

UNCLASSIFIED

AD NUMBER: AD0466319

LIMITATION CHANGES

TO:

Approved for public release; distribution is unlimited.

FROM:

Distribution authorized to U.S. Gov't. agencies and their contractors; Administrative/Operational Use; 30 Jul 1961. Other requests shall be referred to the Army Quartermaster Research and Engineering Command, Natick, MA 01760

AUTHORITY

USAGMREC LTR, 12 NOV 1965

466319

Department of Physics

WORCESTER POLYTECHNIC INSTITUTE

Worcester 9, Massachusetts

CATALOGED BY: DDC

AS AD NO. _____

FINAL RESEARCH PROJECT REPORT TO:

HEADQUARTERS QUARTERMASTER RESEARCH AND ENGINEERING COMMAND, U.S. ARMY

Quartermaster Research and Engineering Center

Natick, Massachusetts

Contract No.: DA 19-129-QM-1647

O.I. No.: 5166

Project No.: 7-99-01-001

Period: 31 July 1960 - 30 July 1961

Principle Investigators: Dr. A. Herschman, Assistant Professor of Physics

D. W. Howe, Jr., Associate Professor of Physics

DDC AVAILABILITY NOTICE
QUALIFIED REQUESTORS MAY OBTAIN
COPIES OF THIS REPORT FROM DDC.

JUL 20 1965

TISA B

NOTICE: When government or other drawings, specifications or other data are used for any purpose other than in connection with a definitely related government procurement operation, the U. S. Government thereby incurs no responsibility, nor any obligation whatsoever; and the fact that the Government may have formulated, furnished, or in any way supplied the said drawings, specifications, or other data is not to be regarded by implication or otherwise as in any manner licensing the holder or any other person or corporation, or conveying any rights or permission to manufacture, use or sell any patented invention that may in any way be related thereto.

SUMMARY

During the tenure of this contract, 31 July 1960 to 30 July 1961, an extensive study was made of the feasibility of applying analogue computer techniques to the "Quantitative Analysis of Metabolic Systems", work which has already been extensively reported on in this series of contracts and been the basis of a series of papers by Dr. F. Heinmets of the QMR&E Command and Dr. A. Herschman of the Worcester Polytechnic Institute.

In the first phase of the work, use of an Analogue computer was made to rework most of a paper which had been based on earlier reports. This is presented in the first part of this report and will be published shortly as "Quantitative Analysis of Metabolic Processes, II" by F. Heinmets and A. Herschman in a forthcoming issue of "Mathematical Biophysics". It was the purpose of this phase to obtain numerical data from a given metabolic scheme and compare it directly with experimental results, in this case the work of M. R. Pollock (Brit. J. Exptl. Pathol., 1950 and 1952).

In the second phase, an investigation was made of the combined effect of non-linearity and feedback in a number of simplified systems, by means of the computer, to gain fluency in the behavior of such systems. This was in keeping with the spirit of the present contract which was designed to circumvent the impasse reported in the last contract report. The results of this phase are presented in the second part of this report.

The final phase was devoted to coding several more complex systems for a much larger computer than that available at this Institute, so that this work will have to be completed by another contractor.

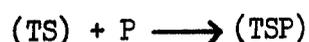
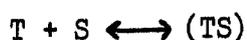
I. Application of Machine Computation

The purpose of this part of the contract was to obtain specific numerical results from general schemes of metabolic processes which did not lend themselves to tractable mathematical analysis. These results were ultimately compared with experimental data obtained by M. R. Pollock (Brit. J. Exptl. Pathol., 1950, 31, 739 and 1952, 33, 587), and found to agree quite well.

The model which was pursued was one in which the Template was made specific by an inducer; the specific Template then combined with elements of the pool to produce the enzyme. In this simple form, there is only a short lag in the synthesis process which was found inadequate to explain the experimental data, so that it was necessary to modify the system to include an intermediate receptor stage making the process one with an extended lag. This latter scheme was found to account for the experimental data quite adequately.

The first scheme considered was:

Scheme I-1



$$\dot{T} = -k_1ST + \bar{k}_1B$$

$$\dot{B} = k_1ST - \bar{k}_1B - k_2BP - k_3C$$

$$\dot{C} = k_2BP - k_3C$$

$$\dot{E} = k_3C$$

where: T = Template concentration

S = Inducer concentration

B = Specific template concentration (TS)

C = Complex concentration (TSP)

E = Enzyme concentration

and the dot over a concentration means a time derivative, and the bar over a rate constant gives the value for the reversed process. In the first model, both the inducer and pool supply was assumed constant either because they were very large

or replenished during synthesis. Thus one has the first integrals of the equations as:

$$S = \text{const.}, \quad P = \text{const.}, \quad T + B + C = T_0 = \text{const.}$$

Introducing the following constants, for simplicity:

$$\alpha = k_1 S, \quad K = k_3 + k_2 P, \quad k = k_3 k_2 P / K, \quad \beta = \alpha - K, \quad \gamma = k_3$$

one obtains directly:

$$T = T_0 e^{-\alpha t}, \quad B = (\gamma T_0 / K) \left[1 - \frac{K(\alpha - \gamma)}{\gamma \beta} e^{-\alpha t} + \frac{\alpha(K - \gamma)}{\gamma \beta} e^{-Kt} \right]$$

$$C = (k_2 P T_0 / K) \left[1 + (K/\beta) e^{-\alpha t} - (\alpha/\beta) e^{-Kt} \right]$$

$$E = k T_0 \left(t - 1/\alpha - 1/K \right) + (k T_0 / \beta) \left[(\alpha/K) e^{-Kt} - (K/\alpha) e^{-\alpha t} \right]$$

Since for most cases of interest α is by far the largest of the rate constants, one may consider that after a reasonable time large compared with $1/\alpha$, all terms in $e^{-\alpha t}$ become negligible, giving as a long time behavior:

$$T = 0, \quad B = (\gamma T_0 / K) \left[1 + (K - \gamma/\gamma) e^{-Kt} \right]$$

$$C = (K - \gamma/\gamma) T_0 \left[1 - e^{-Kt} \right], \quad E = k T_0 \left[t - 1/K + e^{-Kt}/K \right]$$

So that at large times the enzyme is produced at a linear rate with a lag given by the total intermediate time constant and a rate given by the relative intermediate time constant. (Note that in the foregoing one assumed that $\bar{k}_1 \ll \alpha$.) The enzyme production curve is sketched in Fig. 1. The central characteristic of such a linear system as this, is, as has been noted, the ultimate linear increase of the enzyme production after a time lag.

A somewhat more complex system is Scheme I-2, wherein the amount of pool available is depleteable. In this case we must append the equation:

$$\dot{P} = -k_2 B P$$

to the equations of Scheme I-1, and also change the first integrals to:

$$S = \text{const.}, \quad P + E + C = P_0 = \text{const.}, \quad T + B + C = T_0 = \text{const.}$$

If we limit ourselves to long time solutions after which, $T = 0$ for all practical purposes, one has:

$$B = -\dot{P}/k_2P; \quad C = \dot{E}/k_3; \quad \text{and} \quad B + C = T_0$$

giving:

$$P = P_0 \exp(k_2E/k_3 - k_2T_0 t)$$

So that the equation for E becomes:

$$\dot{E} + k_3E = k_3P_0 (1 - \exp(k_2E/k_3 - k_2T_0 t))$$

For small values of the time, this has the solution:

$$E = k_2k_3P_0T_0 t^2/2 - k_2k_3^2 T_0(T_0 + 2P_0) t^3/6 + \dots$$

which may be compared with the result for constant pool, viz.:

$$E = k_2k_3P_0T_0 t^2/2 - k_2k_3^2 P_0(P_0 + T_0) t^3/6 + \dots$$

and is seen to be substantially the same -- i.e., there is very little difference in the lag properties of the two systems. For somewhat larger values of the time \dot{C} and consequently \ddot{E} become zero -- i.e., C has a maximum and E becomes a straight line and since \dot{C} is always rather small after that, E has a quasi-linear appearance. To obtain values of E over this range, one sets \dot{C} to zero in the basic equations and solves the resultant algebraic equation for P to obtain:

$$2P = \left[(T_0 + E - P_0 + k_3/k_2)^2 + 4k_3(P_0 - E)/k_2 \right]^{1/2} - (T_0 + E - P_0 + k_3/k_2)$$

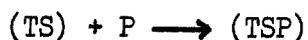
and tabulate P as a function of E from this equation, substituting these values into the equation giving P and E as a function of the time, one obtains t as a function of E, the inversion of which is the desired result. Although strictly

only valid for a single set of values of P, E and t, it is quite accurate over a whole range.

The results of such an analysis will also be found in Fig. 1 and it is quite apparent that there is a fundamental difference between this result and that found earlier, viz., the fact that although the linear region in I-2 is rather long, ultimately the value of E saturates, and as can be seen from the first integrals in this scheme, eventually approaches the constant value P_0 . Thus for small and intermediate values of the time the constancy of the pool is immaterial as long as the pool is rather large and one cannot distinguish between I-1 and I-2 readily; however, for large values of the time, the depletion of the pool is of great importance in that it ultimately causes the saturation of E.

Note that this particular scheme was used in a somewhat different form as an example for a proposed application of the analogue computer to this sort of problem in the previous year's report for this project (DA 19-129-QM-1457, 30 July 1960) where it is referred to as Scheme B of part II of that report, and in which a proposed computer circuit is presented for its analysis. This circuit was, however, never set up, since it was obvious from the foregoing that allowing the pool to be depleted would in no way extend the lag period in the enzyme synthesis, and the experimental evidence was for a longer lag. Hence it was felt necessary to introduce another intermediate step and instead, consider Scheme I-3.

Scheme I-3



$$\dot{T} = \dot{S} = -k_1 TS + \bar{k}_1 B^*$$

$$\dot{B}^* = -T - k_2 B^*$$

$$\dot{B} = k_2 B^* - k_3 B + k_4 C$$

$$\dot{C} = k_3 B - k_4 C$$

$$\dot{E} = k_4 C$$

where we consider the base for constant P and have absorbed P into the constant k_2 . The equations have two immediate first integrals:

$$S_0 - S = T_0 - T = B^* + B + C$$

where S_0 and T_0 represent the initial values of S and T respectively, the other concentrations are assumed to be zero initially.

The long time behavior of the system can be found by setting all the rates but that of E, equal to zero, giving three cases:

$$a) S_0 < T_0, \quad S \rightarrow 0, \quad T \rightarrow T_0 - S_0, \quad \dot{E} \rightarrow k' S_0$$

$$b) S_0 > T_0, \quad T \rightarrow 0, \quad S \rightarrow S_0 - T_0, \quad \dot{E} \rightarrow k' T_0$$

$$c) S_0 = T_0, \quad S \rightarrow T \rightarrow 0, \quad \dot{E} \text{ does not quite become constant.}$$

The quantity k' introduced above is: $k' = k_3 k_4 / (k_3 + k_4)$ and the behavior of \dot{E} in case (c) is not apparent from the procedure just outlined.

To gain a better insight into the general behavior of the system when the (TS) formation is rapid compared with the intermediate steps, it is convenient to use the integrals of the equations to define a quantity U as:

$$U_0 - U = T_0 - T = S_0 - S = B^* + B + C$$

so that we have:

$$TS = (T_0 + S_0)U + (U_0 - U)^2$$

and we have put: $U_0 = T_0 S_0 / (T_0 + S_0)$. Now if we neglect the term in $(U_0 - U)^2$ as being small, a procedure valid where U is not itself small, we obtain a linear equation for U, viz.,

$$\ddot{U} + (K' + k_2)\dot{U} + k_2 K U = 0$$

where we have introduced: $K = k_1(S_0 + T_0)$ and $K' = K + \bar{k}_1$. This equation can be integrated in closed form to give:

$$U = U_1 e^{-\lambda_1 t} + U_2 e^{-\lambda_2 t}$$

where λ_1 and λ_2 are the two roots of: $\lambda^2 + (K' + k_2)\lambda + Kk_2 = 0$, and

$$U_1 = (K - \lambda_2)U_0/(\lambda_1 - \lambda_2), \quad U_2 = (\lambda_1 - K)U_0/(\lambda_1 - \lambda_2).$$

In the limit where K is very large compared with k_2 , the usual case, we have:

$$\lambda_1 = K' + \bar{k}k_2/K', \quad \lambda_2 = Kk_2/K' + K\bar{k}k_2^2/K'^3$$

$$U_1 = K(1 - 2\bar{k}k_2/K'^2)U_0, \quad U_2 = \bar{k}(1 + 2Kk_2/K'^2)U_0/K'$$

so that we see that U consists of a rapidly decaying part and a slowly decaying one, the latter existing only because of the reversibility of the first step in the process. Once U is found the remainder of the terms may be obtained by direct integration, e.g., B^* is given by:

$$B^* = KU_0 (e^{-\lambda_2 t} - e^{-\lambda_1 t})/(\lambda_1 - \lambda_2)$$

Since $B + C$ may be obtained directly from B^* and U , one may easily obtain an equation for C and integrate it and from that obtain an equation for B and also for \dot{E} , this latter is, in terms of $K = k_3 + k_4$ and the k' defined earlier:

$$\dot{E} = k'U_0 \left[1 + \frac{Kk_2 e^{-kt}}{(k-\lambda_2)(\lambda_1-k)} - \frac{\lambda_1 k e^{-\lambda_2 t}}{(\lambda_1-\lambda_2)(k-\lambda_2)} - \frac{\lambda_2 k e^{-\lambda_1 t}}{(\lambda_1-\lambda_2)(\lambda_1-k)} \right]$$

The integral of this is readily found and is seen to contain a linear term and three transients. On the assumption that K is large whereas k_2 and k are small and about equal in magnitude, one obtains:

$$E = k'U_0 \left[t - t_0 + (k^2 e^{-\lambda_2 t} - \lambda_2^2 e^{-kt})/k\lambda_2(k - \lambda_2) \right]$$

with $t_0 = 1/k + 1/\lambda_2 = 1/k + (1 + \bar{k}/K)/k_2$. We have completely neglected the short term transient and the terms in t_0 contributed by it (exactly $1/K$). Note that the reversibility of the first step makes itself felt only in the lag time t_0 and not in the slope of the enzyme production curve. In Fig. 2 this slope obtained from $k'U_0$ and from the infinite time limits considered earlier, as well as those obtained from reading curves obtained from the computer programmed for the exact non-linear equations (see below), are displayed.

In obtaining the curves of Fig. 2 and also Fig. 3, the enzyme production curve for a specific set of values (machine curve), an effort was made, by means of the theory outlined so far, to obtain a consistent set of units with which to describe Pollock's (1952) work. Fig. 3 of Pollock's paper can be considered to give the asymptotic enzyme rate for very small S_0 and hence the slope of this rate as a function of S_0 should give k' directly. This is found to be $23,200 \mu\text{l-CO}_2/\text{unit}/\text{hr}^2$. Where "unit" refers to units of penicillin and $\mu\text{l-CO}_2/\text{hr}$, was Pollock's unit for enzyme production (related to the method of measurement). Presumably the slopes of the E vs t curves are too small since not enough time has elapsed after only one hour to adequately estimate, but Fig. 2 of this paper shows that in the very small S_0 limit, very little error is introduced by inadequate waiting as the two limiting curves are almost coincident. Fig. 6A of Pollock's paper again gives the rate but now for very small T_0 which gives us $k'T_0$ as $660 \mu\text{l-CO}_2/\text{ml}/\text{hr}^2$ when 0.05 mg of cells were used. This implies that there are 0.57 units of T_0 in every mg of cellular material. The values of the rate constants are not so easily obtained. One may estimate t_0 from many of the curves given by Pollock and find it between 0.20 and 0.25 hr; however, the analytic expression obtained for t_0 in terms of the rate constants is probably valid only for the case of his Fig. 3 ($t_0 = 0.21$ hr) and in this case, one does not know enough about the relative values of k_1 , \bar{k}_1 , etc., to obtain each of them from a single expression. Extrapolating

the validity of our linear approximation to higher values of S_0 , we may use his Fig. 6A and the assumption (of a heuristic nature) that: $k_2 = k_3 = k_4$, so that we obtain: $k_2 = 7.2/\text{hr}$, $k' = 3.6/\text{hr}$. This yields the conversion factor of 1.55×10^{-4} units of E producing $1 \mu\text{l-CO}_2/\text{hr}$. In the machine calculation T_0 was taken to be 5V. which for 0.5 mg of cellular material implies that one unit will be represented by 17.5 V. Also the constants k_2 , etc., were taken as 1/sec, so that 1 sec of machine time represents 8.4 min of laboratory time, alternatively, 1 hour of laboratory time is 7.2 sec of machine time.

Thus for example, Fig. 6B of Pollock's paper gives a rate which in Pollock's units is 5650. We may convert this to our standard base as 0.875 units/hr and for the purpose of comparison with the machine's calculations as 2.12 V/sec. This point is indicated in Fig. 2 of this paper along with similar points taken from Pollock's Fig. 3 (his 1950 paper cited above). Unfortunately, Pollock did not use any values of S intermediate between 0.1 and 1.0, so that we cannot show any points in this region.

In Fig. 3 we show one of the machine curves obtained with the constants:

$$k_2 = k_3 = k_4 = 1/\text{sec}; \quad \bar{k}_1 = 5/\text{sec}; \quad k_1 = 5/\text{sec-V}$$

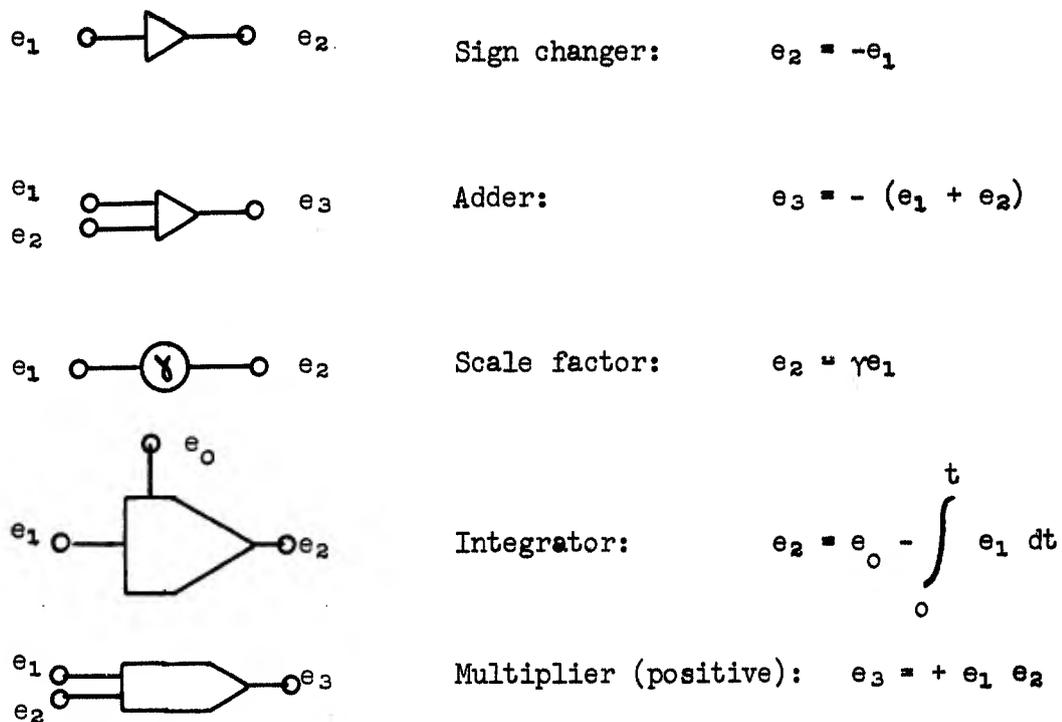
$$T_0 = 5 \text{ V and } S_0 = 16 \text{ V}$$

On the curve are indicated, as experimental points, Pollock's Fig. 6B points, which, as we have seen, would represent 17.5 V for S_0 , but the difference is not considered significant since there is little change of shape with S_0 in this region. The very good fit of this data is the best confirmation of this approach since the scale factors et al which were used were obtained exclusively from Pollock's other curves and independently of this one. Modification of the machine values of k_1 and \bar{k}_1 would also have improved the fit at the lower points, but

this is not considered worthwhile since more accurate experimental measurements would also be necessary.

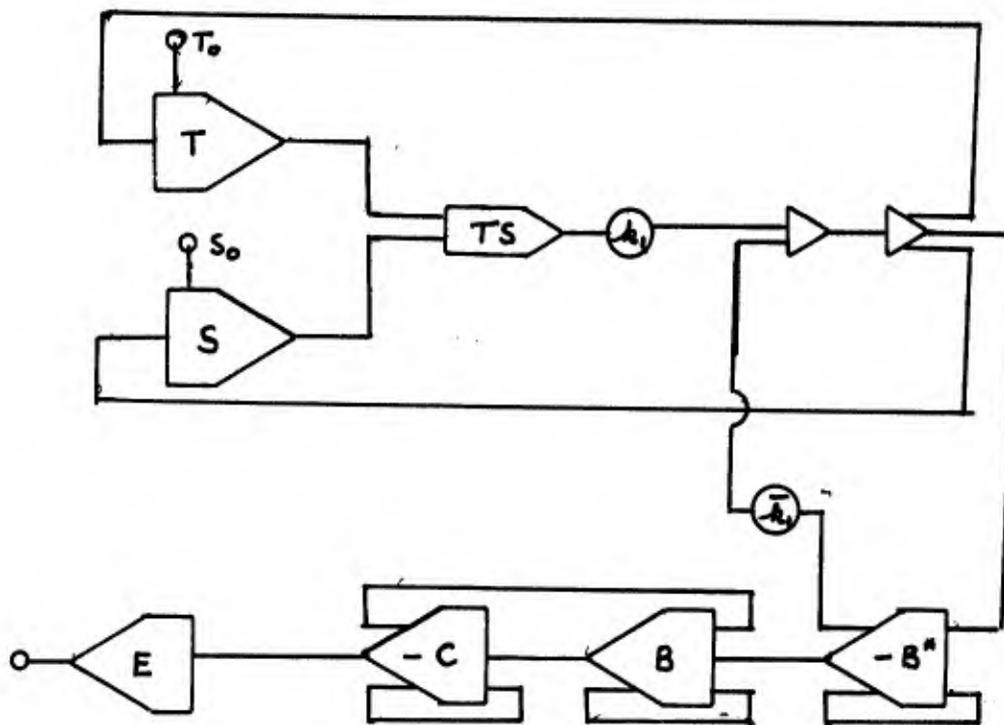
We would now like to investigate in more detail, the manner in which such a system is set up on the computer to obtain the sort of results which we have used to fit Pollock's data. This will also serve as an introduction to the next part of this report where the computer is used as a more active tool.

Briefly, the computer consists of a circuit whose elements add, subtract, multiply, integrate and change the sign of voltages which are applied to them. Thus each such element has an input and an output voltage (designated by e_1 , e_2 , respectively, or with additional subscripts as needed, in the following table), and one distinguishes the nature of the operation on the input voltage to obtain the output, by means of the shape of the element in the diagram. The following is then a "dictionary" of computer elements (see report on DA 19-129-QM-1457, 30 July 1960 for a similar table and reference):



In all of the following analysis, we shall inscribe within the appropriate symbol, the value of its output, in the case of integrators and multipliers; scale factors will simply be denoted by the factor they represent and sign changers and adders will not be marked.

Using this dictionary, it is then a slight step to the following circuit diagram for Scheme I-3:



To see this, we compare the circuit with the equations for the scheme as given earlier, one need merely note that the output of the integrator is indicated on it, so that the multiplier has T and S going into it, and (being a positive multiplier) will put out the product TS , and because of the scale factor,

k_1TS and also $-\bar{k}_1B^*$ are fed into the adder; therefore, with the sign changer, the input on both T and S integrators is $k_1TS - \bar{k}_1B^*$ which is found to be the negative of \dot{T} as it should be. Similarly, one sees that the chain of integrators are labeled as they should be, with the final output the enzyme.

The circuit diagram is then, itself, the analogue of the (chemical) kinetic equations for the system, with each integrator playing the role of the appropriate unit in the system so that with a little practice one may set up the analogue circuit directly from the schematic equations. The advantages of using the analogue computer for this sort of work are thus seen to be many-fold, not only is the accuracy of such a computer more than enough for the data available, but the circuits lend themselves readily to direct interpretation, making it possible to "read out" the biological process involved directly.

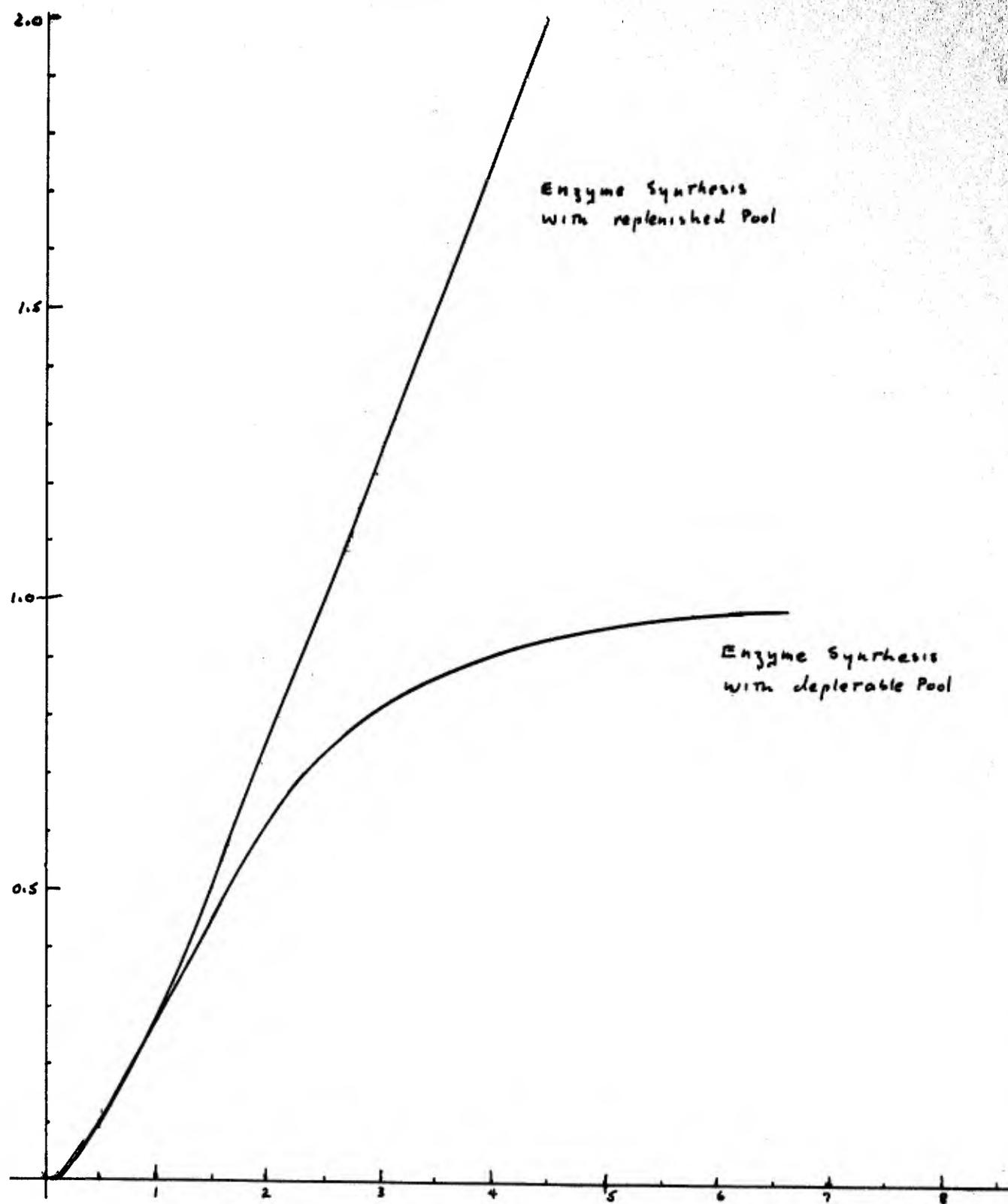


Fig. 1: Comparison between Enzyme synthesis with depletable and replenished pools. The units are taken arbitrarily as $P_0 = T_0 = k_2 = k_3 = 1$.

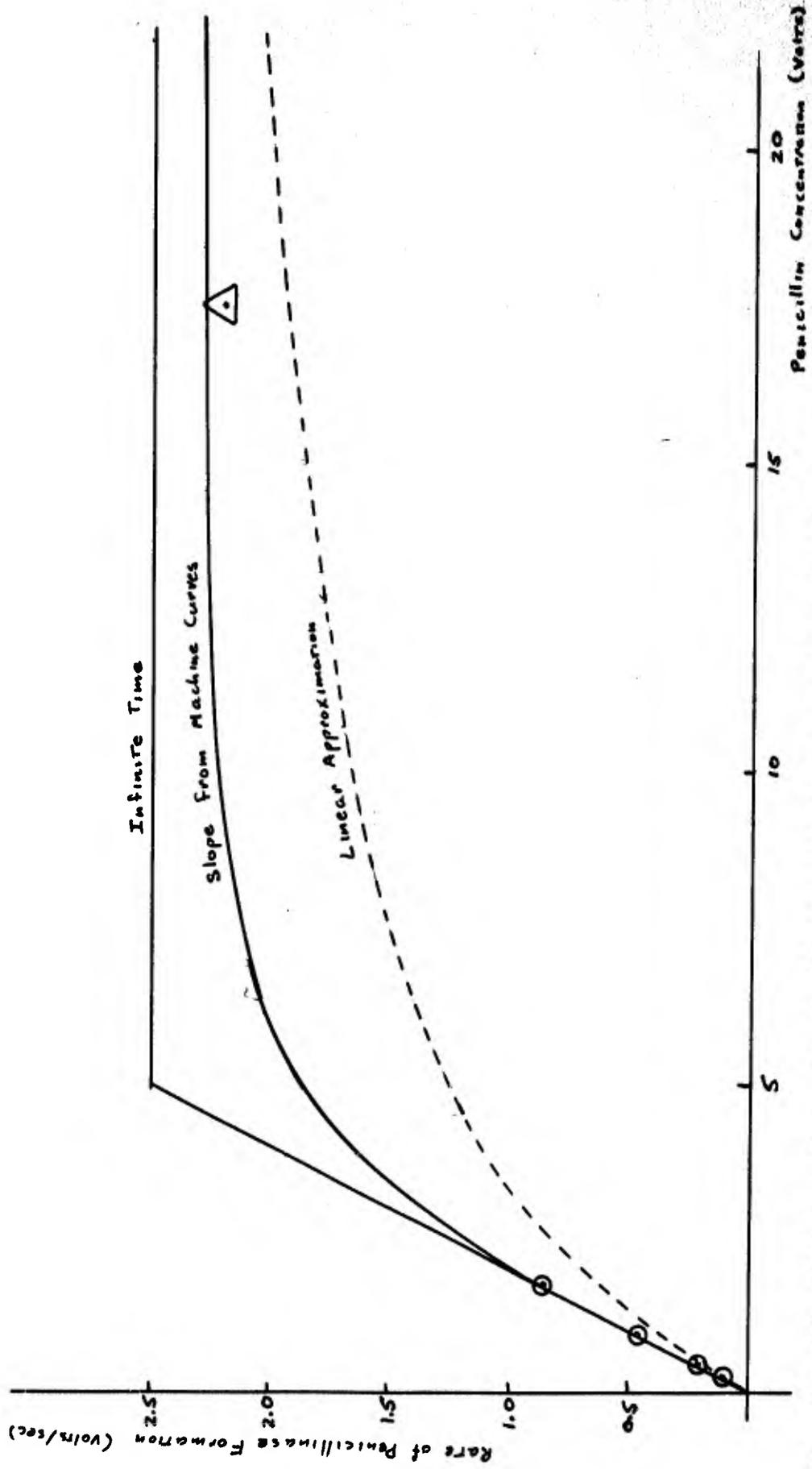


Fig. 2: Rate of Enzyme formation (in linear region) as a function of inducer concentration. The circled points are from Pellock's Fig. 3 (the triangled one is from his Fig. 6B). The curves marked infinite time and linear approximation are derived in the text. The third curve was obtained from the machine curves for individual cases, to the same accuracy as Pellock's points.

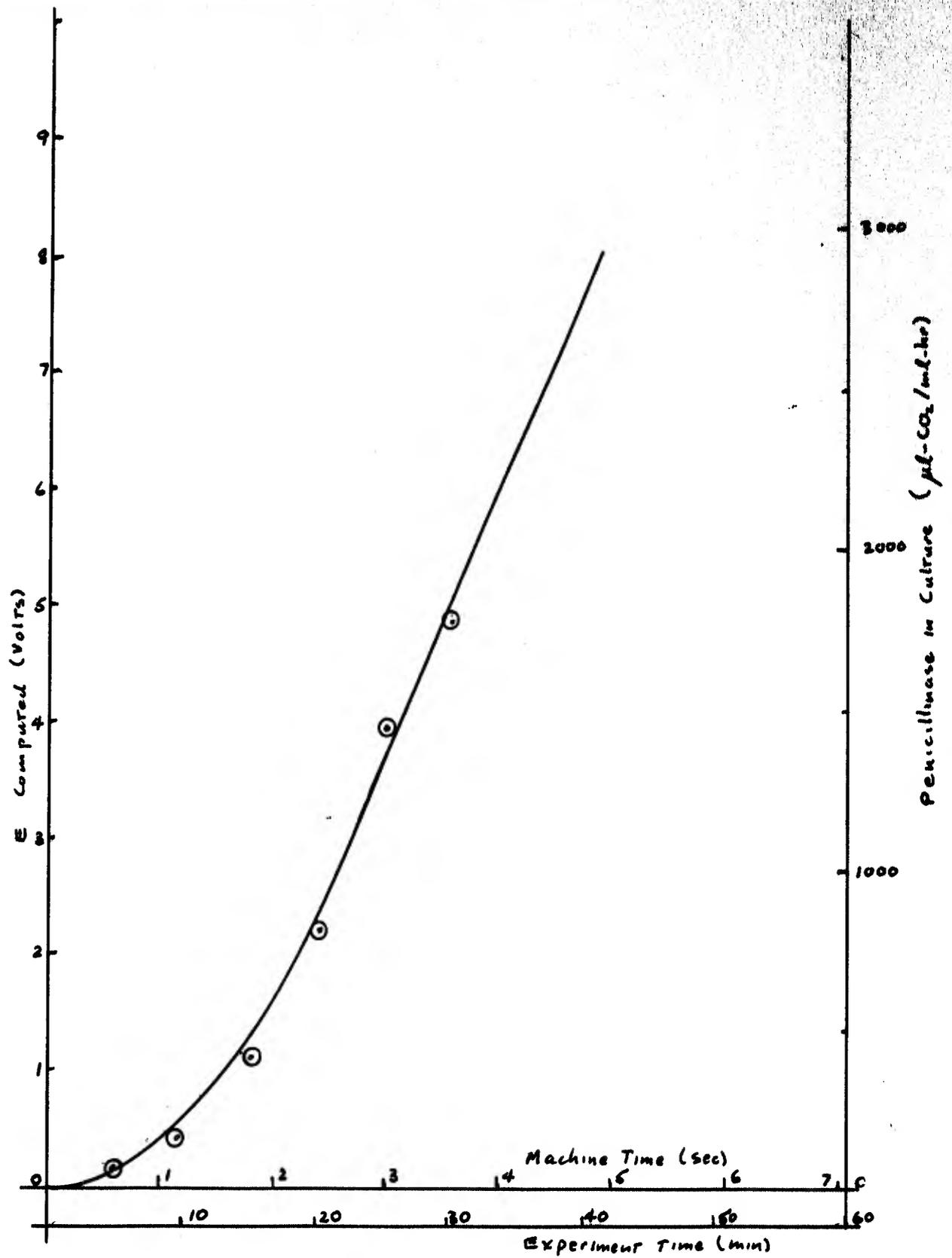
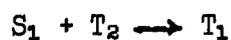
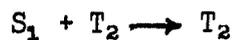


Fig. 3: An actual machine computation (full curve) with $T_0 = 5$ V, $S_0 = 16$ V, on which are placed the experimental points of Pollock's Fig. 6B in circles. The scale factors were obtained from Pollock's other data. See explanation in text.

II. Studies in Non-Linear Synthesis

In this section variations on a non-linear scheme were tried, with the view toward obtaining an intuitive feeling for the behavior of such a system. In the variations, not only were the initial conditions changed, but also intermediate rate constants and the number of intermediate steps taken were also changed. The computer was an invaluable tool in this study, since it was non-linearity of just this sort which caused the bog-down of the previous contract, due to the intractability of the equations, a factor which is completely irrelevant to the machine.

The first system considered was II-1, viz.:



$$\dot{S}_1 = \dot{P} = 0$$

$$\dot{S}_2 = k_1 S_1 E = k_3 S_2 T_1$$

$$\dot{T}_1 = k_4 S_1 T_2 - k_3 S_2 T_1 = -\dot{T}_2$$

$$\dot{E} = k_2 P T_1$$

Since an obvious first integral is $T_1 + T_2 = T_0 = \text{const.}$, and one may choose as a time unit the rate of the first reaction, 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. 4A (Fig. 4B shows the

same circuit as it might be used with all the resistors and capacitors needed to produce the requisite rate constants and scale factors, in place. In practice somewhat different values might be found more convenient.) 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 Fig. 5A and Fig. 5B.

In Fig. 5A, the machine computation for E is plotted for values of S_0 (the initial value of S_2) of: 0, 4, 10, 20 volts. In this scheme T_0 was arbitrarily taken as 10 volts and β was fixed at 0.1 whereas γ was taken as 1.0. In Fig. 5B, 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 significant, 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 non-linear system, will show itself in all the future variations as well. Briefly it can be stated as: 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:

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

So that 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 has almost no radius of convergence. E.g., one may obtain the following formal series for S:

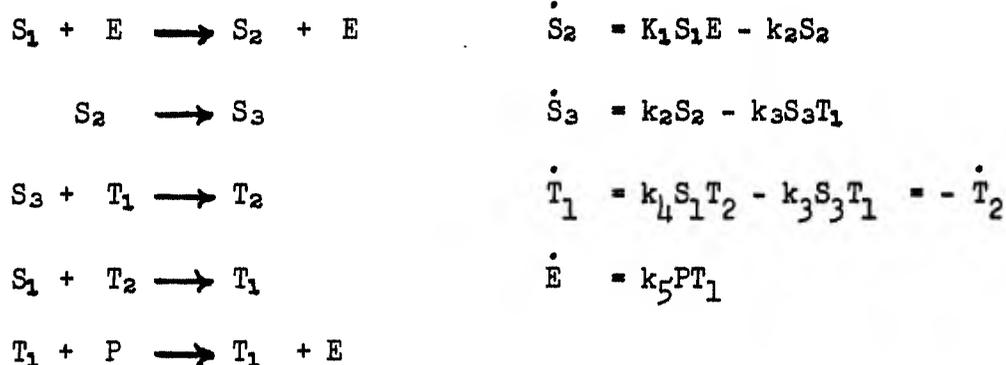
$$S = (\beta E / \gamma \dot{E}) - \beta^2 (E / \dot{E}) / \gamma^2 \dot{E} + \dots$$

so that:

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

implying that S ultimately saturates as does E (to the value T_0/β , i.e., in this case, about 100 volts). 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 Schemes II-2 in which an additional S is placed in an intermediate position, i.e.,



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:

$$k_2 = \alpha, \quad k_5^P = \beta, \quad \text{and } k_3 = \gamma$$

so that with the first integral: $T_1 + T_2 = T_0$, we have the simplified set:

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

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

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

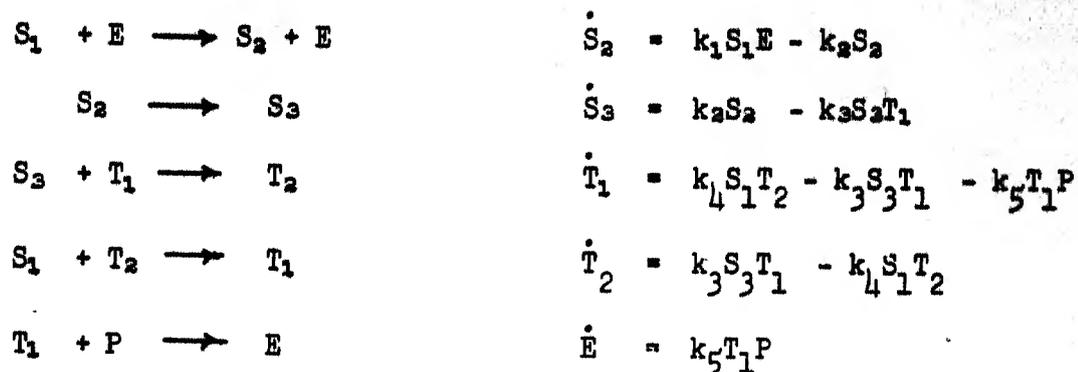
$$\dot{E} = \beta T_1$$

In Fig. 6A, the computer circuit for this set of equations is indicated. And Fig. 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). Fig. 7B gives T_1 for the same parameters, and S_3 and S_2 are given by Fig. 8A and Fig. 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 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 as given way, in II-2, to a very sharp crossing point at $t = 4.8$ sec and $T_1 = 7.5$ volts. Also the S_2 curves exhibit a similar structure at $t = 3.0$ sec and $S_2 = 1.9$ volts, as does the S_3 curves at $t = 4.0$ sec, $S_3 = 0.3$ volts. This "focusing" property is not an unusual property of non-linear systems.

In the final version of this scheme, II-3, we make the final step in the

enzyme synthesis non-soluble, so that the amount of active template available, i.e., 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 γ as before. In this case, however, there are no simple first integrals, but the computer circuit is straightforward and will be found in Fig. 6B. 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 born out by Fig. 9A and Fig. 9B which give the values of E and T_1 , respectively, for the same parameters used in II-2. In Fig. 10A and Fig. 10B are plotted the values of S_3 and S_2 , respectively, for the same conditions. Investigation of these curves shows that the same structure 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 cases $S_0 = T_0 = 10$ volts in which α and γ were varied independently, keeping β fixed at 0.1. In Fig. 11A, 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. 11B) are also unaffected and that there is only some short time fluctuations from the standard value. In the case 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. 6B) has a large and inverse effect on that inducer as can be seen from Figs. 12A and 12B, 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.

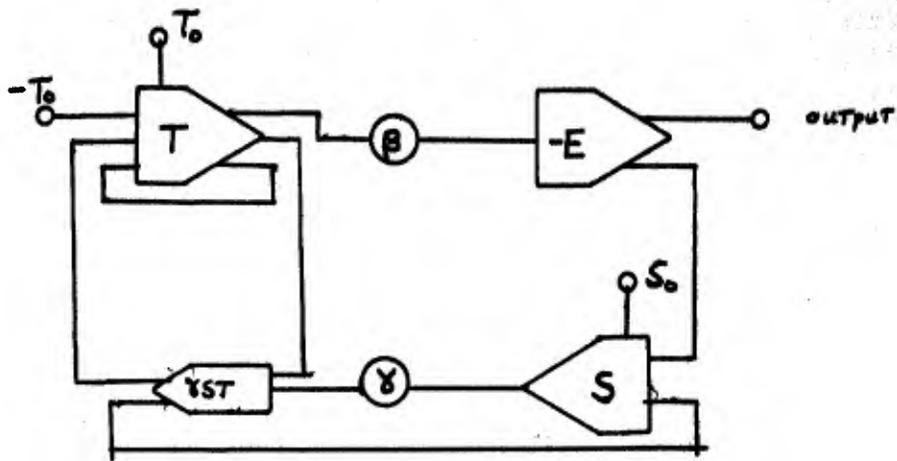
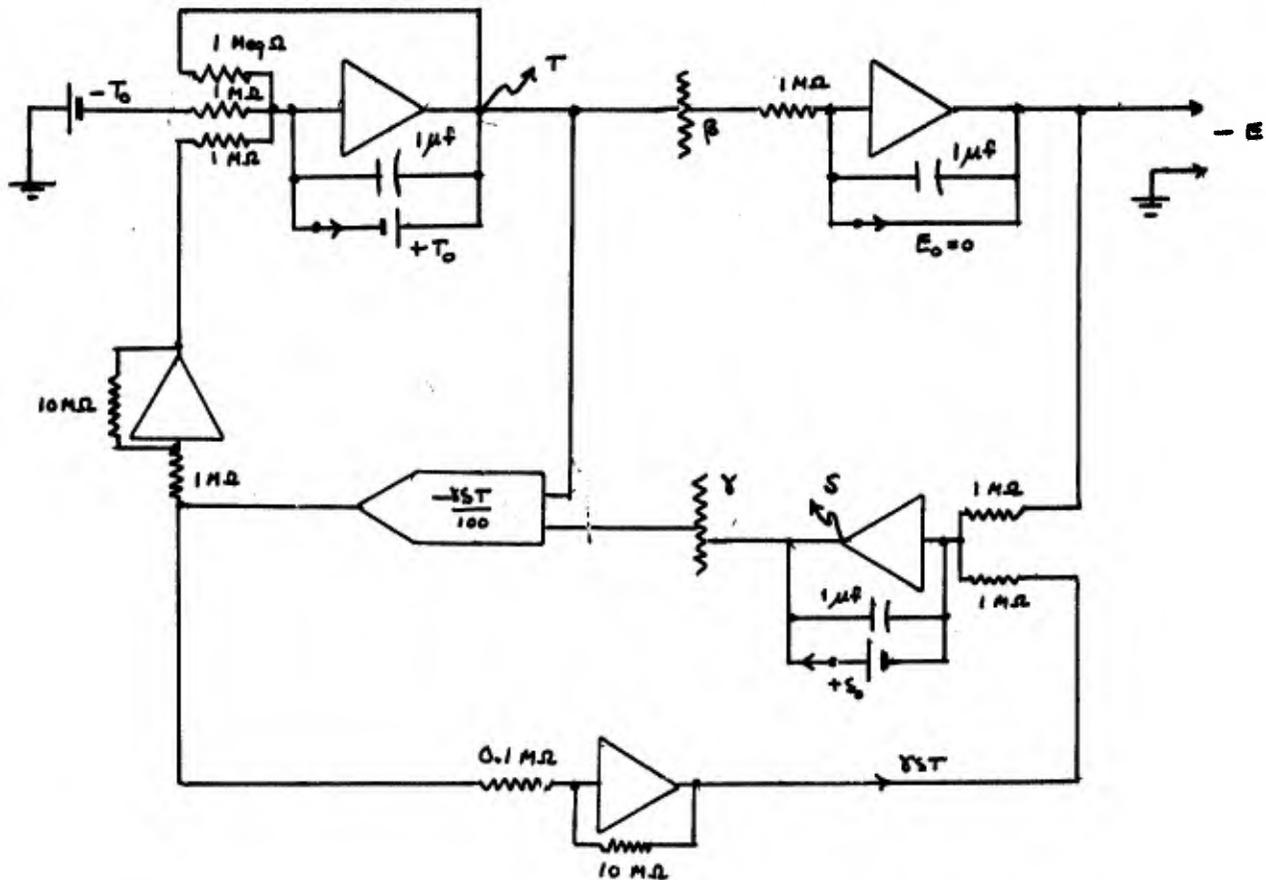


Fig. 4A: Schematic computer diagram for system II-1, as explained in text.



- Initial conditions with switches closed.
- Open switches to start solution.

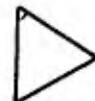
 Represents Amplifier

Fig. 4B: Realistic computer diagram for system II-1, with a possible set of capacitors and resistors although not necessarily the most efficient set for practical operation.

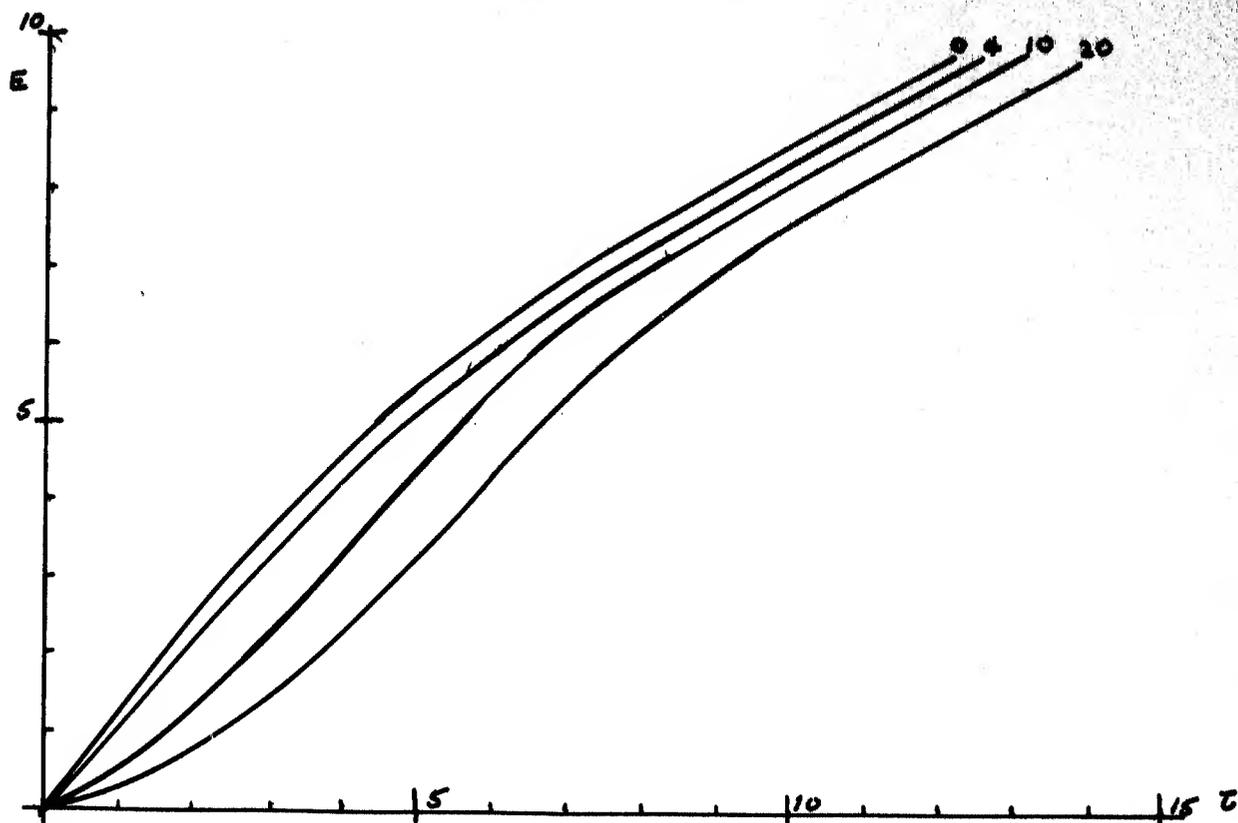


Fig. 5A: Enzyme curves for system II-1, for various values of S_0 as indicated.

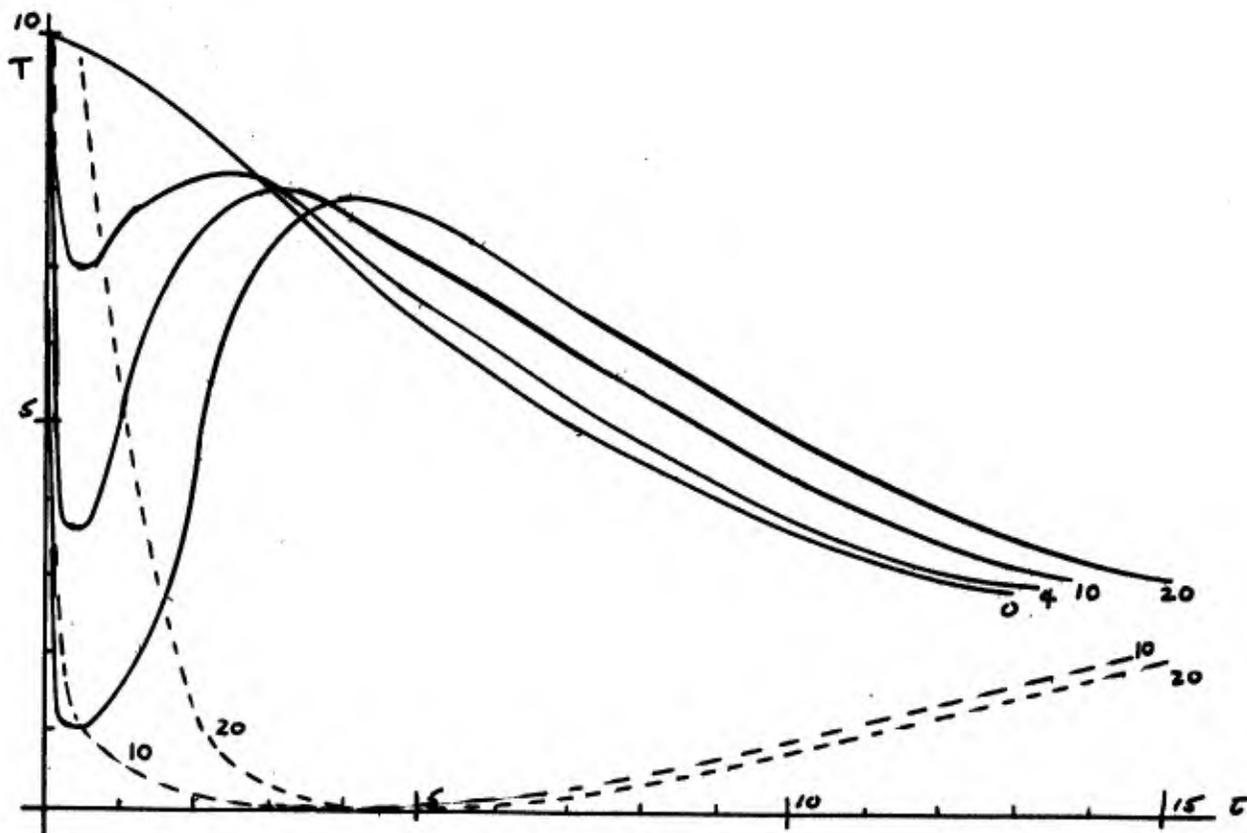


Fig. 5B: Template curves for system II-1, for various values of S_0 as indicated. The dotted curves give the inducer (s) for two representative values of S_0 .

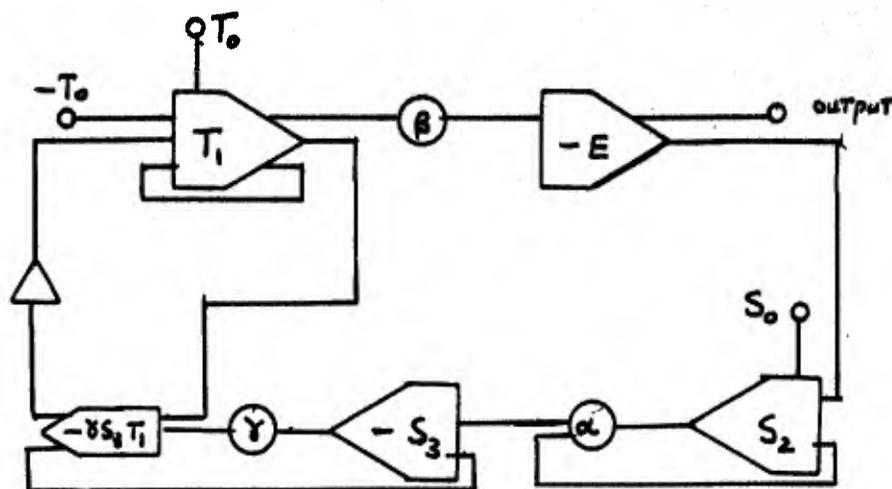


Fig. 6A: Schematic computer circuit for system II-2, as explained in text.

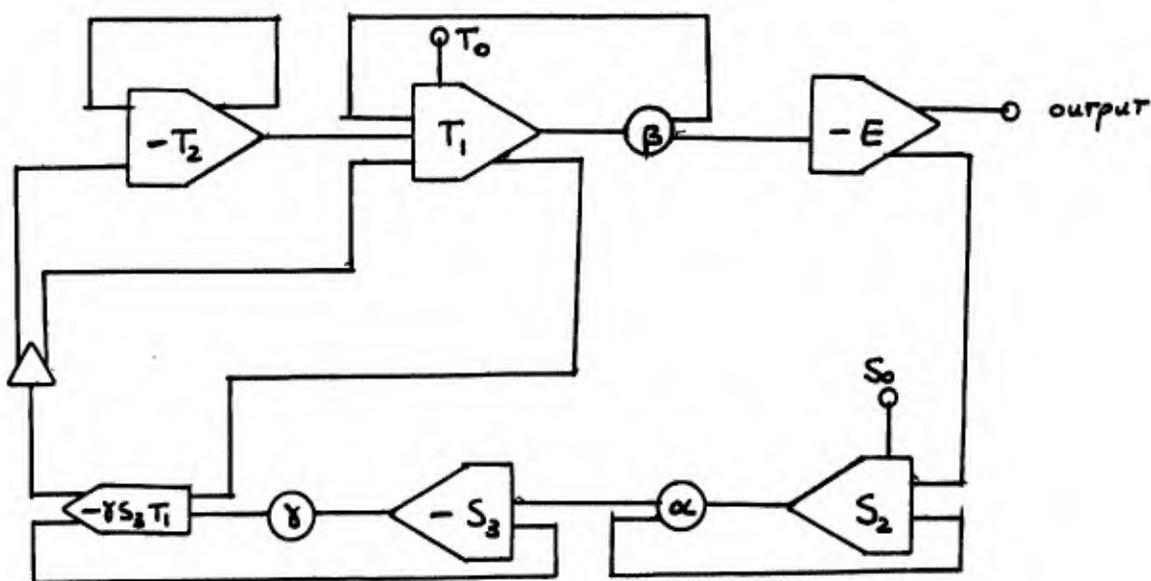


Fig. 6B: Schematic computer circuit for system II-3, as explained in text.

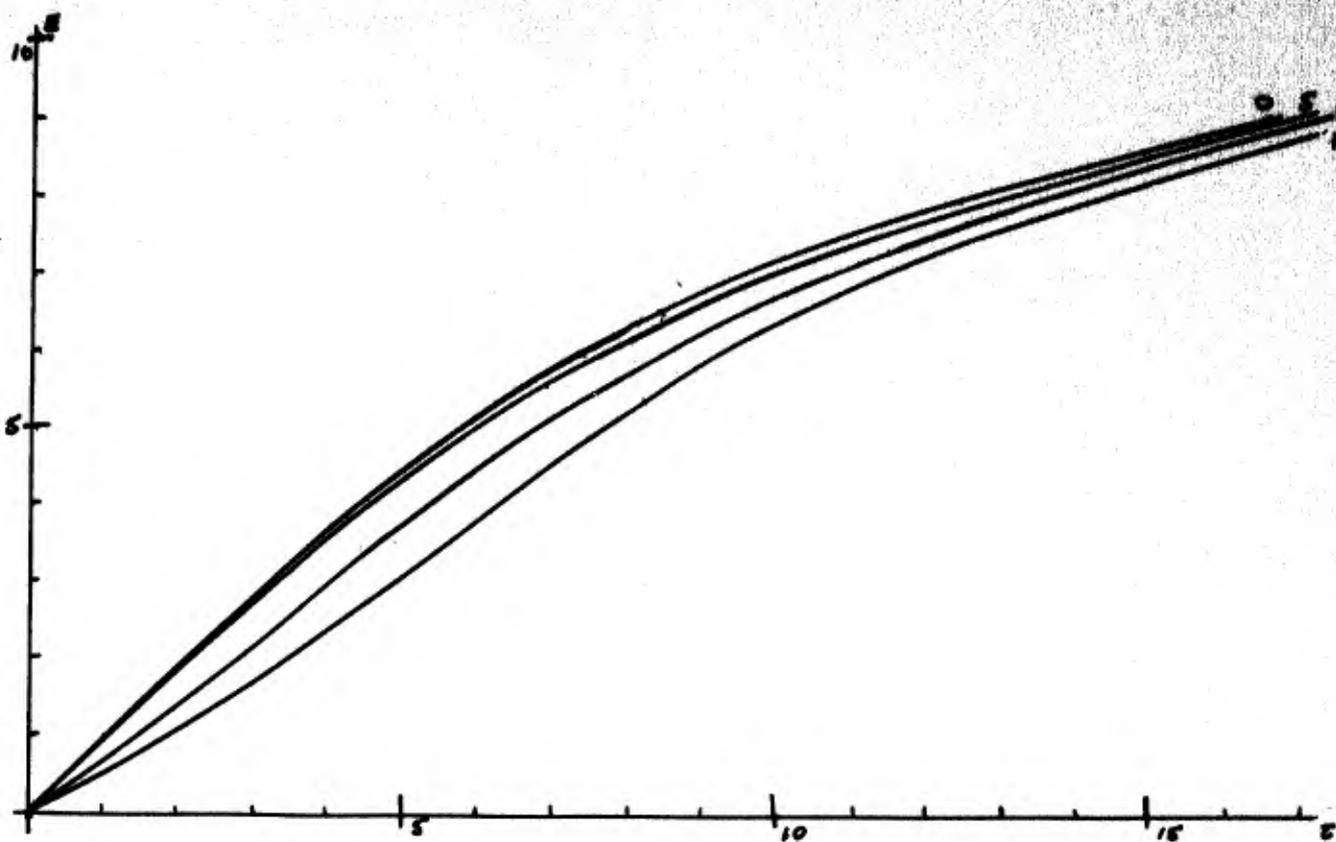


Fig. 7A: Enzyme curves for system II-2, for various S_0 values as indicated.

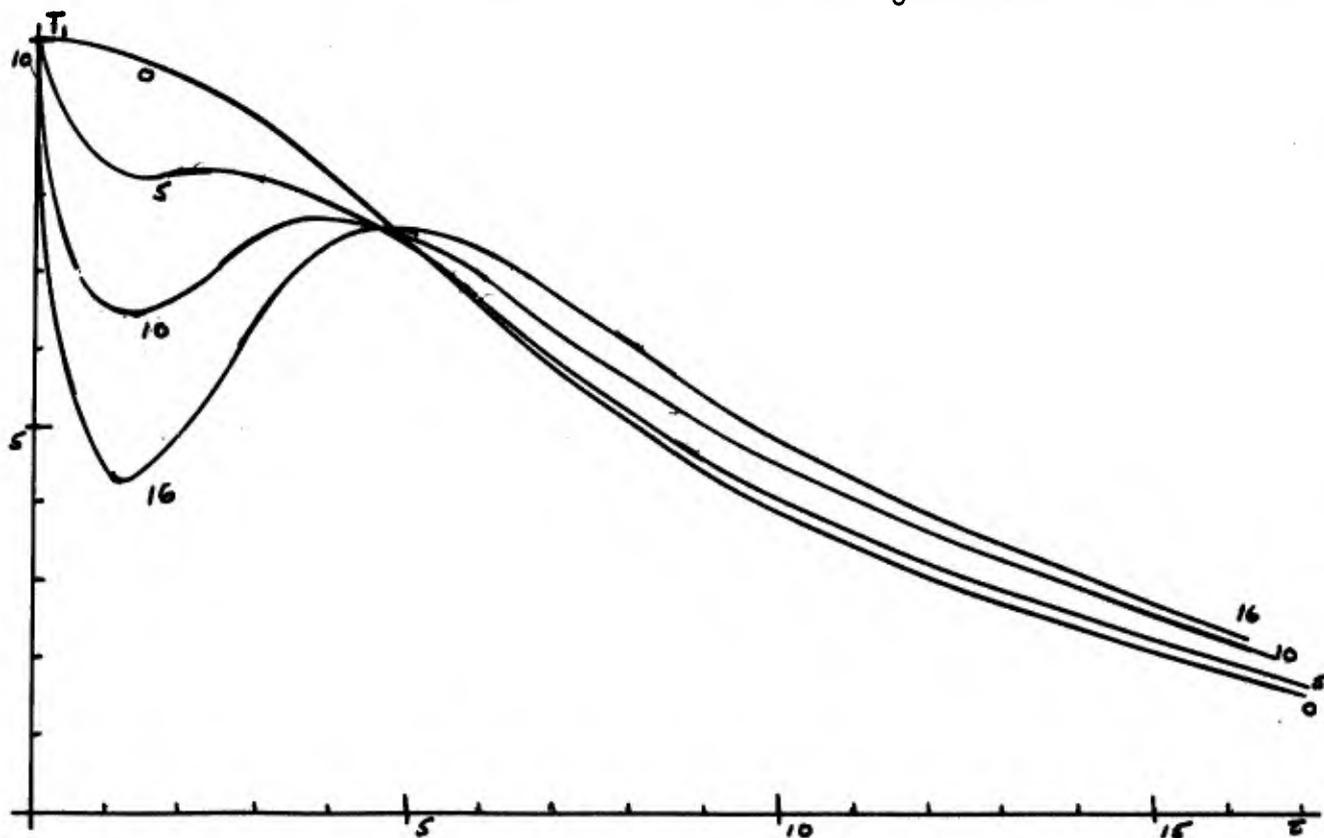


Fig. 7B: Template curves for system II-2, for various S_0 values as indicated.

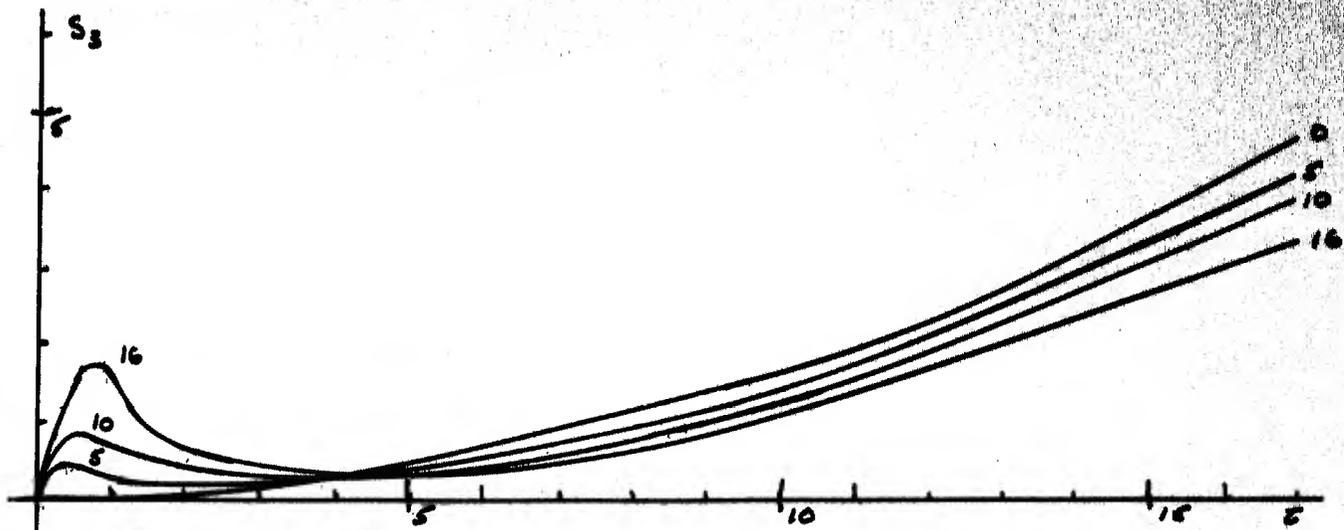


Fig. 8A: The inducer S_3 in system II-2 for various S_0 values as indicated.

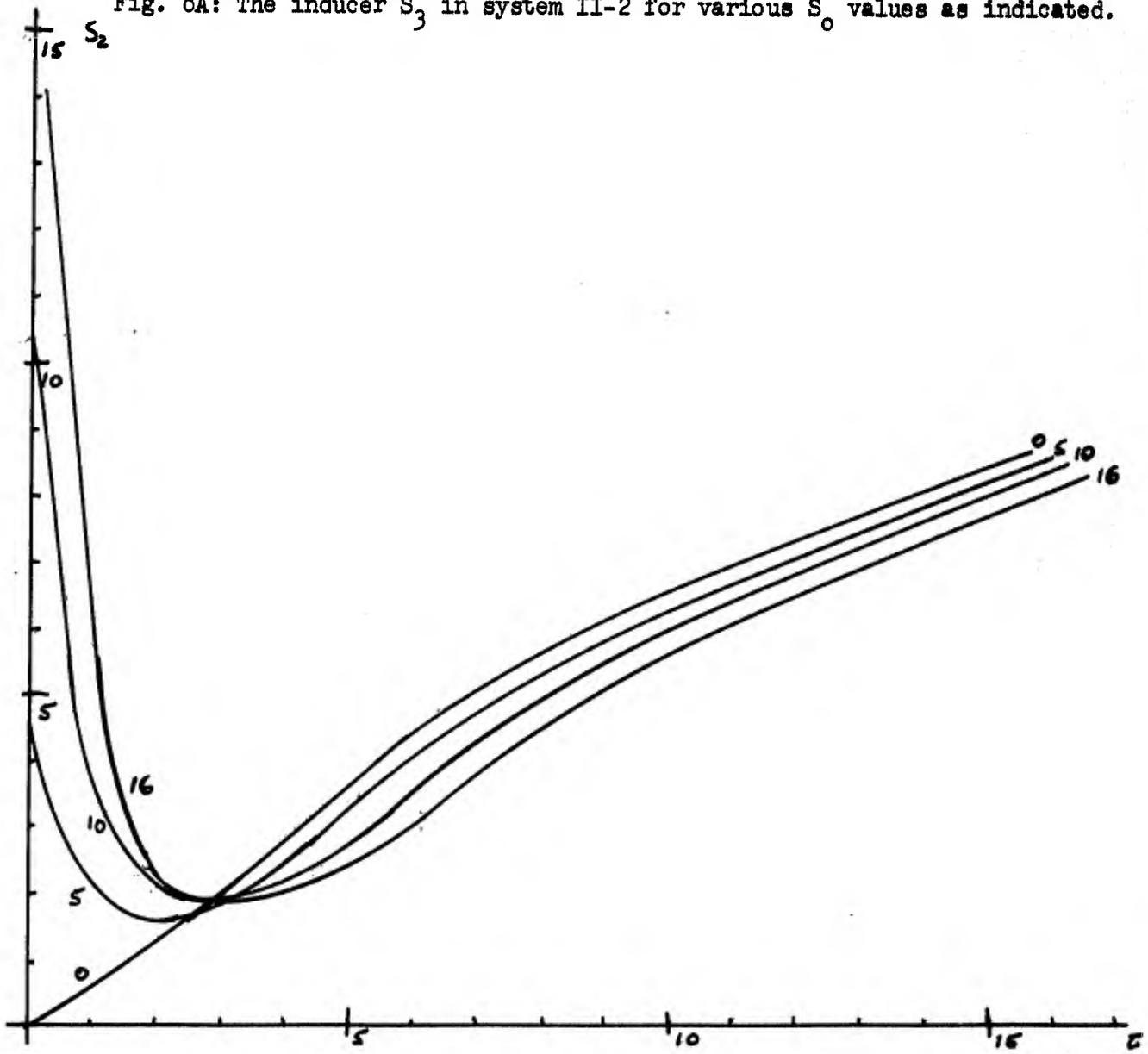


Fig. 8B: The inducer S_2 in system II-2 for various S_0 values as indicated.

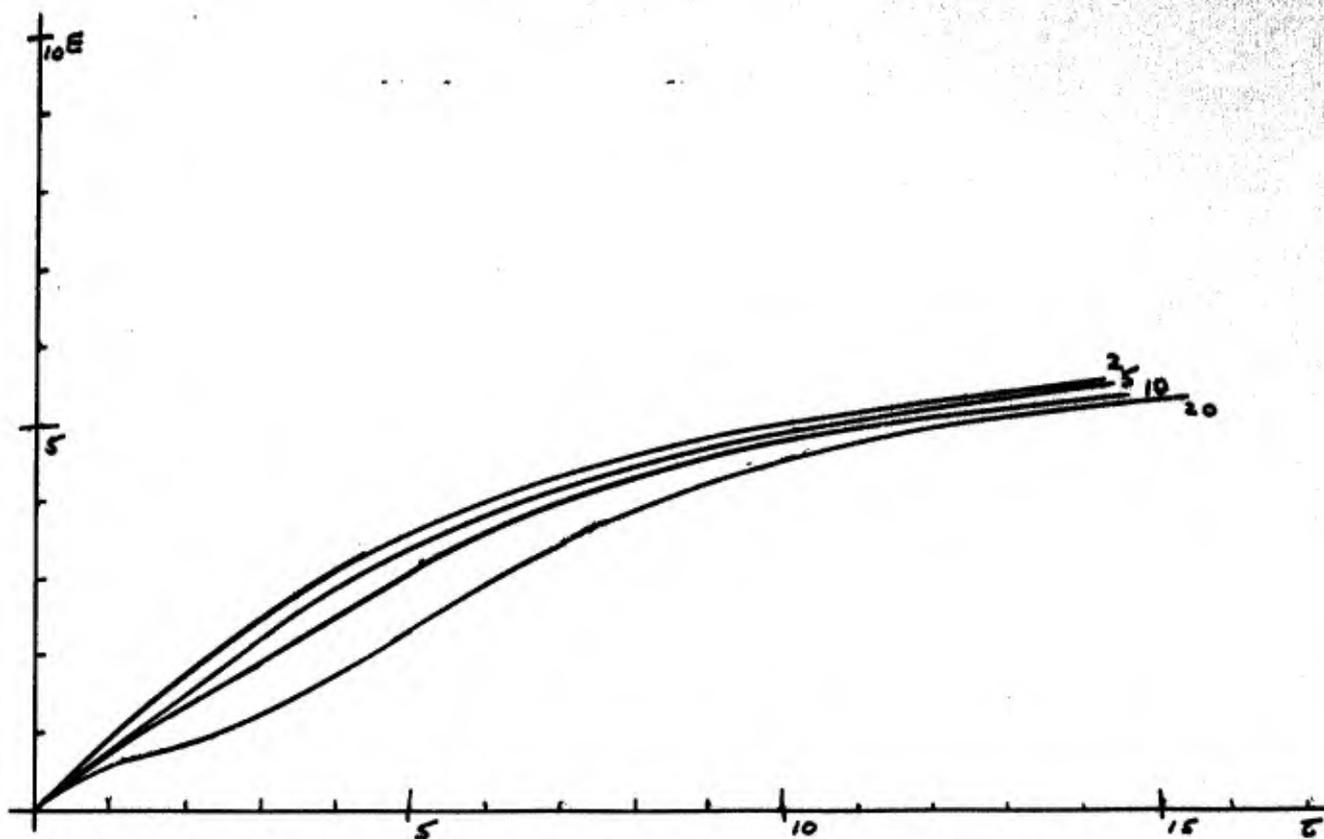


Fig. 9A: Enzyme curves for system II-3, for various S_0 values as indicated.

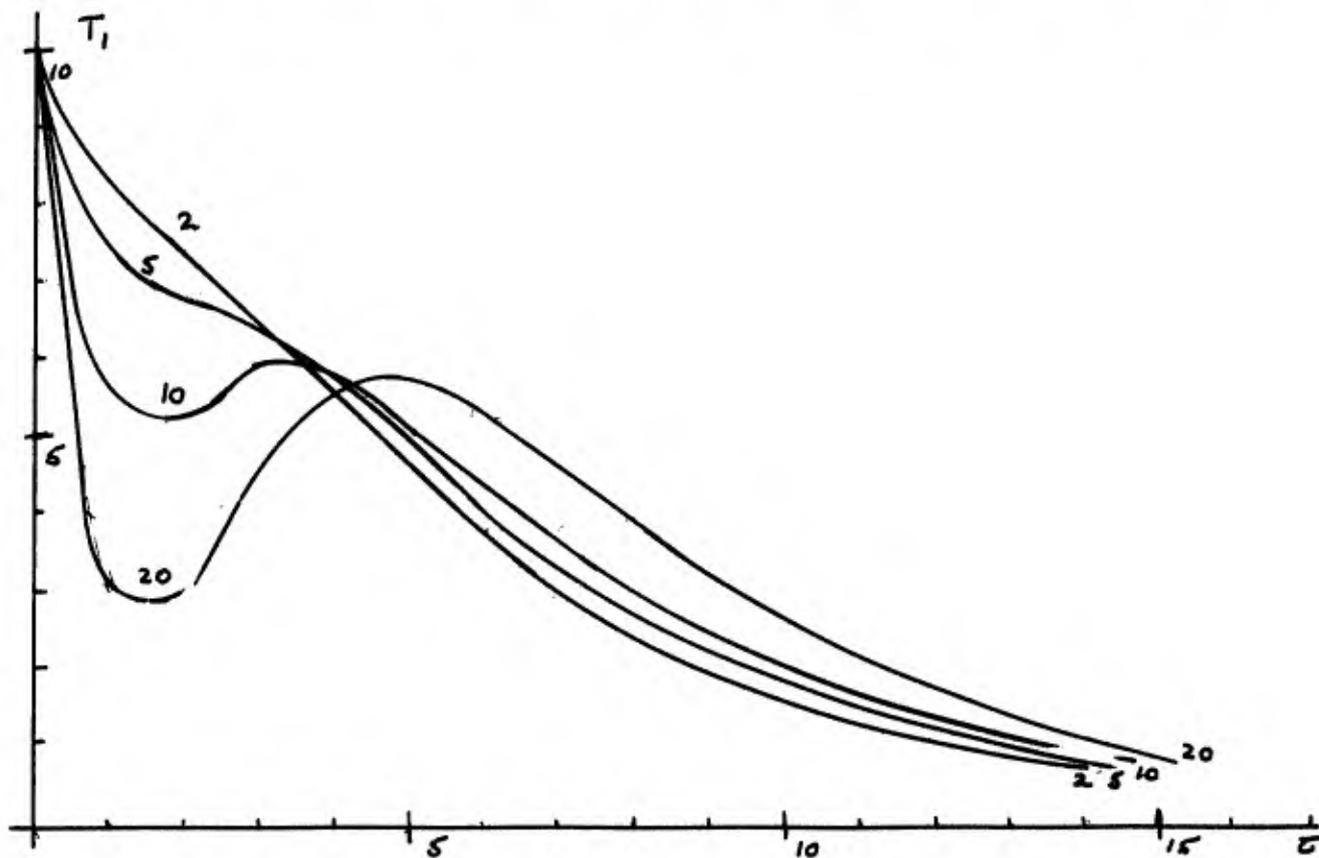


Fig. 9B: Template curves for system II-3, for various S_0 values as indicated.

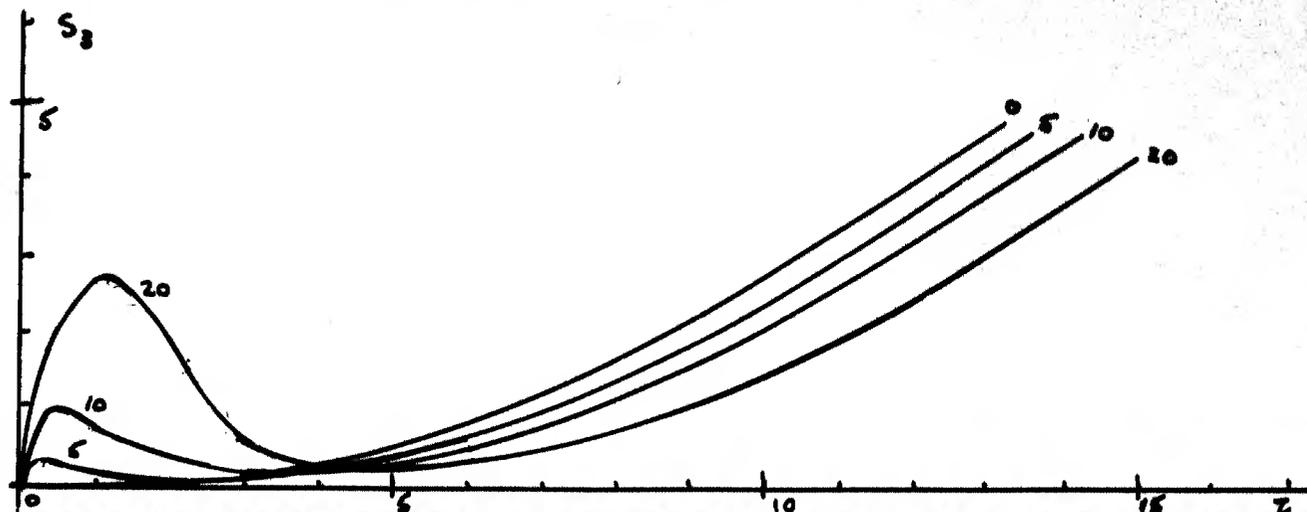


Fig. 10A: The inducer, S_3 , in system II-3 for various values of S_0 as indicated.

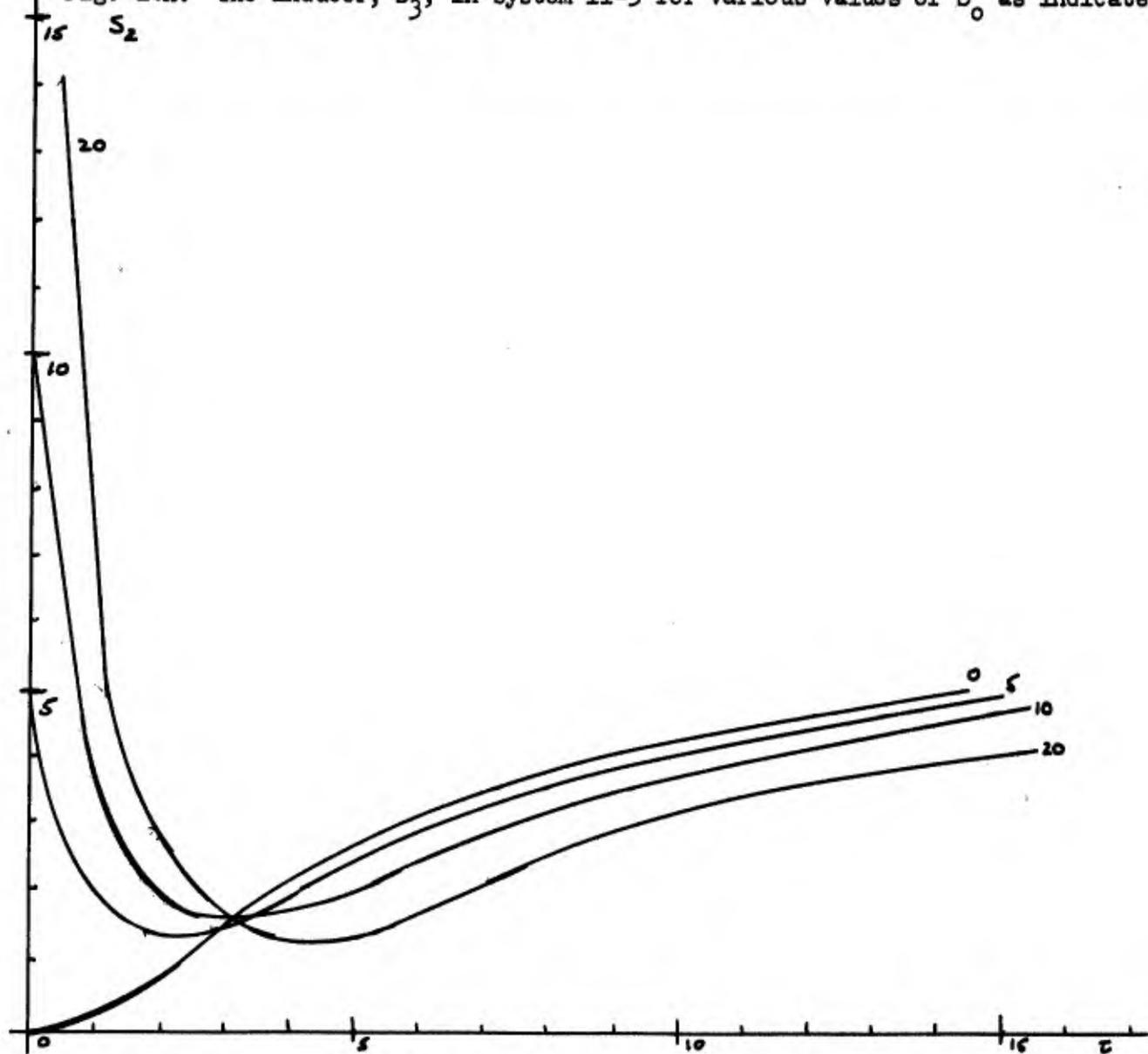


Fig. 10A: The inducer, S_2 , in system II-3 for various values of S_0 as indicated.

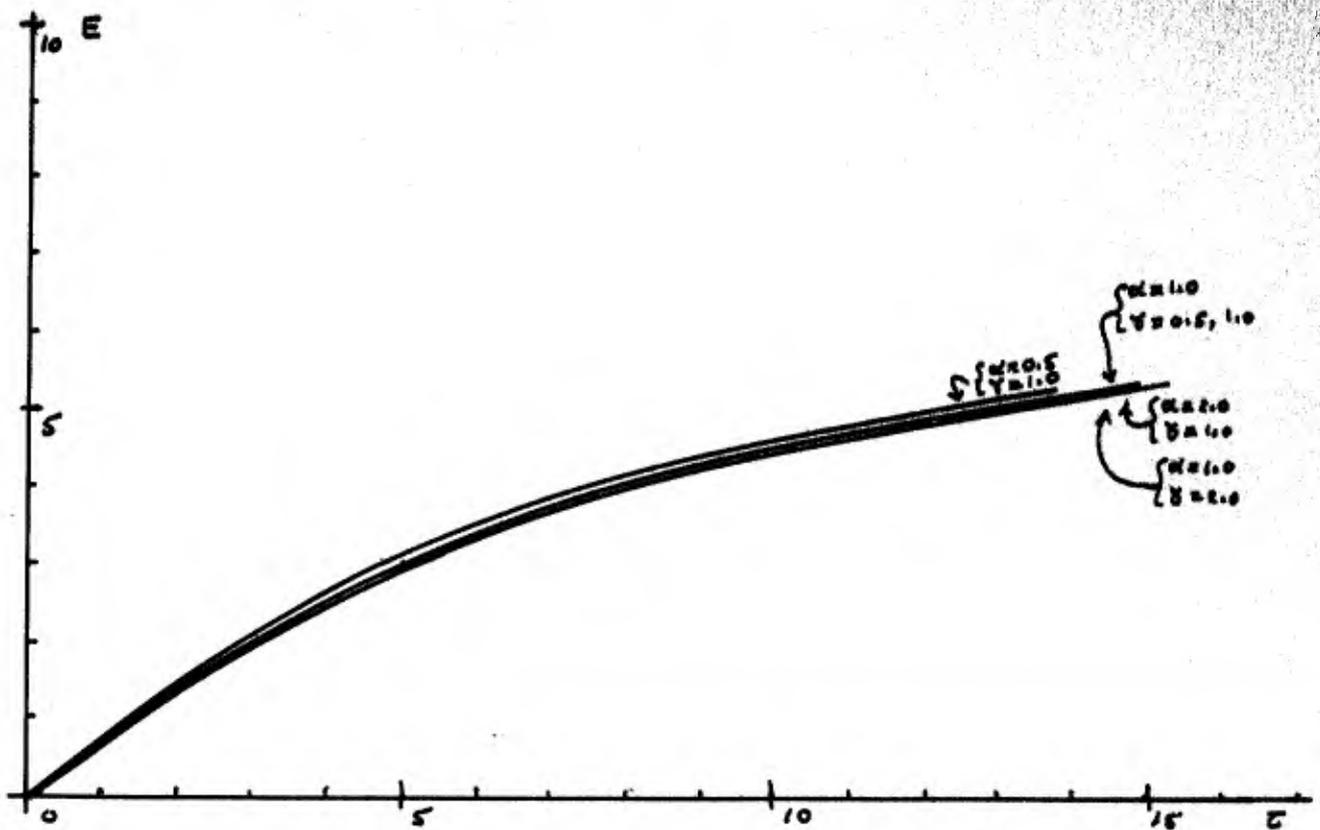


Fig. 11A: Enzyme curves for system II-3 with $S_0 = T_0 = 10$ volts, $\beta = 0.1$ and α and γ varied as indicated.

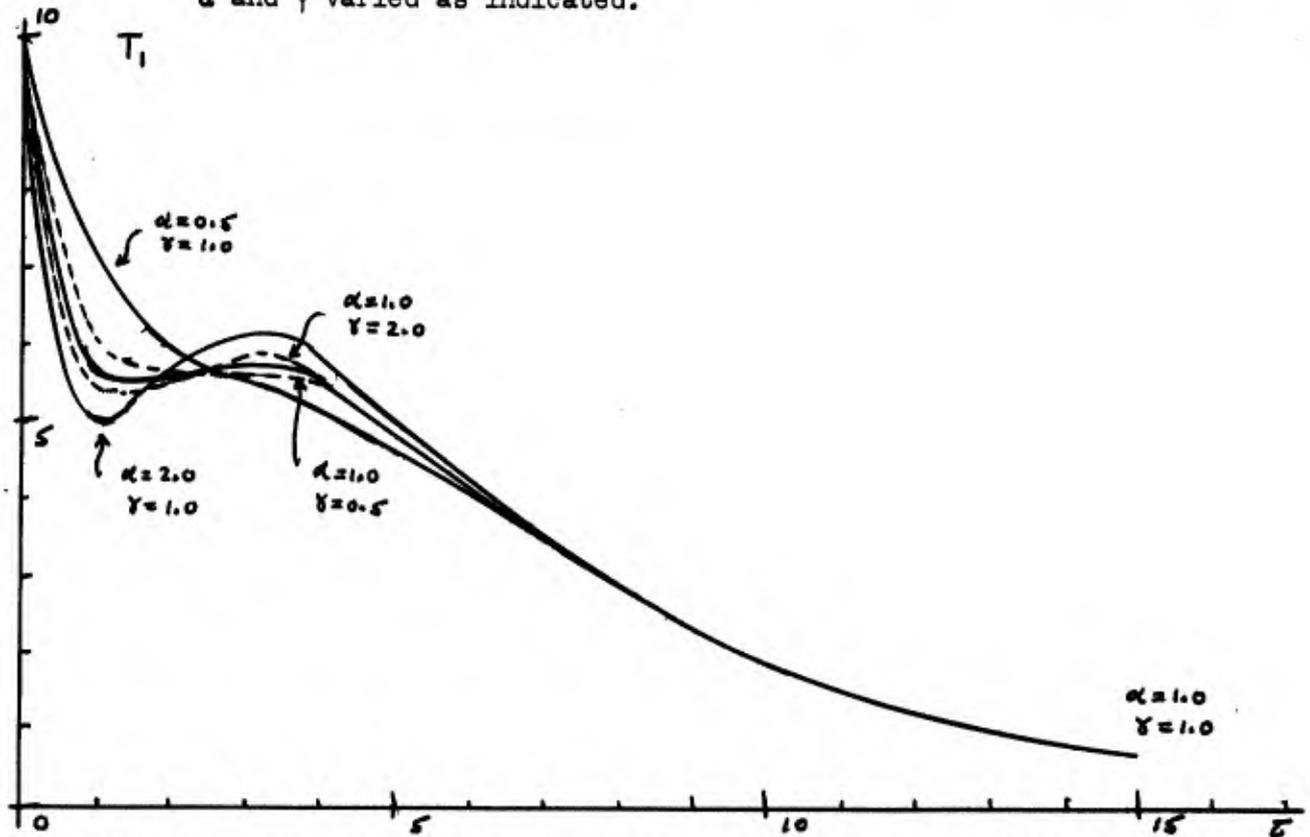


Fig. 11B: Template curves for system II-3 with $S_0 = T_0 = 10$ volts, $\beta = 0.1$ and α and γ varied as indicated.

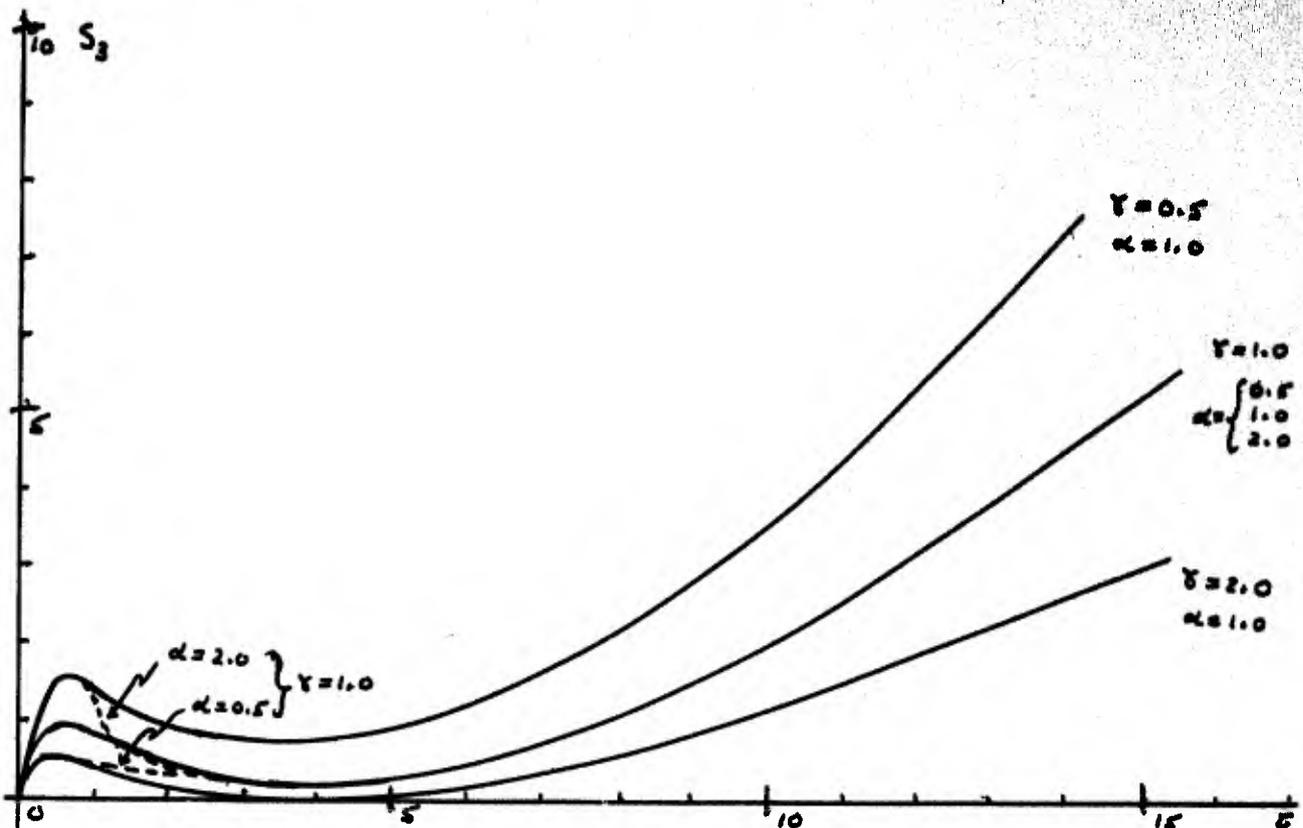


Fig. 12A: Inducer, S_3 , curves for system II-3 with $S_0 = T_0 =$ volts, $\beta = 0.1$ and α and γ varied as indicated.

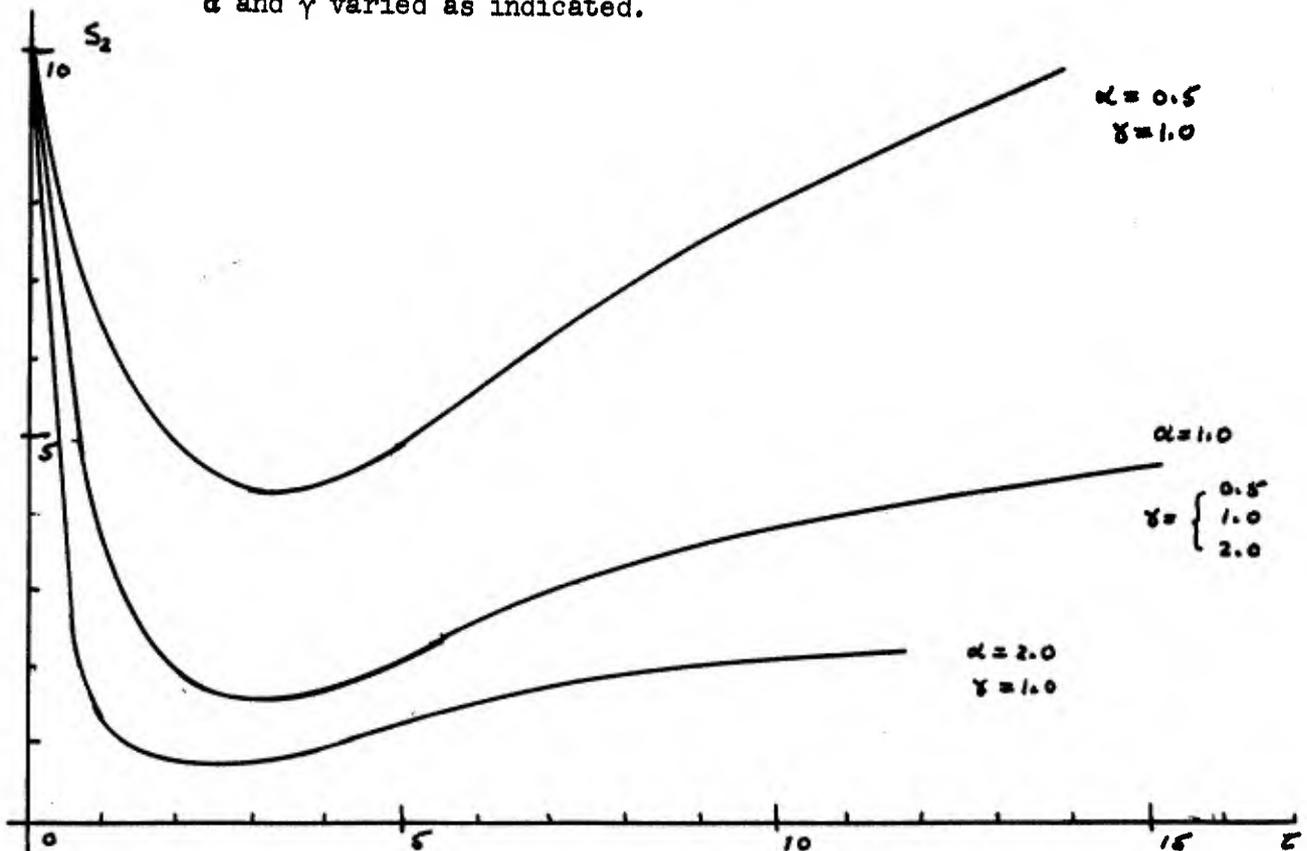
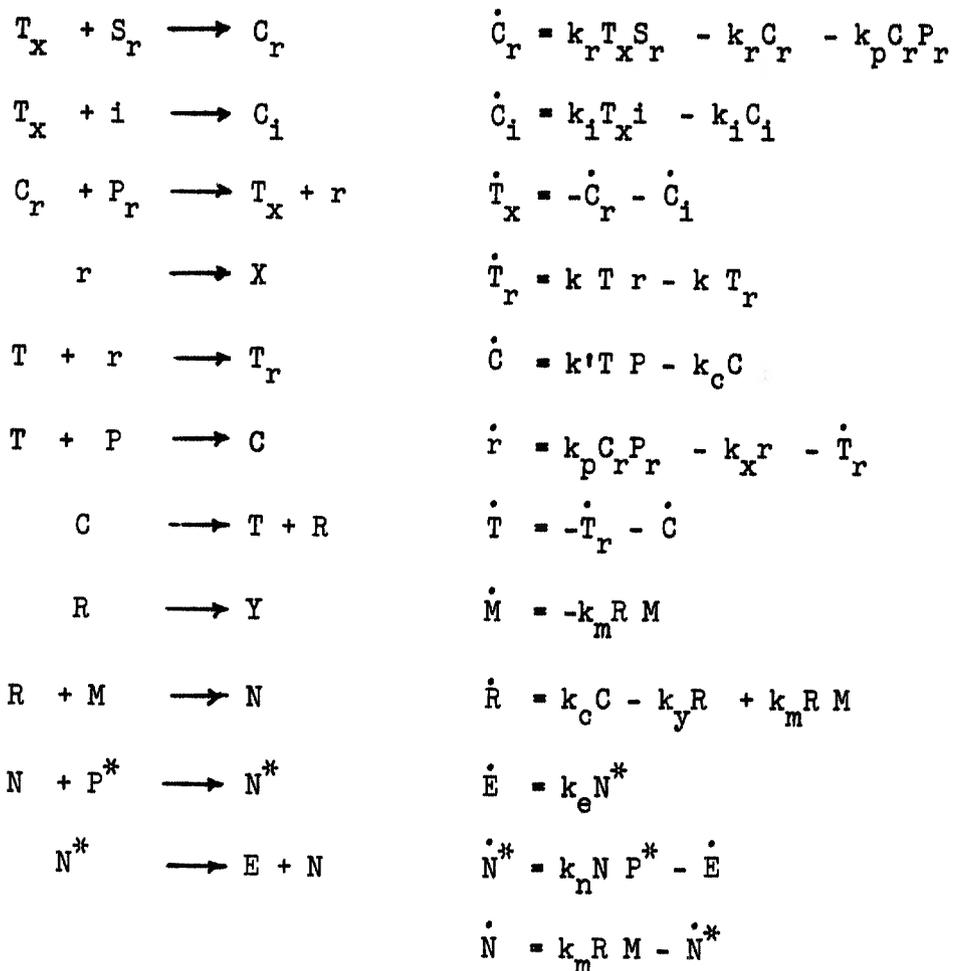


Fig. 12B: Inducer, S_2 , curves for system II-3 with $S_0 = T_0 = 10$ volts, $\beta = 0.1$ and α and γ varied as indicated.

III. Circuits For More Complex Systems

In this phase of the work, the differential equations for three more complex systems were obtained and these were turned into machine circuit diagrams, which because of the large number of components needed in each, would have to be run off on much more elaborate equipment than that available here.

The first of these systems, III-1, is given by the following kinetic equations with their associated differential equations; the machine circuit corresponding will be found in Fig. 13:



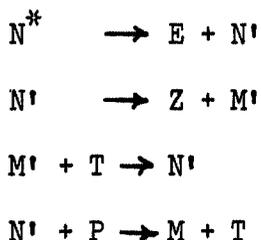
The biological meaning of the symbols are given as follows (the abbreviation, const., in the following chart means that the concentration of the indicated

quantity is assumed constant during the process.):

T_x = unknown template	R = active (RNA)
T = functioning template (DNA)	C = (TP) complex
T_r = repressed template	M = microsomes
r = repressor	N = (RM) complex
i = inducer (const.)	P^* = enzyme synthesis pool (const.)
C_i = ($T_x i$) complex	N^* = (RMP *) complex
C_r = ($T_x S_r$) complex	M' = related microsome
S_r = precursor for repressor (const.)	N' = related (RM) complex
P_r = repressor formation pool (const.)	E = enzyme
P = active formation pool (const.)	X, Y, Z = inactive products

The system breaks down into four units, as can be seen from Fig. 13. The upper line synthesizes the repressor; the middle right, the active RNA; the middle left, the enzyme precursor; and the lower line synthesizes the enzyme itself.

In the next system, III-2, we utilize the same symbols, but introduce a somewhat more complex feedback unit in the last stage, these leave everything but the last stage of III-1 unchanged, and make it:



All of the differential equations in III-1 remain valid except for the following:

$$\dot{M} = -k_m R M = k'_n N' P^*$$

$$\dot{M}' = k_z N' - k'_m M' T$$

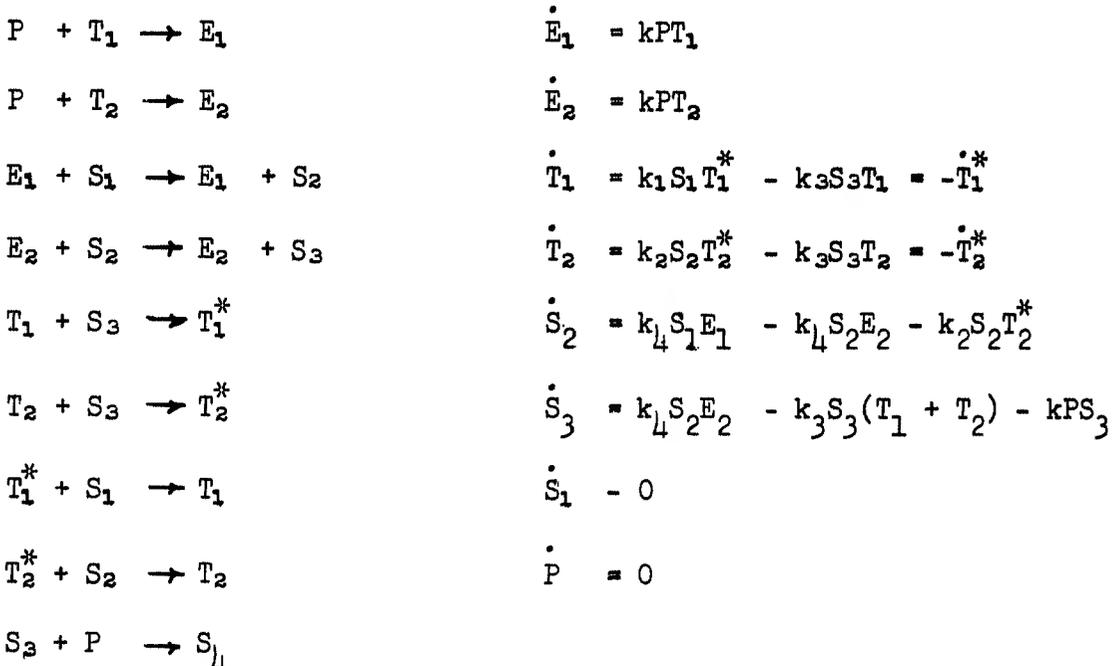
$$\dot{T} = -\dot{T}_R - \dot{C} + \dot{M}'$$

$$\dot{N}' = \dot{N}^* - \dot{M}'$$

$$\dot{N} = k_m R M - k'_n N P^*$$

The machine circuit for this system will be found in Fig. 14, and it will be noted to possess one additional final loop linked into the original system.

In the final case, we consider an example of chain synthesis with repression. Here, since we are only interested in the qualitative effects of repression on the chain, we limit ourselves to direct repression as opposed to the indirect variety considered in the preceding two examples. Thus we consider the system, III-3:



We see in this case that the S_3 itself acts as the repressor, producing the inactive T^* forms of the template, which are only reactivated by the other substrates. The rate constant in the spontaneous decay of the S_3 is arbitrary and may be varied from the value given. The computer circuit corresponding to this system is given in Fig. 15.

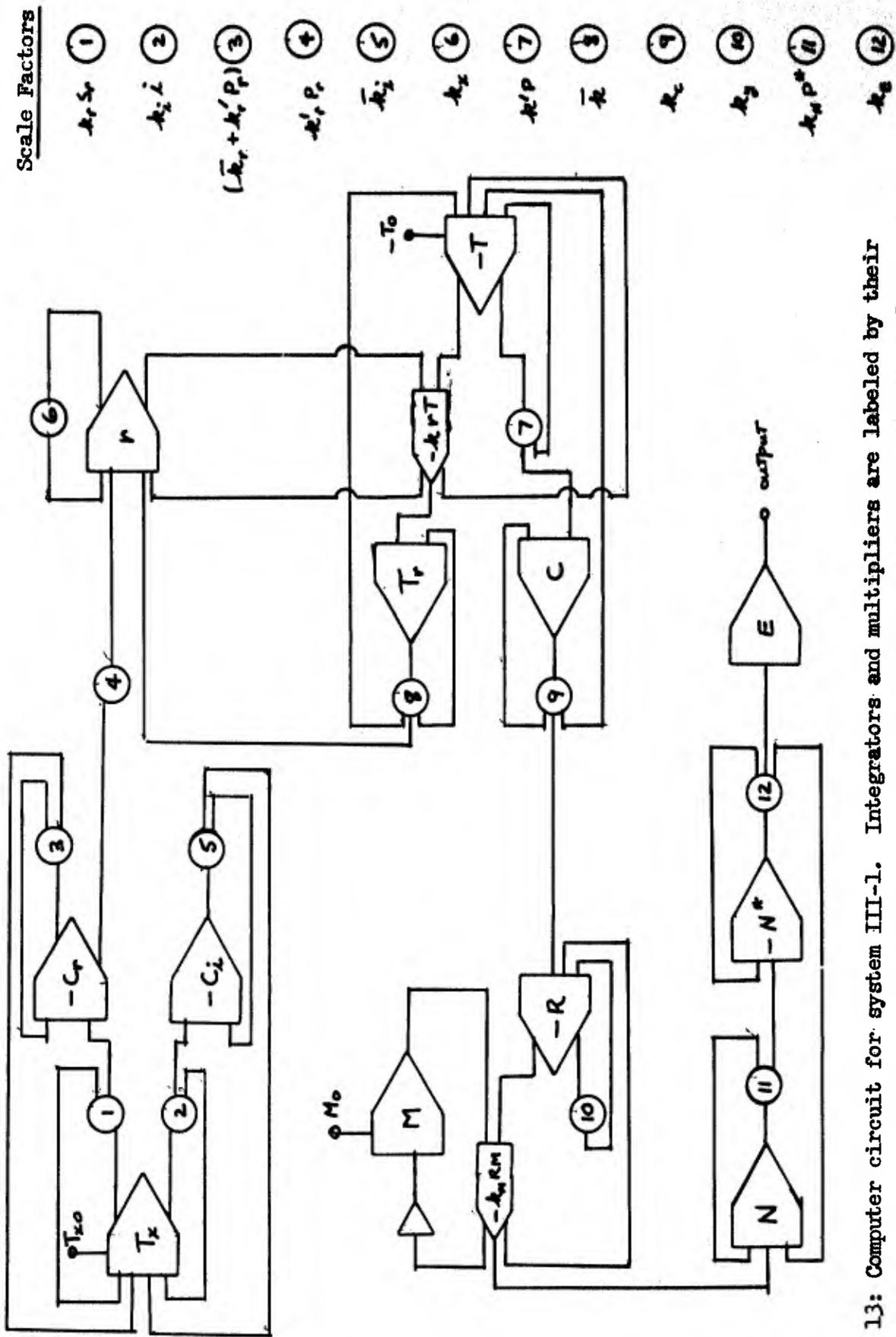


Fig. 13: Computer circuit for system III-1. Integrators and multipliers are labeled by their outputs. Multipliers are positive with scale as indicated and the circuit scale factors are given in numerical order on the right.

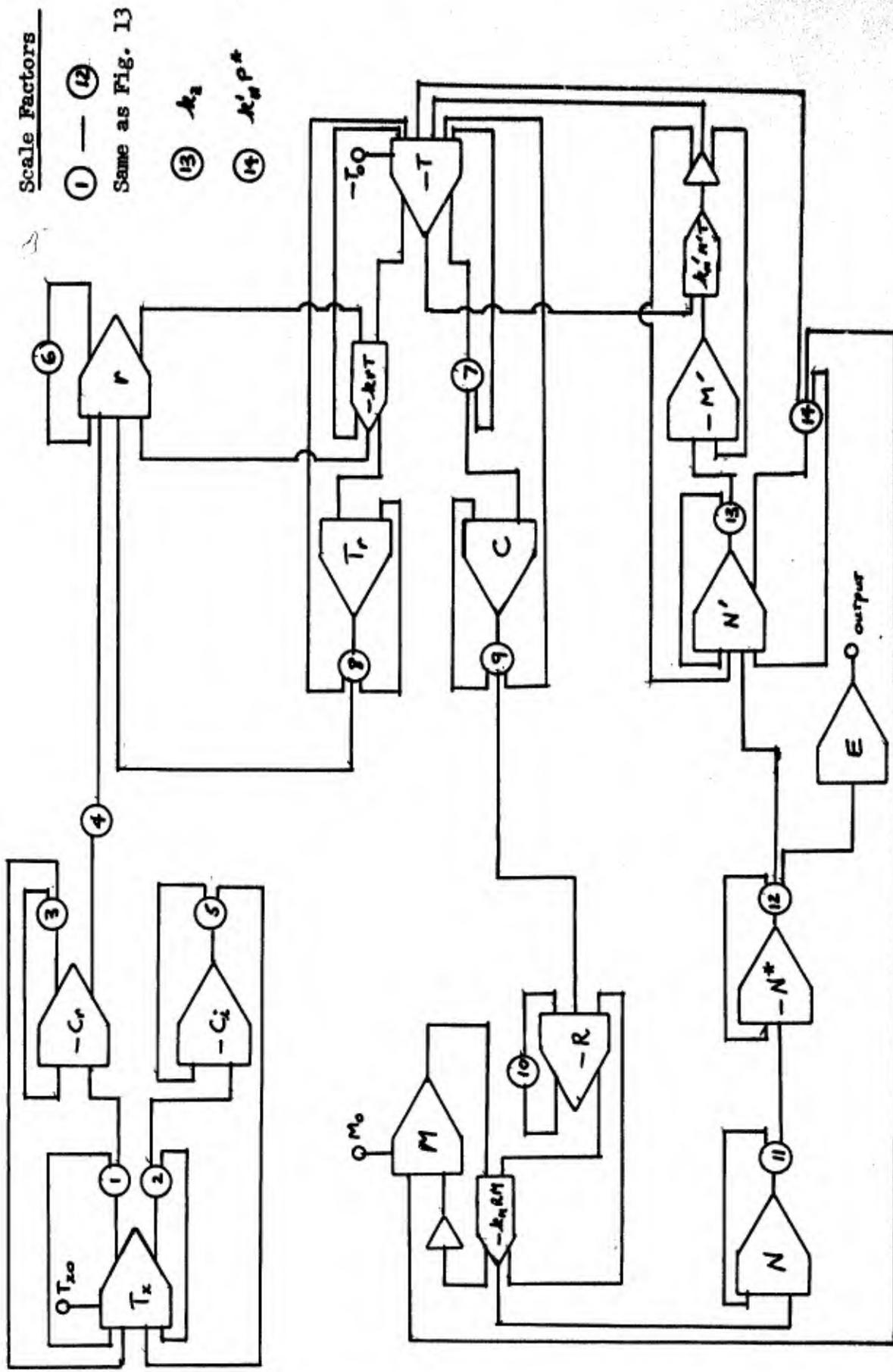
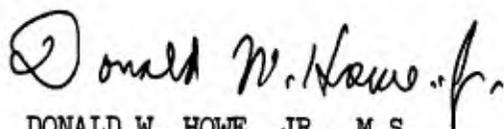


Fig. 14: Computer circuit for system III-2. The same conventions hold as for Fig. 13.

Conclusion

On the basis of the work done during the tenure of this contract, we conclude that the use of analogue computers for the analysis of mathematical schemes for biological processes, is not only feasible but both practical and desirable. The matter of obtaining the differential equations for the various biological schemes envisaged, is straightforward and has been amply analyzed in previous reports in this series; it then becomes a matter of transferring these equations into computer circuitry -- a process which we have shown at length in this report, to be straightforward as well. Once the circuits have been established, the computer may be programmed according to recipes which can be found in most manuals -- one such program is given in Fig. 4B. The results of the computation are as accurate as the experimental evidence warrants and can be used to verify the mathematical schemes by direct comparison with such evidence, as we have seen in the first section. These results may also be used to analyze systems which do not lend themselves to direct intuition, showing, for example, that a given non-linear system is relatively insensitive to variation of its parameters, a result which had been assumed for purely heuristic reasons in the first part of the report and justified more extensively in the second. Finally, we would conclude that the use of these computers warrants an extension of this phase of the research to set up more complex systems such as those considered in the last part of the report on a computer of greater capacity, perhaps one available commercially.


DONALD W. HOWE, JR., M.S.
Associate Professor of Physics


ARTHUR HERSCHMAN, Ph.D.
Assistant Professor of Physics