

A Model-System for the Induction of an Enzymatic Transport System by an External Substrate

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ABSTRACT

A model-system has been developed to analyze the formation of an adaptive enzyme system for the transport of a substrate into the cell. Flow schemes and differential equations are established and the system is programmed for the analog computer. Kinetic data on enzyme formation are obtained during various modes of substrate inducer introduction into the system. The model-system presented reveals highly flexible and well-controlled operational characteristics in a highly variable chemical environment.

INTRODUCTION

In the early phase of enzyme induction studies it appeared that only one enzyme could be induced by one inducer. It soon became apparent, however, that several enzymes are involved, including permeases and several transport enzymes. Subject matter has been analyzed and reviewed [5, 10] and it appears that a substrate type of enzyme induction represents a complex process. Furthermore, a sequential type of enzyme induction can take place, which is essential for the enzymatic metabolism of the inducer substrate. Among many studies in the field, we can refer to an elegant set [9, 11] that reveal an intricate complex of control and regulatory mechanisms operating in an inductive enzyme system. It is apparent that inductive systems are very basic for the adaption of cells to cope with environmental changes. These changes might be modifications in the nutritional medium or of other environmental factors producing

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compensatory physiological processes. It is evident that the inductive phenomenon is very basic, common to all kinds of species and cells, such as fungi, plants, bacteria, and mammals [1, 5-8].

The question can be posed, how does the cell recognize, and how is it aware of, environmental changes? How does a cell sense its environment and react when that environment's molecular composition is altered, as when there is a change in either nutritional source, macromolecular environment, or cell-to-cell relations? More fundamentally, how does a cell know that there are changes in cellular environment? If the mechanisms on the cell surface and its environment provide the information that there are changes in that environment, what are the mechanisms behind those reactions to produce some compensatory processes in the cell to deal with the altered conditions? The extensive data available regarding the functional elements and synthetic processes that occur in the cell should be adequate to permit postulation of mechanisms for the cellular sensing of its environment. There is no need to postulate new mechanisms or search for new elements to serve that purpose. Therefore, it is considered that the basic functional mechanisms that are operative in all cells can, in specific combinations, provide some new activity patterns that are consistent with a particular type of cell function. These have been gained during the course of development and differentiation.

We consider that the basic elements that participate in providing information from external environments are:

- (1) exoenzymes (a) bound on the cell surface, (b) loosely bound in the colloidal matrix around the cell, and (c) dissolved in the medium;
- (2) transport enzymes that operate in the confines of cell surface and membranes;
- (3) small molecular species that interact with enzymes or enter by diffusion and thus provide information about composition of environment.

The role of substrates transported into the cell may be manifold. For example, a molecule could be used as an item of nutrition, or it could represent a foreign substance, in which case cellular reaction has to be different. Reaction to a nutritional element is a direct one when this molecule can be processed via an existing operational system. However, if the operational system cannot metabolize or handle this type of molecule, then new compensatory measures have to be taken by the cell. There could be the development and synthesis of new functional elements to provide an operational pathway to either use the new element or get rid of it. In a multicellular system and in a complex organism, differentia-

tion can provide specialized cells where the functions of various individual cells are correlated with the total operational mechanism of the system. Consequently, the operational procedure in the complex organism reveals a cooperative and integrated activity among various cells.

We consider that exoenzyme systems in the external environment of the cell are the *basic receptors* that provide structural information from the environment and thus represent *cellular sensing devices*. Usually, the basic informational molecules are small and interact directly with the enzyme system. However, when new macromolecular species appear in the cellular environment, then the primary reaction for gaining information about that macromolecular entity is via a degrading process. If a cellular interaction involves other cells, then cellular sensing has to provide information about its neighbors. We assume that all structural species in a cellular environment can be recognized and that information can be processed by the same general type of mechanism. Therefore, we consider that once a proper theory and concept have been developed as to how a cell senses its environment, how it processes this information, and how it sets up a mechanism to react properly to environmental changes, then we will be in a position to interpret many perplexing biological phenomena concerned with cell-environment interrelationships. The basic processes of cellular sensing could provide information in the following areas: (a) induction and development of the exoenzyme system by small substrate molecules or by the degradation products of macromolecules; (b) induction and formation of antibodies; and (c) cell recognition and cell-to-cell interaction.

We consider that the basic mechanisms for these processes have similar operational features. However, integration of specific mechanisms into multiple patterns will characterize different cellular processes. In all cases, the primary sensing mechanism is essential for the degradation or sampling of environmental species. Consequently, the exoenzyme system provides continuous information about cellular environment. When these species are altered, however, the cell in the framework of genetic background makes the proper adjustment to new conditions. We plan eventually to analyze and propose models for all three basic phenomena. However, the scope of the problem is so extensive as to require that each of the phenomena be treated separately [3, 4]. Therefore, we will analyze here only the primary induction of an enzyme system, which is prerequisite for the entry and metabolism of a new substrate. In order to elucidate cellular adaptive processes and gain insight into operational

features, we must develop model-systems. These can be formulated mathematically and analyzed by simulation techniques on computers. Thus the system can be studied in a quantitative manner and kinetic processes can be followed as a function of time.

MODEL-SYSTEM AND MATHEMATICAL FORMULATION

Since the induction of a new enzyme system for transferring a new external substrate into the interior of the cell represents a very common phenomenon, it would be instructive to develop a general model-system that contains many functional characteristics known to operate in such enzyme systems. Thus the operational characteristics could be studied in a variety of states and the effects of specific elements or regulatory features could be elucidated. The entry of an inductive substrate into the cell is prerequisite for the enzyme induction process. In general,

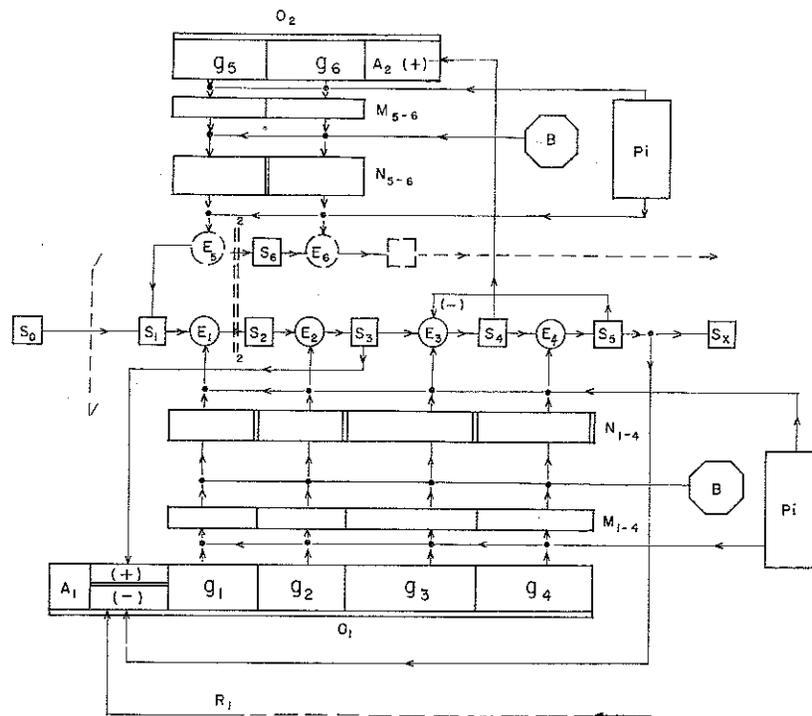


FIG. 1. A schematic model for induction of transport enzyme system. Symbols are given in Table I.

the principal modes of entry are those in which: (a) a substrate interacts with a permease; (b) a substrate enters by diffusion via cell membrane and subsequently interacts with an enzyme; (c) a polymeric or insoluble solid substrate is degraded, after which its degradation products enter the cell.

TABLE I
SYMBOLS FOR MODEL-SYSTEM

$O_1 (G_1, G_2, G_3, G_4)$	Slightly active operon for genes producing messenger RNA M_{1-4} ; (O_1^* highly active state for operon)
A_1	Activating site for operon O_1
A_2	Activating site for operon O_2
$O_2 (G_5, G_6)$	Inactive operon; O_2^* is active state and messenger RNA M_{5-6} is produced.
M_{1-4}	Messenger RNA for the synthesis of enzymes $E_1, E_2, E_3,$ and E_4
M_{5-6}	Messenger RNA for the synthesis of enzymes E_5 and E_6
N_{1-4}	Template for the synthesis of enzymes $E_1, E_2, E_3,$ and E_4
N_{5-6}	Template for the synthesis of enzymes E_5 and E_6
E_1	Exoenzyme for transporting substrate S_1
E_2	Enzyme that converts substrate S_2 into substrate S_3
E_3	Enzyme that converts substrate S_3 into S_4
E_4	Enzyme that converts substrate S_4 into S_5
$S_1, S_2, S_3, S_4, S_5, S_6$	Substrates
S_0	External substrate introduced into the system that passes through diffusion barrier
E_5 and E_6	Second induced enzyme for substrate S_1 transport; it is in inactive state; active state E_5^* is produced via activation process (k_{34})
R_1	A repressor that converts operon O_1 completely into inactive state
k_1, \dots, k_n	Various rate constants
E_3'	Inactive state of E_3
P_i	Internal pool for enzyme synthesis
B	Ribosomes
X	Breakdown products
1-----1	Diffusion barrier
2-----2	Membrane barrier

In all three cases, for the system to become operational it is essential that a small residue of enzymes be present to initiate the first reactions. This means that in case (a) there would be present a surface enzyme

TABLE II
FLOW EQUATIONS

1. $O_1 + P_i \xrightarrow{k_1} M_{1-4} + O_1$	16. $E_2 + S_2 \xrightarrow{k_{21}} E_2 + S_3$
2. $O_1 + S_3 \xrightarrow{k_2} O_1 \xrightarrow{k_2^*} O_1 + X$	17. $E_3 + S_3 \xrightarrow{k_{22}} E_2 + S_4$
3. $O_1 + P_i \xrightarrow{k_3} M_{1-4} + O_1^*$	18. $E_3 + S_5 \xrightleftharpoons[k_{-23}]{k_{23}} E_3'$
4. $M_{1-4} \xrightarrow{k_5} X$	19. $E_4 + S_4 \xrightarrow[k_{-24}]{k_{24}} E_4 + S_5$
5. $O_2 + S_4 \xrightarrow[k_6]{k_6^*} O_2 \xrightarrow{k_7} O_2 + X$	20. $E_1 \xrightarrow{k_{25}} X$
6. $O_2 + P_i \xrightarrow{k_8} M_{5-6} + O_2^*$	21. $E_2 \xrightarrow{k_{26}} X$
7. $M_{5-6} \xrightarrow{k_9} X$	22. $E_3 \xrightarrow{k_{27}} X$
8. $O_1 + S_5 \xrightarrow{k_{10}} O_1 + X$	23. $E_4 \xrightarrow{k_{28}} X$
9. $M_{1-4} + B \xrightarrow{k_{11}} N_{1-4}$	24. $E_5 \xrightarrow{k_{29}} X$
10. $N_{1-4} + P_i \xrightarrow{k_{12}} E_1 + E_2 + E_3 + E_4 + N_{1-4}$	25. $S_3 \xrightarrow{k_{30}} S_X$
11. $M_{5-6} + B \xrightarrow{k_{13}} N_{5-6}$	26. $E_5 \xrightarrow{k_{31}} E_5'$
12. $N_{5-6} \xrightarrow{k_{14}} X$	27. $S_0 \xrightarrow{k_{31}} S_1$
13. $N_{1-4} \xrightarrow{k_{15}} X$	28. $E_5' + S_1 \xrightarrow{k_{35}} S_6 + E_5'$
14. $N_{5-6} + P_i \xrightarrow{k_{16}} E_5 + E_6 + N_{5-6}$	
15. $E_1 + S_1 \xrightarrow{k_{20}} [E_1 S_1] \xrightarrow{k_{20}} E_1 + S_3$	

that acts as a sensor enzyme, thus providing the genetic system with information about micromolecular changes in environment. In case (c) small residues of exoenzymes degrade polymeric substrates into the products that can be transported into the cell. In case (b), although the first entry of substrate is by diffusion, subsequent transport steps are considered to be enzymatic. The case where a substrate would have access to genetic elements only via diffusion processes is improbable and is not considered here. Thus in all three cases the role of a substrate-inducer is to trigger an already existing genetic mechanism into operation, where part of the system may already be operational, but at very low level of activity.

Figure 1 represents (see also Table I) a model-system that contains the following main operational features. (1) Operon O_1 is slightly active and consequently enzymes E_1 - E_4 are present at low concentrations; (2) operon O_2 can be activated via a repressor gene or it could be activated directly. Since we are dealing with a conceptual system, for reasons of

TABLE III
DIFFERENTIAL EQUATIONS FOR THE SYSTEM

1. $\dot{M}_{1-4} = k_1 P_i O_1 + k_4 P_i \dot{O}_1 - k_5 M_{1-4} - k_{11} B M_{1-4}$
2. $\dot{O}_1 = k_3 \dot{O}_1 - k_2 S_3 O_1 + k_{10} S_5 \dot{O}_1$
3. $\dot{O}_1 = -\dot{O}_1$
4. $\dot{O}_2 = k_7 \dot{O}_2 - k_6 S_4 O_2$
5. $\dot{O}_2 = -\dot{O}_2$
6. $\dot{M}_{5-6} = k_8 P_i \dot{O}_2 - k_9 M_{5-6} - k_{13} B M_{5-6}$
7. $\dot{N}_{1-4} = k_{11} B M_{1-4} - k_{16} N_{1-4}$
8. $\dot{E}_1 = k_{12} N_{1-4} P_i - k_{25} E_1 - k'_{20} E_1 S_1 + k_{20} [E_1 S_1]$
9. $\dot{E}_2 = k_{12} N_{1-4} P_i - k_{25} E_2$
10. $\dot{E}_3 = k_{12} P_i N_{1-4} - k_{27} E_3 - k_{23} S_5 E_3 + k_{-23} E'_3$
11. $\dot{E}_4 = k_{12} P_i N_{1-4} - k_{25} E_4$
12. $\dot{N}_{5-6} = k_{13} B M_{5-6} - k_{14} N_{5-6}$
13. $\dot{E}_5 = k_{19} P_i N_{5-6} - k_{34} E_5$
14. $\dot{S}_1 = k_{31} S_0 - k'_{20} S_1 E_1 - k_{33} E'_5 S_1$
15. $\dot{S}_2 = k_{20} [E_1 S_1] - k_{21} E_2 S_2$
16. $\dot{S}_3 = k_{21} E_2 S_2 - k_{22} S_3 E_3 - k_2 S_3 O_1$
17. $\dot{S}_4 = k_{22} S_3 E_3 - k_{24} S_4 E_4 - k_6 S_4 O_2$
18. $\dot{S}_5 = k_{24} S_4 E_4 + k_{-23} E'_3 - k_{23} S_5 E_3 - k_{23} S_5 E_3 - k_{32} S_5 - k_{10} S_5 \dot{O}_1$
19. $\dot{E}'_3 = k_{23} S_5 E_3 - k_{-23} E'_3$
20. $\dot{E}'_5 = k_{34} E_5 - k_{30} E'_3$

simplicity we assume that operon O_2 is completely inactive, but becomes operational when a substrate enters the cell via enzyme E_1 - E_4 system. (3) Enzyme concentration in the operon O_1 system is regulated by a negative feedback loop, whereby end substrate S_5 acts as operon O_1 repressor. (4) Activity of enzyme chain E_1 - E_4 is also controlled by S_5 via a negative feedback loop at the E_3 level. (5) Operon O_1 activity is increased when the substrate enters the enzymatic pathway; enzyme level is consequently a function of the concentration of external substrate-inducer. (6) The end products of the enzyme reaction chains will be used for the metabolic and synthetic processes.

The model-system in Fig. 1 was designed to study the most elementary processes in substrate-type induction. It is evident that the operon O_1 system alone can provide substrate S_0 with a proper enzyme system.

In order to diversify the induction concept, however, a parallel enzymatic system (O_2) is induced, thus providing means for a cell to increase drastically the transport and metabolism of a new substrate. In cellular metabolic systems, many patterns of parallel operations occur. For example, besides a constitutive pathway, there is also an inductive pathway for a substrate absorption [8]. In actual cellular systems, which are complex, many simultaneous regulatory mechanisms operate on enzyme activity and enzyme synthesis level. In essence these mechanisms entail the basic features that are incorporated into our model-system. We do not consider here competitive actions of various substrates and dominance of various basic metabolites in the inductive processes. In the symbolic sense a dominant inductive regulation is exercised on operon O_1 level by an inducer product R_1 , which represses O_1 activity.

Operation of the model-system can be studied in detail in conjunction with Table I and the flow schemes in Table II. A set of differential equations (Table III) has been established and programmed for the analog computer, which is used as a mathematical tool [2].

RESULTS OF COMPUTER ANALYSIS

Once an operational model has been established on the analog computer, kinetics of the system can be studied under various experimental conditions as function of time. In Fig. 1 elements of the operon O_1 system are presented. Since O_1 is slightly active in the normal state, messenger M_{1-4} and enzymes E_1-E_4 are always present. The steady level of sensor enzyme E_1 concentration in the absence of an external inducer is indicated in Fig. 2 at the time S_0 is introduced (\downarrow). (All curves represented in Figs. 2-9 are recorded directly on the analog computer.) Subsequently, after a temporary decline, there is a drastic increase in E_1 concentration. This increase is also reflected in messenger M_{1-4} and active operon O_1^* concentration levels. After an initial transient phase, all three entities establish a steady concentration level. Figure 3 reveals the activation of the operon O_2 system. All entities are initially at zero concentration level, but after a certain delay, active operon O_2^* is produced and subsequently messenger M_{5-6} and enzyme E_5' appear. After a certain interval of time, concentration of these entities reaches a constant level. However, the level depends on substrate S_0 concentration. This is demonstrated in Fig. 4, in which levels of enzymes E_1 and E_5' are presented at different substrate S_0 concentrations.

The regulatory effect of an end product of an enzymatic chain reaction is illustrated in Fig. 5. It is evident that substrate S_5 can effectively reduce the level of enzyme E_2 . Since substrate S_5 acts on operon O_1^* all enzymes in the chain E_1 - E_4 are coordinately controlled.

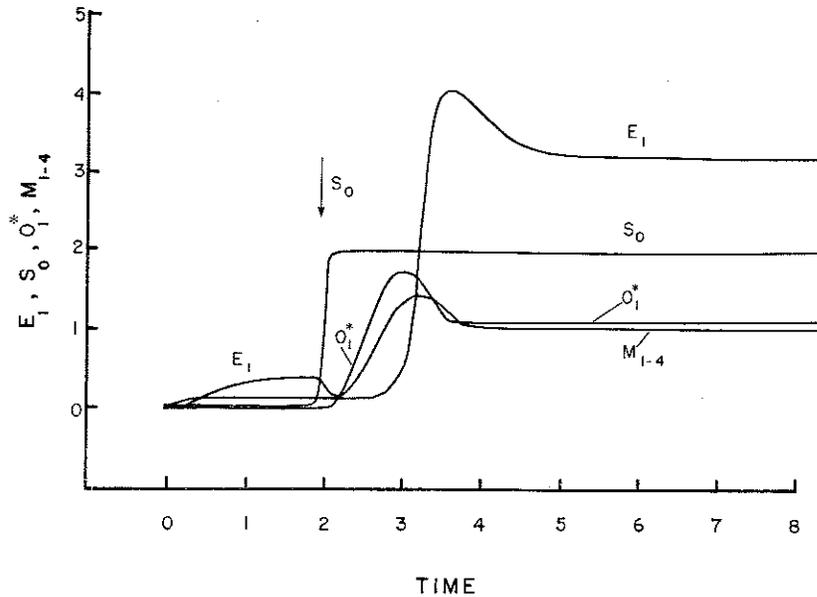


FIG. 2. The level of operon O_1^* , enzyme E_1 , and messenger M_{1-4} after introduction (\downarrow) of substrate S_0 .

Two operons that provide two parallel enzymatic systems are both controlled by single substrate-inducer S_0 and are firmly coupled. The question can be posed: What happens when the inducer S_1 is a substrate for enzyme E_1 but not for the enzyme E_2' ? Figure 6 shows the concentrations of both enzymes in conditions where rate constant $k_{33} = 0$ and $k_{33} = 1.0$. It is evident that there is only minor reduction of E_1 , while E_2' establishes itself after a certain time lag at a slightly lower level.

It is of interest to observe how an enzymatic transport system performs in a transient induction condition. Previous experiments revealed that the level of enzyme concentration was coupled to the concentration of external substrate S_0 . So long as the S_0 level was constant, enzyme concentration was also maintained at a definite level. In cellular growth,

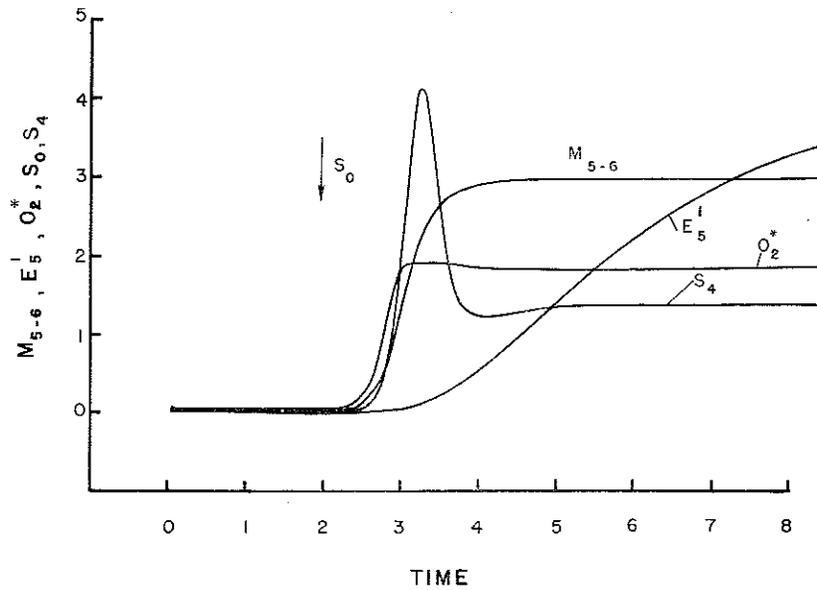


FIG. 3. The level of operon O_2^* , messenger M_{5-6} , substrate S_4 , and enzyme E_5' after introduction of substrate S_0 .

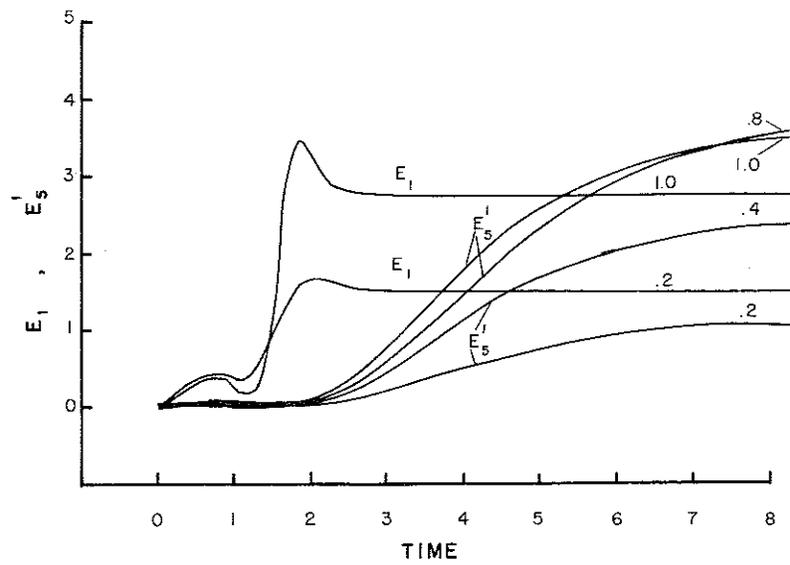


FIG. 4. The effect of substrate S_1 concentration on enzyme E_1 and E_5' synthesis. Relative S_0 concentrations are indicated on curves.

inductive enzymes provide means for cells to adapt themselves to the variable environmental conditions. For example, a cell may be placed in an environment where only a new substrate is present, but the amount of the substrate is limited and it will be exhausted. Figure 7 shows the effect of S_0 concentration on substrate S_1 and enzyme E_5 when a narrow pulse of S_0 is introduced into the model-system at different concentration levels. This is equivalent to a laboratory experiment where cells are

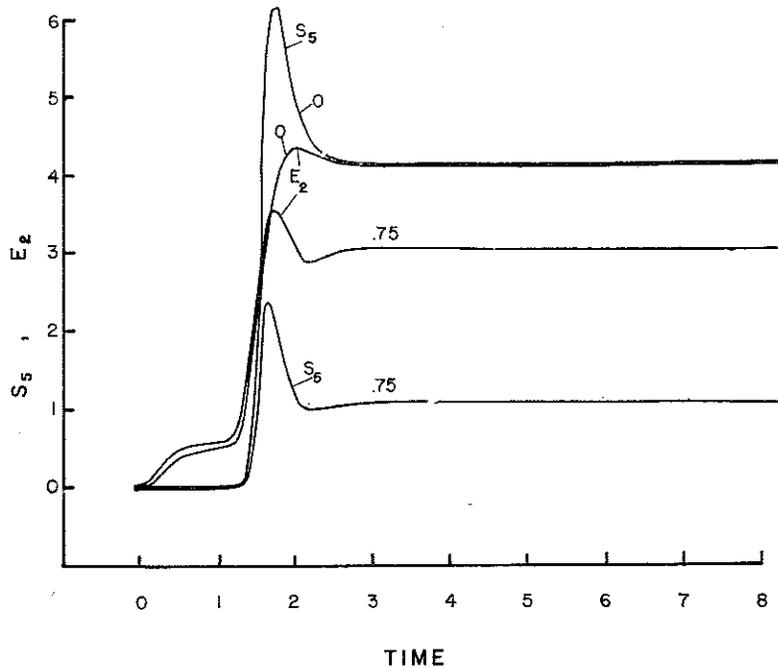


FIG. 5. Demonstration of the regulatory effect of end substrate S_5 on operon O_1^* (Table II, Eq. (8)). Enzyme E_2 and substrate S_5 recorded at $k_{10} = 0$ and $k = 0.75$ values.

exposed for the same time to a substrate-inducer at different concentration levels. It is evident that a transient pulse of S_1 is produced (Eq. (27), Table II), followed by E_5 production. Enzyme appears at different concentration levels. Since enzyme E_5 is unstable, its concentration declines, after reaching a peak value, to zero level. In Fig. 7 only the maximum concentration levels of E_5 are presented. Other functional entities of the system reveal similar kinetic characteristics.

During the experiments on the computer it was observed that in the range of certain rate constant values, the enzyme concentrations exhibited oscillatory characteristics, even when the external substrate S_0 is maintained at constant level. Figures 8 and 9 demonstrate enzymes E'_5 and E_1 , respectively. Enzyme E_1 concentration level is constant when the relative value of rate constant $k_{31} = 0.3$, but has an oscillatory ripple when $k_{31} = 0.1$. However, it appears that the average value of enzyme con-

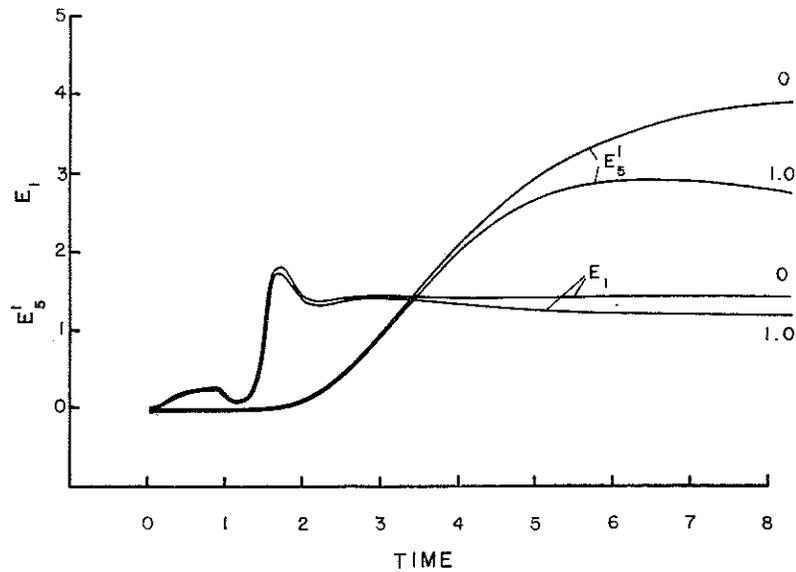


FIG. 6. Concentrations of enzymes E_1 and E'_5 at two different rate constant k_{31} values (0 and 1.0).

centration as a function of time is constant. What is the significance of such oscillatory characteristics of enzyme concentration levels in biological processes? There is no particular advantage for a cell to have an oscillatory enzyme concentration at a constant substrate level. It appears that the oscillatory phenomenon is basically caused by regulatory feedback features. The system is normally fairly "damped," but at certain parametric values oscillations may become pronounced. Since average enzyme concentration in an oscillatory state is constant, the transport system should function normally in these conditions also.

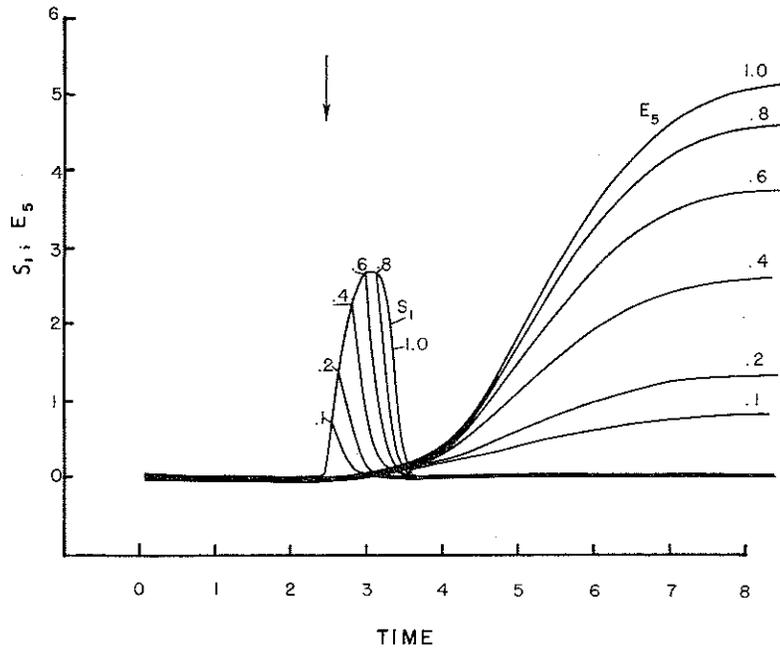


FIG. 7. The effect of substrate S_0 concentration. Enzyme E_5 and substrate S_1 levels after introduction of a pulse-type dose of S_0 . Arrow indicates the start of the pulse and numbers on curves indicate relative concentration level of S_0 .

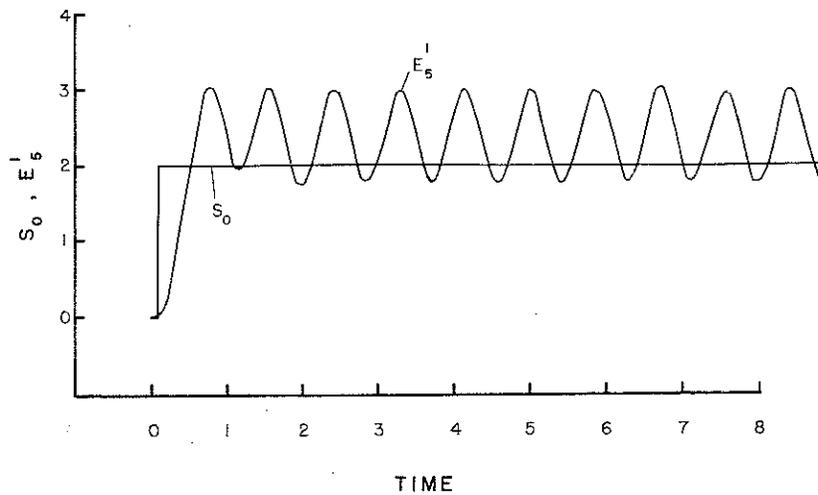


FIG. 8. Oscillatory state in enzyme E_5' concentration level. After the introduction of substrate S_0 it is maintained at constant concentration level.

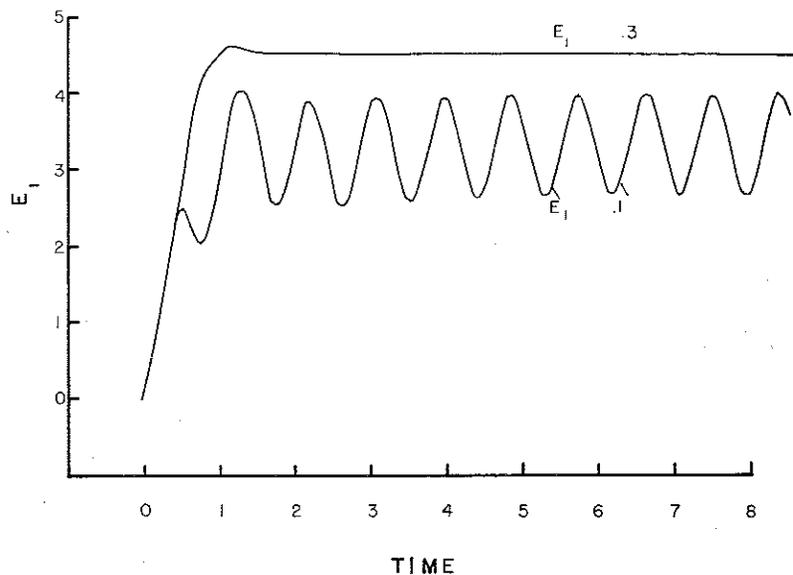


FIG. 9. The effect of rate constant k_{31} on enzyme E_1 on level. Numbers on curves indicate relative k_{31} values.

CONCLUSIONS AND SUMMARY

Analog computer analysis of an adaptive enzyme system reveals that model-systems can yield useful information about operational characteristics of the system. It is evident that the level of enzyme concentration is dependent on the substrate-inducer concentration. Therefore, the cell is able to avoid excessive or insufficient enzyme formation. Tight coupling between enzyme level and substrate-inducer level provides means for the cell to optimize its synthetic processes according to the environmental stimuli. Furthermore, regulatory features protect the cell by limiting maximum enzyme levels when concentrations of substrate-inducer become very high. The presence of low-level sensory enzymes guarantees that the cell is able to detect the presence of a new substrate and thus initiate further synthesis of enzymes, provided that other substrates present are not inhibitory. Thus the cell is able to cope with extreme variations with its chemical environment.

The model-system proposed herein was used to analyze the adaptive process from a conceptual point of view. The principles and methods used to establish and analyze the model-system can, however, be used

for the analysis of actual metabolic and synthetic schemes in a variety of cells.

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