

THEORETICAL ANALYSIS OF MODELS FOR ENZYME SYNTHESIS

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Biological processes at the molecular level are extremely complex and descriptive analysis alone is completely inadequate for evaluation of the relationships between individual functional units. Therefore, it is essential that formalized relationships be established between the operational elements of the system and that the problem be solved in a quantitative manner. In order to facilitate the analysis, it is often useful to set up a functional model of the system. The validity of such models depends essentially on the available experimental data and the ingenuity of the model builder. Since the performance of a realistic model will be compared with that of the normal system, its success depends essentially on the soundness of the model. We assume, of course, that adequate mathematical procedures are used. In contrast, highly sophisticated mathematical treatment does not produce any significant results when applied to a poor model.

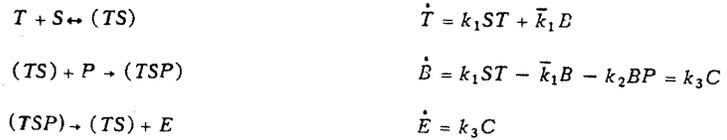
At the current level of information, the models representing biological processes are, of necessity, simplified and simulate the system in terms of some general parameters. This way, only the cross-behaviors of the system will be presented, but when more detailed experimental data become available, a more refined model system, leading to a more detailed description of the system, can be developed. Complex metabolic processes can be analyzed on such premises. In the current work we will consider the formation of enzymes on a model-system basis. Previous work in this area (Heinmets, 1960; Heinmets and Herschman, 1960, 1961a, 1961b) revealed that the conventional mathematical approach was not adequate for treatment of systems composed of an aggregate of operational parameters. Computer analysis seems to be suggested. However, in order to analyze complex biological processes, it is essential that methods should be developed for treatment of such systems. The purpose of this paper is to demonstrate the enzyme synthesis in systems where regulatory and feedback mechanisms are operative. In mathematical terms, the output of such a system is nonlinear and accurate solutions are unobtainable. These difficulties can be overcome by use of a suitable computer. An analysis will be carried out with the aid of an analog computer and models will be presented in sequence of increasing complexity. At the current phase, the main objective is to develop methods of treating the complex synthetic processes. In addition, it serves to demonstrate for the experimental biologist that metabolic schemes can be analyzed in terms of transient-state end products and intermediates.

It is our opinion that such a quantitative approach to synthetic and other cellular processes is necessary, since only by understanding of normal cellular processes can one gain insight into abnormal processes, such as malignancy

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and alteration of cellular characteristics during the aging of species, etc. Furthermore, the action of agents (drugs, antibiotics, antimetabolites, etc.) which interfere with the normal metabolic process and synthesis can be understood wholly when metabolic systems are presented in terms of quantitative relations and interactions. This, of course, is a prerequisite for rational therapy.

Scheme I-1 demonstrates the notation and the use of operational elements (in systems involving multiple elements, subscripts are used):



where:

T = Template concentration
 S = Inducer concentration
 B = Specific template concentration (TS)
 C = Complex concentration (TSP)
 E = Enzyme concentration
 P = Pool

This notation is used throughout the paper. When new symbols are added, they will be mentioned specifically. A systematic study of various schemes for synthesis will be carried out. Special emphasis will be placed on variations of various parameters and their effect on the end product of the system.

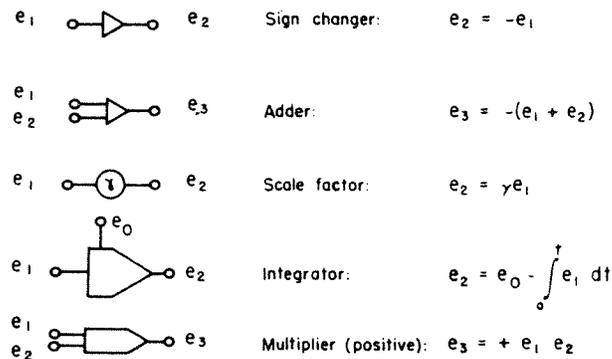
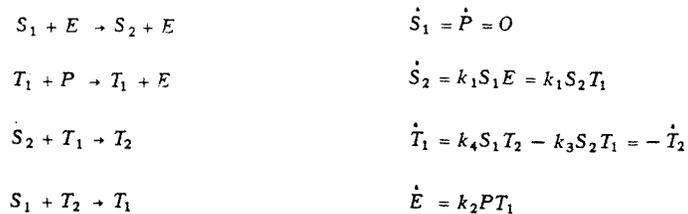


Fig. 1. Symbols for analog computer circuit. (From: Jackson, A. S. 1960. Analog computers. McGraw-Hill, New York.)

The first system considered was II-1 (see Fig. 2):



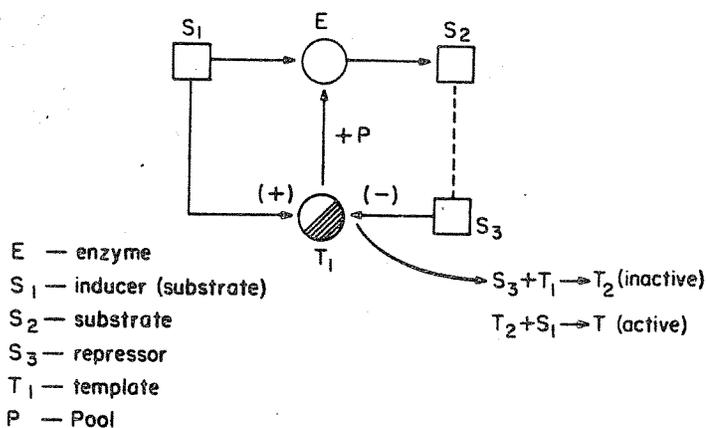


Fig. 2. Single-step enzyme synthesis with repression by the end product (S₂) on template level.

Since an obvious first integral is $T_1 + T_2 = T_0 = \text{const}$, and one may choose the rate of the first reaction as a time unit, viz., assume $k_1 S_1 = k_4 S_1 = 1$, giving only the intermediate and final reaction times as parameters,

$$k_2 P = \beta \quad \text{and} \quad k_3 = \gamma$$

one obtains the following simplified form of the equations,

$$\dot{T}_1 = T_0 - T_1 - \gamma S_2 T_1$$

$$\dot{S}_2 = E - \gamma S_2 T_1$$

$$\dot{E} = \beta T_1$$

which set may be readily coded for the computer as in Fig. 3. In the computer circuit, we have dropped the subscripts on T_1 and S_2 , since in the final equations these are the only quantities of this form involved. This convention is also adhered to in the output curves shown in Figs. 4a and 4b.

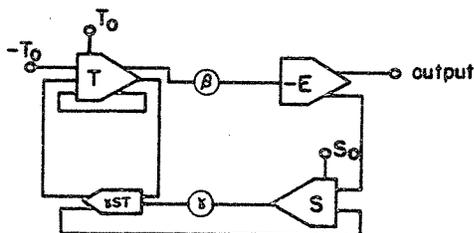


Fig. 3. Schematic computer diagram for system II-1 (see Fig. 1 for symbols).

In Fig. 4a, the machine computation for E is plotted for values of S_0 (the initial value of S_2) of 0, 4, 10, and 20 v. In this scheme T_0 was arbitrarily taken as 10 v and β was fixed at 0.1, whereas γ was taken as 1.0. In Fig. 4b, T is plotted for the same conditions. Also indicated on this plot, as dotted lines, are two representative S curves. We first note that since E is essentially the integral of T , the extreme fluctuations in T for small times give rise only to a slight displacement of the various E curves, one from another. What is more signif-

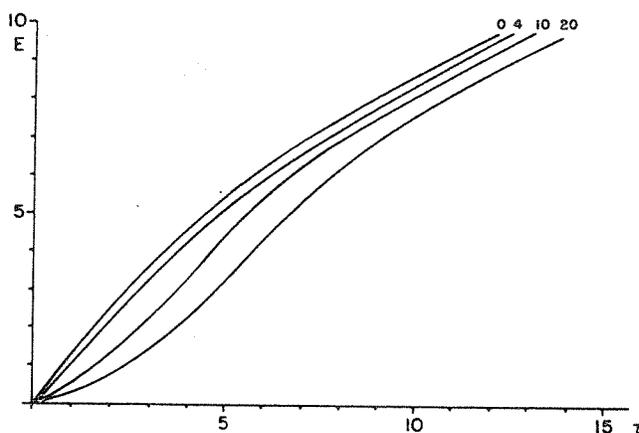


Fig. 4a. Enzyme curves for system II-1, for various values of S_0 as indicated.

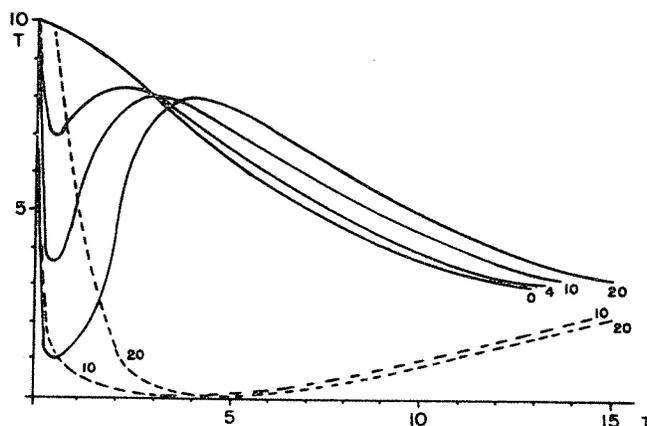


Fig. 4b. Template curves for system II-1, for various values of S_0 as indicated. The dotted curves give the inducer (S) for two representative values of S_0 .

icant, however, is that the various T curves, which begin in such a widely different manner, all approach the same general form, except for a slight relative displacement. This, one of the most important properties of the nonlinear system, will show itself in all the future variations as well. Briefly, it can be stated as follows: even though the initial conditions play a large role in the initial behavior of the systems, ultimately the behavior becomes substantially independent of them, being a function, primarily, of the form of the equations. Mathematically this can be seen from the following formal integral of the equations

$$S = \exp\left(\frac{-\gamma E}{\beta}\right) \left[S_0 + \int_0^t E \exp\left(\frac{+\gamma E}{\beta}\right) dt \right]$$

Thus, since E is an apparently increasing function of the time, the first term in the brackets ultimately damps out to zero, leaving the asymptotic behavior independent of the initial condition in S_0 .

Attempts to solve the equations by successive approximation, starting from an asymptotic series for this integral, are not wholly consistent since the series

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has almost no radius of convergence. For example, one may obtain the following formal series for S :

$$S = \frac{\beta E}{\gamma \dot{E}} - \frac{\beta^2 (E/\dot{E})}{\gamma^2 \dot{E}} + \dots$$

so that

$$\gamma S \dot{T} = E - \frac{\beta (E/\dot{E})}{\gamma} + \dots$$

implying that S ultimately saturates, as does E (to the value T_0/β , in this case, about 100 v). The difficulty of this line of approach, however, is that the perturbation term in the series is not really small, and, in fact, diverges (the presence of a time derivative of a constant in the denominator).

The saturation properties in the solution become much more accentuated when intermediates are placed into the circuit. This becomes apparent in Scheme II-2, in which an additional S is placed in an intermediate position (see Fig. 5):

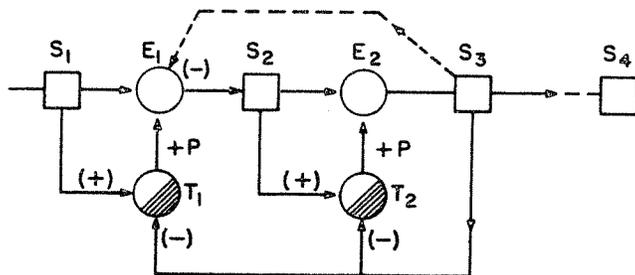
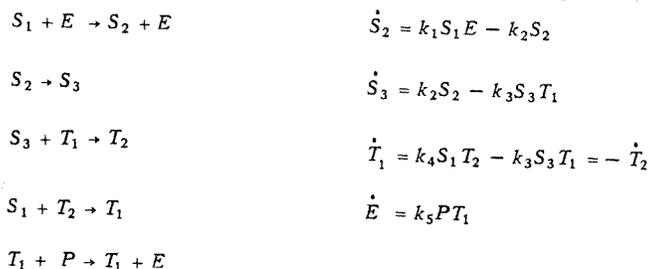
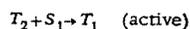
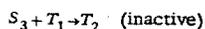


Fig. 5. Single-step enzyme synthesis with repression by the converted end product (S_3) on template level.



Again we choose as our time unit the time of the first process and set $k_1 S_1 = k_4 S_1 = 1$, taking for the other rate constants $k_2 = \alpha$, $k_5 P = \beta$, and $k_3 = \gamma$, so that with the first integral $T_1 + T_2 = T_0$, we have the simplified set

$$\dot{S}_2 = E - \alpha S_2$$

$$\dot{S}_3 = \alpha S_2 - \gamma S_3 T_1$$

$$\dot{T}_1 = T_0 - T_1 - \gamma S_3 T_1$$

$$\dot{E} = \beta T_1$$

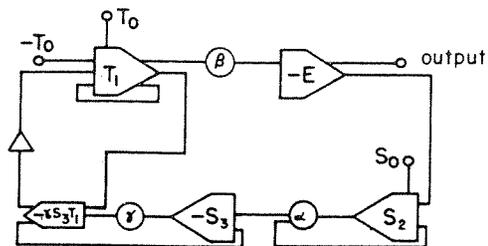
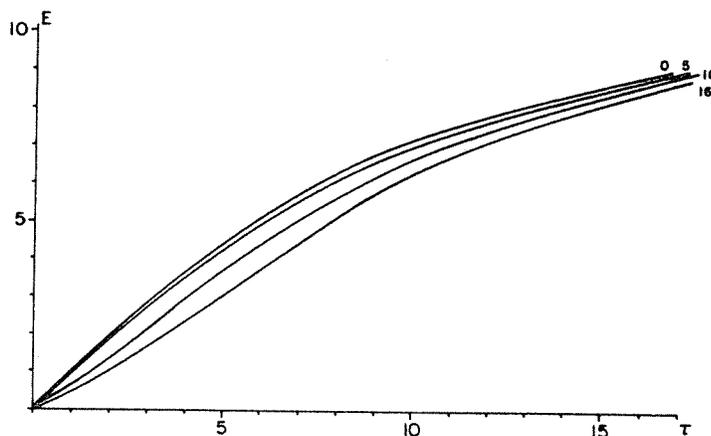
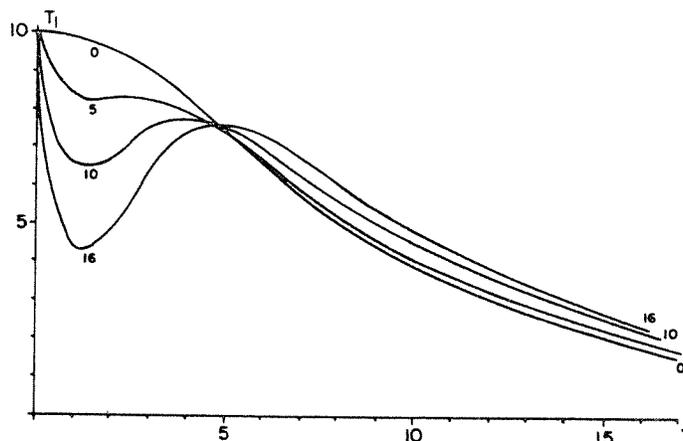


Fig. 6. Schematic computer circuit for system II-2.

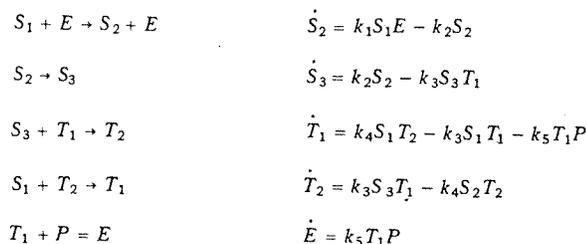
The computer circuit for this set of equations is indicated in Fig. 6. Figure 7a gives the resultant value of E for the choice $\alpha = \gamma = 1.0$, $\beta = 0.1$, $T_0 = 10$, $S_0 = 0$, 5, 10, 16 (the initial value of S_2). Figure 7b gives T_1 for the same parameters, and S_3 and S_2 are given by Figs. 8a and 8b, respectively.

One readily sees that the presence of the additional intermediate step merely modifies the sharpness of the initial T_1 response and has little effect on the long-time behavior; however, in this case one does see a much more pronounced

Fig. 7a. Enzyme curves for system II-2, for various S_0 values as indicated.Fig. 7b. Template curves for system II-2, for various S_0 values as indicated.

saturation effect in E than in the first case considered. Both E and S_2 apparently saturate to the value T_0 . A perturbation expansion similar to II-1 can also be made in this case, but would be on just as shaky grounds. Another conspicuous point of structure in these results is the sharpness of the focus of the T_1 and S_2 families of curves. In Scheme II-1, the diffuse region in which the T curves apparently crossed has given way, in II-2, to a very sharp crossing point at $t = 4.8$ sec and $T_1 = 7.5$ v. Also, the S_2 curves exhibit a similar structure at $t = 3.0$ sec and $S_2 = 1.9$ v, as do the S_3 curves at $t = 4.0$ sec, $S_3 = 0.3$ v. This "focusing" property is not an unusual property of nonlinear systems.

In the final version of this scheme, II-3, we make the final step in the enzyme synthesis nonsoluble, so that the amount of active template available, T_1 , is steadily depleted by the production of enzyme. This scheme has the form



And again we choose $k_1 S_1 = k_4 S_1 = 1$ and define α , β , and ν as before. In this case, however, there are no simple first integrals, but the computer circuit is straightforward and will be found in Fig. 9. We expect the results of Scheme II-3 to be essentially the same as those for II-2, except for a tendency of T_1 to fall off to zero more rapidly and, therefore, for E to saturate more rapidly (since it is essentially the integral of T_1). This is borne out by Figs. 10a and 10b, which give the values of E and T_1 , respectively, for the same parameters used in II-2. In Figs. 11a and 11b are plotted the values of S_3 and S_2 , respectively, for the same conditions. Investigation of these curves shows that the same points are maintained, except that the crossover point in T_1 of II-2 has given way to a region declining in a manner similar to T_1 itself. We see that our qualitative conclusion, arrived at earlier, of the asymptotic weak dependence on parameters appears to be upheld in this case as well.

As a final investigation of this conclusion, we have obtained results for the case $S_0 = T_0 = 10$ v, in which α and γ were varied independently, keeping β fixed at 0.1. In Fig. 12a, we see that the effect on E is completely unimportant, i.e., the final output is virtually unaffected by the intermediate rate constants. We also see that the asymptotic values of T_1 (Fig. 12b) are also unaffected and that there are only some short-time fluctuations from the standard value. In the case

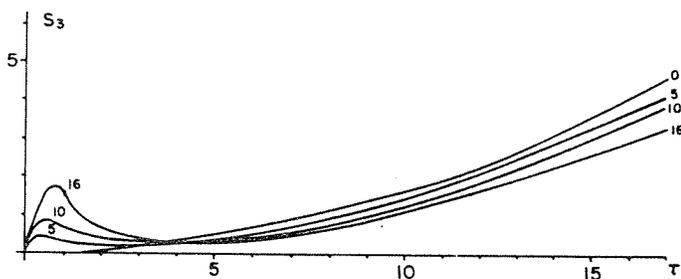


Fig. 8a. The inducer, S_3 , in system II-2, for various S_0 values as indicated.

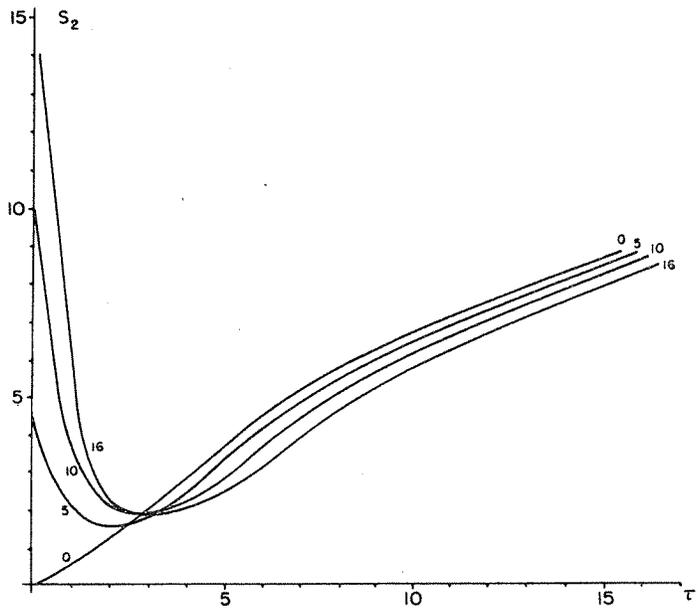


Fig. 8b. The inducer, S_2 , in system II-2, for various S_0 values as indicated.

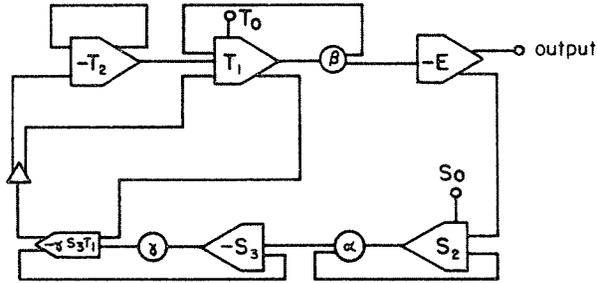


Fig. 9. Schematic computer circuit for system II-3.

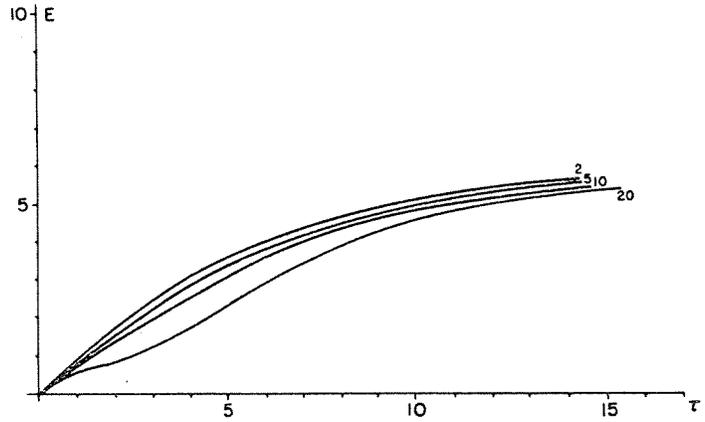


Fig. 10a. Enzyme curves for system II-3, for various S_0 values as indicated.

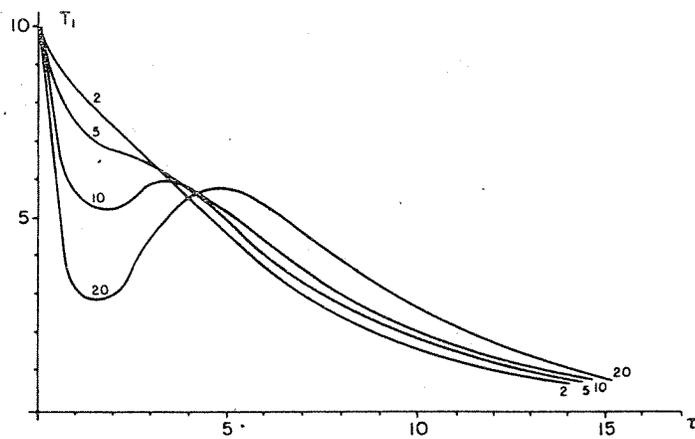


Fig. 10b. Template curves for system II-3, for various S_0 values as indicated.

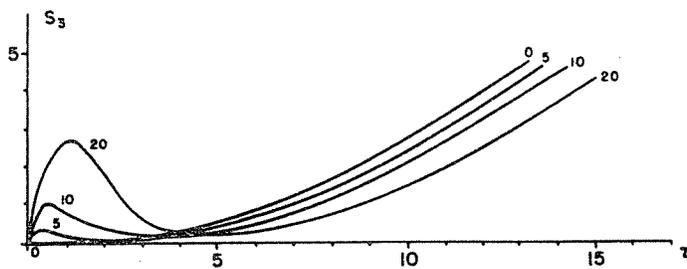


Fig. 11a. The inducer, S_3 , in system II-3, for various values of S_0 as indicated.

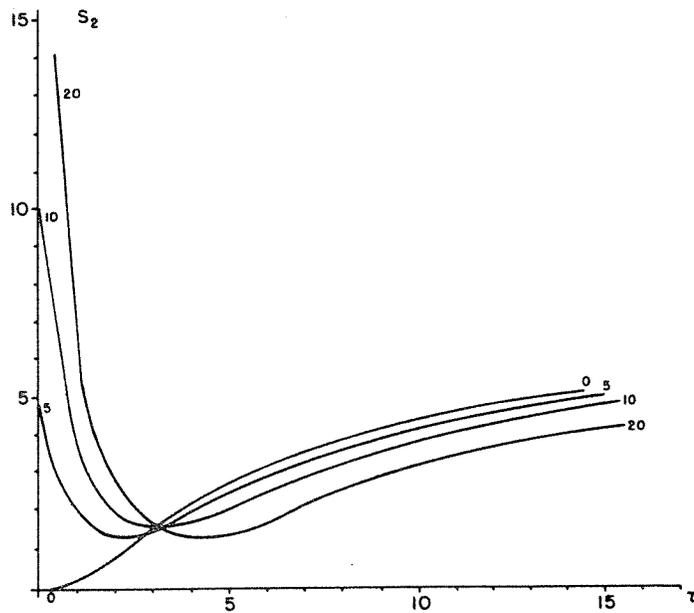


Fig. 11b. The inducer, S_2 , in system II-3, for various values of S_0 as indicated.

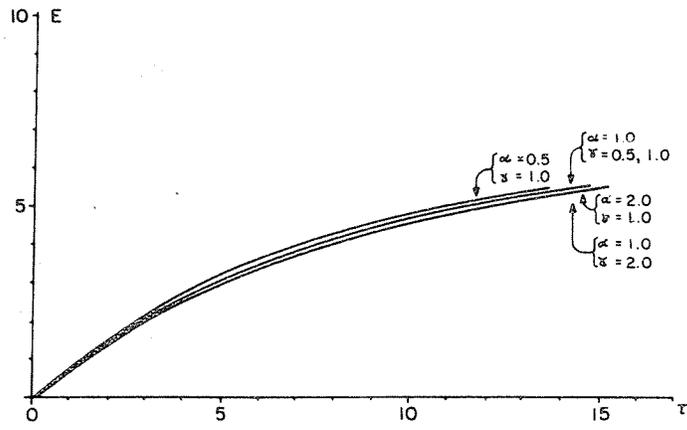


Fig. 12a. Enzyme curves for system II-3, with $S_0 = T_0 = 10$ v, $\beta = 0.1$, and α and γ varied as indicated.

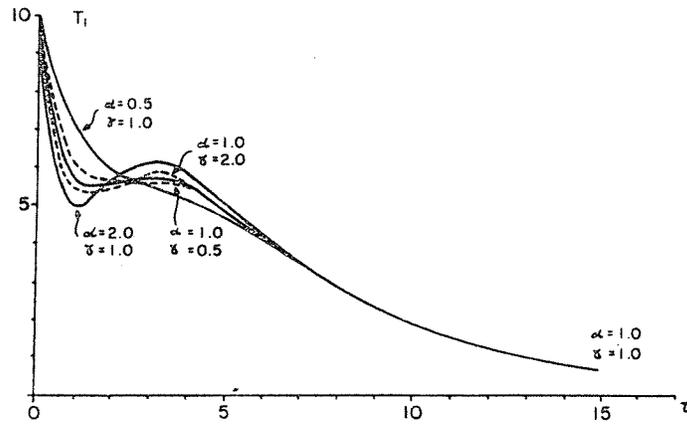


Fig. 12b. Template curves for system II-3, with $S_0 = T_0 = 10$ v, $\beta = 0.1$, and α and γ varied as indicated.

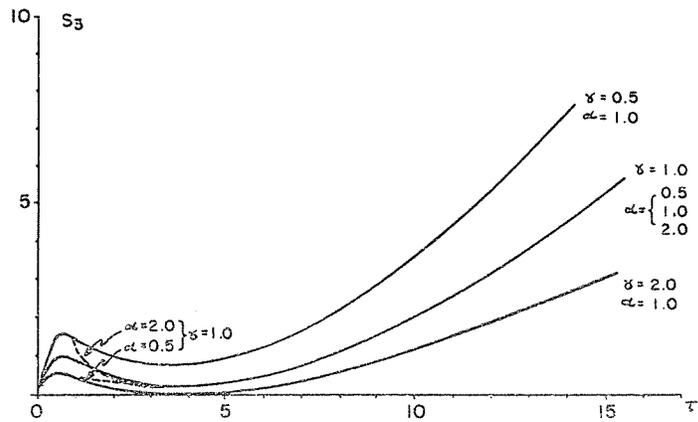


Fig. 13a. Inducer (S_3) curves for system II-3, with $S_0 = T_0 = 10$ v, $\beta = 0.1$, and α and γ varied as indicated.

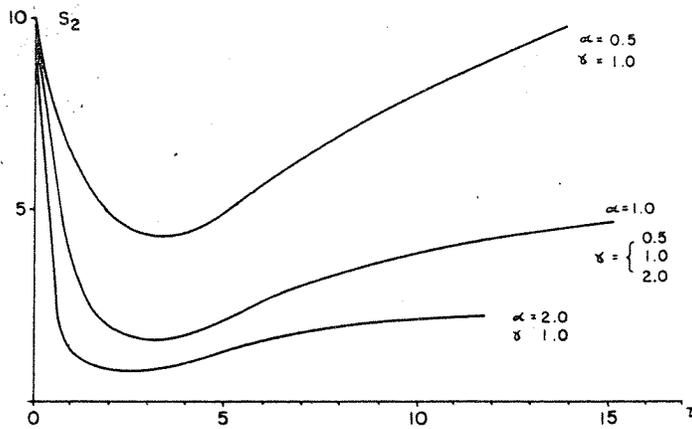


Fig. 13b. Inducer (S_2) curves for system II-3, with $S_0 = T_0 = 10$, $\beta = 0.1$, and α and γ varied as indicated.

of the inducers, however, there is a rather pronounced effect. Variation of the rate constant which governs the feedback to a specific inducer (see Fig. 9) has a large and inverse effect on that inducer, as can be seen from Figs. 13a and 13b, but variation of the other rate constant has substantially no effect. Thus we see that the output of the circuit is substantially stable against variation in its parameters, at least as far as its asymptotic behavior is concerned.

REFERENCES

- Heinmets, F. 1960. An analysis of the concept of cellular injury and death. *Int. J. Rad. Biol.* 2, 341-352.
 Heinmets, F. and Herschman, A. 1960. Quantitative analysis of metabolic processes. *Phys. Med. Biol.* 4, 238-253.
 Heinmets, F. and Herschman, A. 1961a. A model-system for enzyme synthesis by sequential induction and mathematical formulation of the process. *Bull. Mat. Biophys.* 23, 69-89.
 Heinmets, F. and Herschman, A. 1961b. A model-system for the synthesis of dissociable enzyme and mathematical formulation of the process. *Bull. Math. Phys.* (in press).