related to abnormalities in the growth of tissue. Moreover, it is essential that the manufactured food products, beverages, drugs, vaccines, etc., on which contemporary society so largely depends should be free from microbiological contamination, and this requires knowledge of how to obtain conditions of sterility. From the abstract point of view, the study of cellular viability as a phenomenon of the living state in terms of some basic parameters is fascinating but difficult. Only by understanding the basic processes in the single can the more complex multicellular processes be comprehended.

During the past few decades, many theories have been proposed to interpret the cellular 'killing process'. These differ from each other mainly in methods of analysis or modifications in mathematical treatment, but the essential foundation is common, namely, population statistics. Of these theories the most prominent is the well-known target theory, in which the rate-curves for cellular 'inactivation' or 'killing' provide the information in regard to the

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killing process and from which 'single hit' and 'multi-hit' mechanisms are derived (Timofeef-Ressovsky and Zimmer 1947, Lea 1947, Wood 1953, Rahn 1945).

Despite its shortcomings, the target theory is still widely accepted as a satisfactory way to interpret the process of cellular death. There are many obvious difficulties in applying it to the interpretation of complex experimental data and, accordingly, in the past two decades, a few scientists have raised objections to the theory; some have suggested modifications and others have insisted that a revision is essential (Zirkle and Tobias 1953, Zirkle 1952, Roberts and Aldous 1949, Allsopp 1948, 1951, Hinshelwood 1951, Hall 1953, Gonzalez and Barron 1956). These criticisms, however, merely reveal the weaknesses of the theory and fail to offer basically new solutions.

A few years ago, while analysing the process of cellular death and injury, we came to the conclusion that a rational approach to the problem should be made via cellular physiology. Experimental data showed various changes in the physiological pattern of the cell as a result of ultraviolet irradiation (Heinmets and Kathan 1954, Heinmets and Lehman 1955). Since a cell is a highly complex functional system, it seems apparent that it could be killed or injured in a variety of ways. However, no attempt will be made at present to review the numerous references in the literature to various physiological changes which occur in the process of cellular injury.

At the same time as our work was being carried out and new ideas proposed for the interpretation of cellular viability (Heinmets 1954), it appears that the problem had been considered elsewhere (Meissel 1956) and essentially similar proposals for exploring cellular injury were suggested. In our publication (Heinmets and Kathan 1954) we summarize: "In order to achieve a rational interpretation of the viability concept, it is evident that comparative (normal and injured cell) physiological and cytological (micro) studies form the foundation for the future work . . ." In another section of the same paper we further stated, ". . . because the metabolic injury pattern produced by the action of ultraviolet irradiation is so complex and is dependent on such a variety of factors . . . a large number of simultaneous measurements on cellular activity have to be made . . . to find a relation between viability and specific alterations in metabolic and synthetic processes. Such facilities were not available . . ." The point of view expressed by Meissel (1956) is that "The character of these changes (caused by radiation) can be revealed and understood only if *simultaneous* and *parallel investigations* of the structural, physiological and biochemical changes and disturbances arising under the influence of radiations are carried out on the same organisms. As far as we know, such complex investigations in microbiology still have not been carried out".

It is interesting to note that two independent groups of scientists have not only offered similar criticisms of an obsolete theory, but also proposed strikingly similar approaches to the solution of the problem.

It may be expected that considerable progress in the problem of cellular injury and death will be made when experimental research is based on cellular physiology and not on the target theory. However, it is to be noted that, in such an approach, the physiological and biochemical changes in an injured cell are so complex as to offer no apparent and simple way to correlate them directly with viability (Heinmets and Lehman 1955, Meissel 1956). It appears to us

desirable to analyse cellular processes from a functional point of view, in which cellular injury and death could be interpreted via parameters that are measurable experimentally.

Research in cellular biology, either on the macro or micro level, is usually confined to the specific mechanisms within the isolated systems, obviously because experiments in complex systems would become extremely difficult to perform and would require scientific knowledge from various areas. However, summation of the experimental data derived from simple systems does not provide the solution to the problem of obtaining information and understanding of cellular processes at higher levels of integration. It seems to be essential that experiments be planned and designed with definite postulates and theoretical speculations. A multi-component system containing various elements which interact internally or with the external agents may yield results which cannot be estimated by guessing or by observation. It is essential that a system be formulated mathematically as far as possible. It could then be analysed quantitatively and the products of interactions established. Of necessity, the problem could be treated, at the present time, essentially from the point of view of abstraction and symbolism, but the approach should be based on fundamental elements of cell physiology.

In this paper a descriptive cell model is presented to facilitate the discussion. The effect of various interfering agents on the model system will be analysed in a general manner.

2. A SKELETON OF A FUNCTIONAL CELL MODEL AND ITS RELATION TO THE BASIC METABOLIC PROCESSES

Here, no attempt will be made to analyse views and speculations published in the literature on viability concepts or on the cell models. The author's views have been formed by the reading of various (often not related) data in the literature, and, in the background of the accumulated information, an attempt is made to formulate the basic principles of a functional cell model for a simple type of cell. It can be considered, for example, to be a micro-organism, and our considerations are at present limited to this type of cell. However, the basic principles are considered to be valid also for a more complex type of cell, but for these a more elaborate treatment is required which is not attempted in this work.

One of the reference bases for viewing the organizational characteristic of a biological system, such as a cell, is the exploration of its functional patterns in terms of interactions between the basic operational components, such as enzymes, organizers (templates), substrates, inducers, co-factors, etc. No attempt will be made to consider cellular organization and its changes from the point of view of thermodynamics, since such a treatment would be highly general, and no coherent pattern could be derived to analyse specific functional processes.

A simple skeleton model is considered to be founded on the following basic considerations.

1. The cell is an entity, confined in volume, containing an aggregate of functional units which operate, essentially, according to a definite average pattern.
2. Within the entity the number of operational units is duplicated or increased many fold during the activity of the entity; this represents the synthetic process.

3. There are many synthetic processes proceeding simultaneously and, in general, synthetic processes are interdependent, but may be competitive. There are, however, some phases of synthesis which are independent of each other; certain specific processes accomplish separation of the entity into two or more parts (cell division or multiplication).

4. Synthetic processes are made possible by a flux of specific 'nutrient molecules' from the environment into the cell.

5. 'Nutrient molecules' must have a large enough content of inner energy to permit a certain number of molecular transformations and interactions to take place. These processes can take place only at certain environmental temperatures and conditions.

6. After the 'nutrient molecules' have passed through a number of transformations, they acquire certain configuration characteristics, with the result that they may be used as elementary units by interacting with a template system for the synthesis of various operational units.

7. A template system may be considered to contain (a) a basic and relatively permanent set of templates which serve as organizers of synthesis, (b) an inducible template system (which is an integral part of the permanent system), which becomes functional only by interaction with an inducer. The latter may be of external or internal origin.

8. In certain environmental conditions, operational units may decompose as a function of time.

A schematic functional cell model is represented in figure 1. As an initial step, 'nutrient molecules' and co-factors enter into the cell via enzymatic-transfer mechanisms or by diffusion. Metabolic intermediates or substrates proceed by a series of interactions with various enzymes. The pathways may be described as linear, parallel, cyclic, etc., and various cross-linkages may exist between the pathways. The terminal metabolic products, metabolic intermediates and co-factors from a 'metabolic pool'. Only one type of general

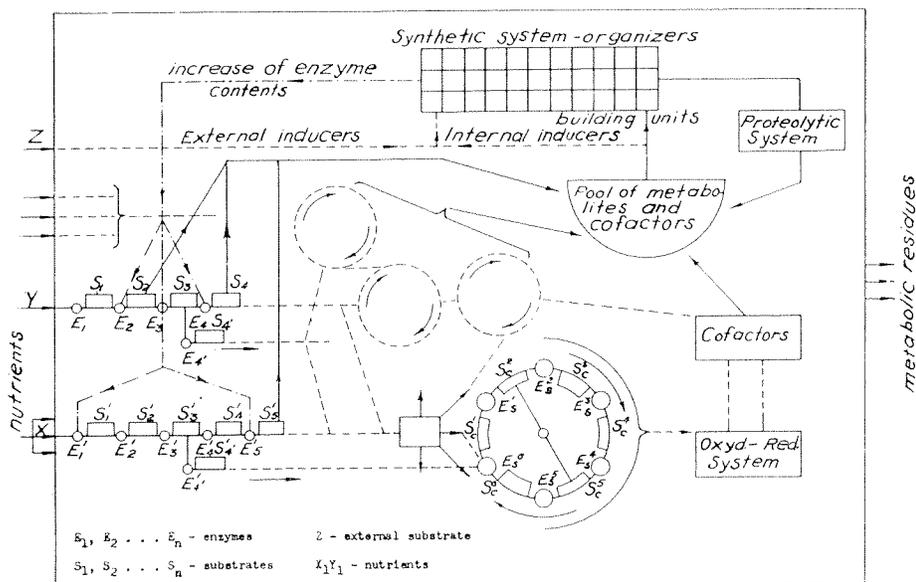


Figure 1. A scheme of functional cell model.

pool is considered without separating it into subdivisions (Cowie and Walton 1956), since the problem is complex enough when analysed with a single pool.

It must be emphasized that the functional scheme, at the present level of development, does not deal with the geometry of the system and the distribution of the functional units. It represents a continuously growing system, but it can acquire a periodicity when a limited pool concept is introduced, and specific synthetic mechanisms are provided for the division of the system. Without limitation such a schematic system would be characterized by steady-state growth. Indeed, when the measurements are made on a mass of bacteria growing in random fashion, one can observe only average steady-state synthesis (Cowie and Walton 1956). However, work with synchronized bacteria (Maruyama 1956, Burns 1959) has revealed that synthesis of major structural components per division cycle seem to proceed in distinct phases.

3. FUNCTIONAL DISORGANIZATION AND ITS RELATION TO CELLULAR VIABILITY

Factors that interfere with or excessively modify cellular organization and reactions can be considered, in a biological sense, to injure or kill the cell. Such interference may result from the interaction of cell molecules with a multitude of agents, i.e. absorption of photons, collision with elementary particles, other molecules. Changes in the structural molecules of the cell may be described in terms of ionization, dissociation, complex-formation, configuration alterations, etc. In the *terms of the functional sub-units* of the cell, for example, the following changes may take place: (1) inactivation or alteration of enzymes, (2) modification of templates for synthetic processes, and (3) alteration and decomposition of metabolites, intermediates and co-factors.

Since various agent molecules interact differently with the cell molecules, a multitude of modes of functional disturbance may develop. This phenomenon can be designated as a multiple-injury pattern (Heinmets and Kathan 1954, Heinmets 1954). From the chemical point of view, the molecular alterations produced within the cell may be reversible or irreversible. When the cell is injured by a reversible type of chemical reaction, this injury may be removed, at least in principle, when the reaction is reversed by chemical or other means. When the injurious reaction is chemically irreversible and cannot be neutralized by other means (i.e. photon-action, catalytic agents, etc.), then, if the damage is extensive, the cell will disintegrate and its viability will be lost. However, at certain levels of injury, the cell may survive when it can compensate for the injury via the metabolic process. Experimentally, various modes of cellular reactivations and recovery phenomena have been observed (Heinmets and Lehman 1955 Errara 1953).

The general statement that a chemical or physical agent interacts with cellular constituent elements and thereby injures the cell is only a superficial description and does not specify the changes taking place within the cellular processes. We can define cellular injury as a phenomenon in which the normal functional processes of the cell are altered by the interfering agent. Various operational units of the cell have specific molecular compositions and organizations. The chemical interaction-specificity determines which of the operational units are altered by a particular agent. For example, an oxidizing agent may affect free

reducing sites, whereas an increase in the environmental temperature affects all thermally-unstable molecules and structural units. The functional processes can also be affected indirectly by factors that interfere with the cellular integrity as a unit. Extensive alterations of membrane and surface structures represent such injury.

Cellular processes are so complex and so interwoven that it is very difficult to perform a clean-cut analysis of the system, especially when several factors within the system are altered simultaneously. It seems advisable to dissect some of the principal parts of the system for separate analysis and scrutinize them more closely, later analysing the system, if possible, in totality. For this reason, an attempt will be made at first to analyse enzyme synthesis *per se* and, later, its relationship to other metabolic processes.

4. SCHEMATIC FORMULATION OF ENZYME SYNTHESIS AND INTERFERENCE OF SYNTHETIC PROCESSES

In order to analyse the problem of cellular growth on the basis of the functional cell model, synthetic processes have to be formulated in such a way that interrelationships between various operational units can be established. In order to simplify the analysis, a descriptive formulation will be presented.

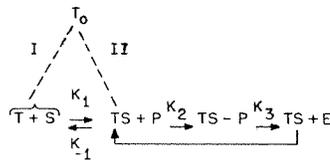
Reviews (Borsook 1956, Cohn 1957) indicate that enzyme and protein synthesis has been the subject of extensive experimental studies, but only a few attempts have been made to formulate it. The early work of Spiegelman (1948) proposed an autocatalytic mechanism, and Yudkin (1938) suggested a mass-action concept for enzyme synthesis. An extended mass-action theory was presented by Mendelstam (1956). Since Dixon and Webb (1958) have analysed the subject of enzyme synthesis very thoroughly, no attempt is made to review the subject.

The present attempt to formulate enzyme synthesis and its relation to the other metabolic processes is based in many respects on the work of Cohn and Monod (1953) and Pollock (1953). Recent experimental evidence suggests that the classical model for induced enzyme synthesis may require some revision (Pardee, Jacob and Monod 1958). However, since experimental data are still too limited for a definite revision, the classical model is considered sufficient at present to carry out an analysis.

Our basic ideas and schemes for synthesis were first presented at a symposium (Heinmets 1954) under the title, 'Physiological Concept of Cellular Injury and Death'. Spiegelman (1956) recently proposed a new schematic formulation of enzyme synthesis. His schemes and ours have some common features, but differ in some basic characteristics. No critical evaluation of the two proposals is attempted here since the subject is still too speculative and open for theorizing. Our scheme has mainly been composed with the idea of analysing the process of cellular injury as related to disorganization of the functional pattern. A more detailed analysis of models of enzyme synthesis and mathematical formulation of the process will be presented elsewhere (Heinmets and Herschman 1960).

Fundamental operational units for the enzyme synthesis are considered to be templates, pools (containing amino acid and co-factors), and inducers. A hypothetical scheme for induced enzyme synthesis is presented in figure 2.

The template is a basic structural unit for enzyme formation, which determines the biological specificity of the enzyme. In cell division the characteristics of a basic template system are retained by successive generation (in average). The genetic problem is not considered here. However, a cell may acquire a new template system or increase the existing system when placed in an environment where *inducers* are present. It is convenient to consider in the present analysis that an inducer interacts with an incomplete template (pretemplate) and an inducer-specific template is formed. Pretemplate surfaces have molecular configurations which make possible a competition between various inducers on particular interaction sites. Since the precursor pool for a pretemplate formation is considered limited, there may, consequently, be a competition for the precursor pool between various template-forming systems. It is considered that formation of new and constitutional template systems occur under the influence of a basic template system (T_0). Enzyme synthesis takes place when a template system interacts with an amino-acid pool. The scheme in figure 2 is self-explanatory.



- T_0 - original template system.
- T - pretemplate.
- S - metabolic substrate which acts as an inducer for the enzyme synthesis.
- TS - substrate specific template.
- P - pool, which is an aggregate of amino acids and cofactors.
- (TS-P) - a template and pool complex which can be a non-dissociable enzyme.
- E - a dissociable enzyme.
- I - pathway for template formation by induction.
- II - pathway for constitutional template formation.

Figure 2. A schematic model for enzyme synthesis.

It is well known that in cellular growth and activity, synthetic processes are most vulnerable to various interfering agents. This subject has been discussed in various symposia and publications, but due to the fact that so little basic information on these processes is available the treatment has been only general. It seems advisable to attempt a more detailed analysis, even at the speculative level, using the synthetic scheme in figure 2 as the base.

Why is synthesis so very vulnerable? The answer seems to be because it is such a complicated process and a multitude of specific molecular units and co-factors are involved. One can speculate that the most significant phases of synthesis can be classified as follows.

4.1. Template formation

It is especially important when synthesis of a new enzyme is concerned: it is either induced externally or initiated internally in a particular growth-phase of the cell. The presence of all precursors is required for the template formation. An interfering agent may disorganize the template-forming process by the alteration of: (a) original template (T_0), (b) precursor molecules or processes

by which they are formed, (c) substrates (S), which act as inducers. If in any of these steps a significant change takes place, template formation can be completely stopped or a malfunctional template system is formed. In the latter case the template may be inoperative or it may lead to a non-functional protein formation. The interference with the template-forming system can be considered to be generally 'fatal' to a synthetic system because synthesis is founded on it, and there are no direct alternatives for the process. If the template-forming system is modified partially, at least in principle, the cell may be able to survive via alternate metabolic pathways, but it will survive as an altered cell. However, in the case when the existing template can be used repeatedly, as in our analysis with dissociable enzyme production (Heinmets and Herschman 1960), synthesis will continue but at a reduced rate. This synthesis may be sufficient to maintain cellular growth at a lower rate, but if the cell division takes place, this synthetic system will be diluted out and the cell will lose its viability unless a new template is formed. It is suggested that such a limited enzyme formation could be the explanation for the commonly observed phenomenon that after the injury cells divide for several generations, but finally fail to divide, and die.

4.2. *Metabolic pool*

Since a definite number of amino acids are required for a specific enzyme synthesis, it is essential that they all be simultaneously available, otherwise the synthesis will be stopped. Consequently, any agent that interferes with the completeness of the pool will suppress enzyme synthesis. This can be accomplished by interference with the formation of amino acids, or by modification of already formed amino acids which may act as functional analogues. Analogues may act as inhibitors of the synthesis, or they may be incorporated during the process, but the result may be a non-functional protein. Alteration of various co-factors of the pool may, in general, have similar inhibitory effects.

The main difference between the non-functional template system and the pool system seems to be the following :

When the template system is altered, it is difficult to visualize how it can be restored, especially when extensive alteration of the original template (T_0) system has taken place. On the other hand, when the pool is incomplete or only some of its components are affected, there may be a delay in the synthesis or a partial non-functional protein formation, but the synthesis will be resumed when formation of amino acids takes place via normal or alternative pathways. Only when amino-acid-forming systems are disorganized in such a manner that a new pool cannot be formed could the pool deficiency affect cellular growth and viability. Otherwise there could be only a lag in the division. However, if the lag phase is too long, and thermal and proteolytic decomposition of some operational units is fast, it is possible that functional disturbances appear. It should be pointed out that the characteristics of functional disturbances may depend on the state and the phase of cellular division, since the content of operational units and pools is dependent on such factors. However, more experimental information is required before any fruitful speculations on this subject can be offered. Another important factor is the sensitivity of the various functional units to the interfering agents. Nucleic acids, which are the principal

components of a template, are relatively stable, especially when condensed and polymerized into the larger structural units. Free precursors have many more sites available for interaction with other molecules (agents) and represent a more vulnerable system. This may suggest that the existing template system (T_0) is more stable than the precursors. Injuries to (T_0) could be characterized as 'genetic' and are relatively rare events when compared with the other forms of injury that affect cellular viability. However, if only minor changes have taken place in the template system, it can be expected that in favourable conditions the cell will survive, but with an altered metabolic pattern.

5. ELEMENTS OF METABOLIC PATHWAYS AND THEIR RELATION TO FUNCTIONAL PROCESSES AND FUNCTIONAL DISTURBANCES

Some basic elements of the reaction patterns that can be considered to be operative in metabolic pathways are presented in figure 3. The simplest of the patterns would be a linear chain of sequential reactions in which one substrate-enzyme system feeds into another similar system. Various modifications appear when the linear system can form a positive loop and yield a cyclic process, or form a negative loop (direct or indirect) yielding a feed-back system. Several other complexes can be visualized and are represented in figure 3. By using individual elements, many complicated patterns of metabolic pathways can be composed (similar to a scheme as presented in figure 1, but in much higher degree of complexity).

It may be asked what would happen to the operational performance of a metabolic pathway when some unit suddenly becomes non-functional, or is altered in such a way that it has a negative affect on the system? The following examples could be cited.

1. Inactivation of an enzyme may stop the turnover of the substrate; accumulation of the substrate may induce synthesis of enzymes at a much higher rate than normal and so re-establish the enzyme; this is not so obvious when it is considered that the substrate could be inhibitory to some other enzyme and an excessive substrate concentration may even interfere with the synthesis of other enzymes.

2. A substrate could be converted by an external agent into a substrate analogue, and later may block not only the corresponding enzyme activity but also its synthesis.

3. Cyclic and other complexly-organized pathways may have inherent potentialities of self-disorganization. For example, when altered substrates at excessive concentrations lead to cross-inhibition, the balance between the synthesis and progressive disorganization may determine the outcome.

It is conceivable that in a case where a principal pathway becomes inoperative in the process of injury, a minor alternative pathway may become the principal pathway operating perhaps at lower rate. What are the conditions and requirements for the switch of pathways, and is the switch permanent or reversible? Such questions are all very important for the understanding of cellular injury, but they can be analysed only when quantitative formulations of the system have been established. It seems advisable from the experimental point of view to measure cellular-injury patterns in terms of metabolic reactions. This could provide some quantitative data. Such measurements should be

carried out immediately after the injury in conditions where the synthesis is essentially stopped, in order that the properties measured should not change essentially during the measurements. Such a procedure for estimation of enzyme injury could be carried out while protein synthesis is suppressed by specific inhibitors.

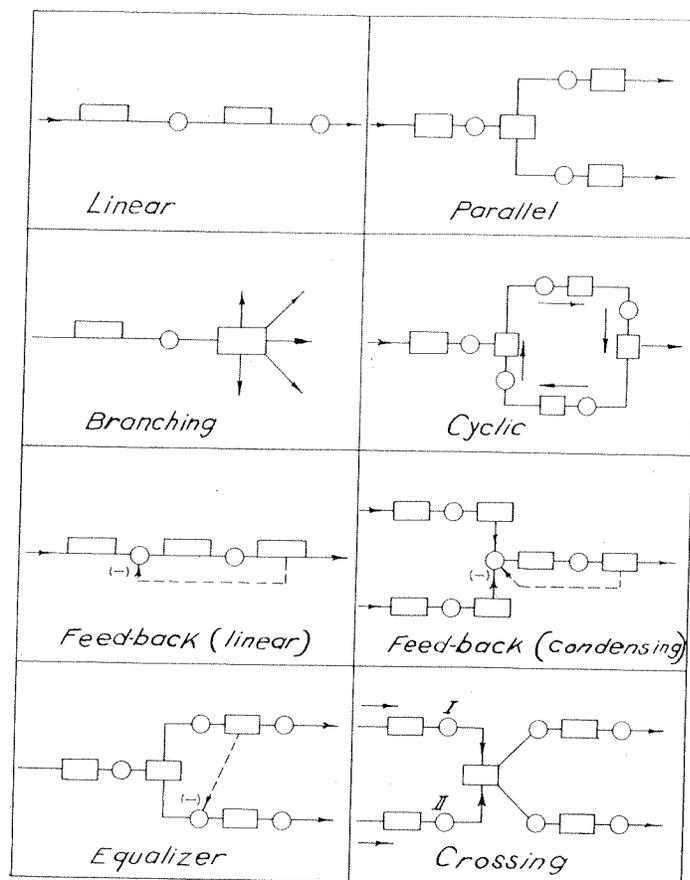


Figure 3. Some elements of metabolic pathways.

The ability of a cell to tolerate or acquire a particular injury pattern may depend on a multitude of factors, i.e. cellular metabolic pattern before the injury, level of metabolic pools available for the synthesis, length of time during which the injury is produced and environmental conditions, treatment of the cell after the injury and the medium of growth, etc. Obviously, in order to obtain quantitative data pre- and post-injury treatments have to be standardized. However, it is not so simple to compare various viability data, since the end-points of cellular viability are not at all definite. Some of the cells, when tested after the injury, may not divide in one media but may divide in another. Cells may not divide at all, or they may divide for a few generations, and then fail. Only detailed quantitative study of metabolic processes may yield information essential to the clarification of the complex viability issue.

Cellular growth and development represents an integrated operation of multiple synthetic processes, and it is essential that there be a balance between

the individual processes. Experimental observations of unbalanced growth leading to fatal and irreversible injury (Barner and Cohen 1956, Dagley and Walker 1956) clearly indicate the importance of synchronization at all levels of cellular processes. It is suggested that a process considered in its totality may fail only because some individual steps are inoperative. Consequently, the functioning of the overall process could be understood only when the elementary but basic steps can be described in detail in a quantitative manner. A series of calculations on sequential enzyme-substrate chain reactions, coupled with enzyme synthesis, have been carried out (in process of publication). Results suggest that metabolic processes, in certain levels, can be analysed quantitatively by using model systems, and new information may be obtained in analysing the process of cell injury.

La conception de la viabilité cellulaire est extrêmement complexe et a été l'objet de beaucoup de spéculation. Une théorie nouvelle est proposée concernant l'interprétation du dommage cellulaire et du procédé fatale. Des facteurs qui entravent l'organisation cellulaire et ses réactions ou qui en modifient d'une façon étendue peuvent être considérées, dans un sens biologique, de dommager ou de tuer la cellule. Une telle intervention peut être le resultat de l'interaction des molecules cellulaires avec une multitude des agents. En termes des *sub-unités fonctionelles* de la cellule, les changements suivants peuvent avoir lieu: (1) l'inactivation ou le changement des enzymes, (2) la modification des templates pour le procédé synthétique, et (3) le changement et la decomposition des metabolites, des intermédiaires et des co-facteurs.

Une analyse est faite afin de déterminer comment le procédé cellulaire peut être touché par des agents extérieures et comment les disorganisations fonctionelles pourrait avoir lieu. Il est proposé qu'il y a une relation entre le phénomène de la disorganisation fonctionelle et le dommage et la mort cellulaire.

Der Begriff der Zellenlebensfähigkeit ist äusserst kompliziert und bildete den Gegenstand vielfacher Spekulation. Eine neue Theorie wird nun vorgeschlagen, um die Verletzung der Zelle und den tödlichen Vorgang auszulegen. Faktoren, die die Zellorganisation und Reaktionen stören oder weitgehend modifizieren können in einem biologischen Sinne im Hinblick darauf, ob sie die Zelle verletzen oder töten, untersucht werden. Eine solche Störung könnte das Resultat einer Zwischenwirkung von Zellulärmolekülen mit einer Vielfältigkeit von Stoffen sein. Ausgedrückt in *funktionellen Untereinheiten* der Zelle können folgende Änderungen stattfinden: (1) Eine Inaktivisierung oder eine Veränderung der Enzyme. (2) Eine Modifizierung der Templatflächen für das synthetische Verfahren, und (3) eine Veränderung und Auflösung der Metaboliten, der Intermediäre und der Co-Faktoren.

Eine Analyse wird vorgenommen, wie die Zellvorgänge von äusseren Stoffen beeinflusst sein könnten und wie funktionelle Organisationsstörungen entstehen können. Es wird vorgeschlagen, dass eine Beziehung zwischen dem Phänomen der funktionellen Organisationsstörung und der Zellenverletzung und des Zellentodes besteht.

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