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A NONLINEAR MODEL FOR TEMPLET REGULATED PROTEIN SYNTHESIS

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SUMMARY

A mathematical model of biological polymerization reactions is
mulated and applied to protein synthesis. Under certain reason-
e assumptions, including that of a nondisassociated enzyme temp-
complex, the resulting set of first order nonlinear differential
ations were solved by integral transform techniques. The case
re the enzyme templet complex disassociates is also treated by
turbation techniques.

The results indicate that the synthesis can be regarded as con-
ting of three stages (assuming constant monomer concentration):
an initial stage in which the concentration of protein M_N is giv-
by $M_N \sim Ct^{2N+1}$ (where N is the degree of polymerization and t,
e) and therefore representing a lag phase; (b) an intermediate
ge in which the protein concentration is determined by an exponen-
l polynomial; (c) a third state of a linear increase in protein
centration with time. Comparisons of these predictions with the
vitro data reported by Nirenberg [5] indicate satisfactory agree-
t.

Methods are also described for determining the rate constants,
ociated with the polymerization, from the in vitro kinetic data.
s important result should facilitate the kinetic analysis of pep-
e synthesis.

A NONLINEAR MODEL FOR TEMPLET REGULATED PROTEIN SYNTHESIS

In a previous investigation [1] a linear model was presented
rotein synthesis by polyribosomes. Some analytical results
nonlinear model are given here.

lation

In the following we consider a system of messenger RNA tem-
bound to ribosomes of total concentration T_o and amino acid
erizing enzyme (i.e., transferase) of total concentration E_o.
sume that T_o and E_o remain constant during the course of the
erization so that denaturation and hydrolysis are assumed not
cur.

The details of complex formation between the polymerase, the
nger RNA and the amino acid-adaptor RNA compound is not known.
e following we assume that the initial complex is formed by
eactions

$$E + T \underset{k_{02}}{\overset{k_{01}}{\rightleftharpoons}} E\,T \qquad ;$$

$$M + E\,T \underset{k_{12}}{\overset{k_{11}}{\rightleftharpoons}} M\,E\,T \quad .$$

irst expression describes the complex formation between the free
erase (E) and the messenger RNA (T) (presumed adsorbed onto a
some) with a rate constant k_{01} for the association and k_{02} for
isassociation. The amino acid initially present as the amino
acyl-adaptor RNA compound, designated by M, then forms the com-
M E T, as is indicated by reaction (2a). If subsequent studies

ate that instead of (1a) and (2a), the significant reactions

$$E + M \rightleftharpoons E.\ M \quad ,$$

$$E.\ M + T \rightleftharpoons M\ E\ T \quad ;$$

$$T + M \rightleftharpoons T\ M \quad ,$$

$$T\ M + E \rightleftharpoons M\ E\ T \quad .$$

he present formalism will be altered in detail only but the
al methods described here are still applicable.
Following the formation of MET, any internal readjustment of
required to initiate the polymerization may be represented by

$$M\ E\ T \xrightarrow{k_{13}} E\ T\ M \quad .$$

such transformation is necessary, then the present analysis
es with $k_{13=0}$. The polymerization reaction subsequent to
may now be written as

$$M + E.\ T.\ M \overset{k_{21}}{\rightleftharpoons} M.\ E.\ T.\ M \quad ,$$

$$M\ E\ T\ M \xrightarrow{k_{23}} E.\ T.\ M_2 \quad .$$

neral, after (1a) we have a sequence of the following pairs
eactions

$$M + E\ T\ M_i \underset{k_{(i+1)2}}{\overset{k_{(i+1)1}}{\rightleftharpoons}} M\ E\ T\ M_i \quad ,$$

$$M.\ E.\ T.\ M_i \xrightarrow{k_{(i+1)3}} E.\ T.\ M_{i+1} \quad .$$

ion (6a) represents the reversible adsorption of the amino
-adaptor RNA molecule while (7a) represents (with the excep-
of i=2) the irreversible formation of the peptide bond. It
be noted that the above formulation assumes that the enzyme-
et-peptide complex remains irreversibly associated during the
e of protein synthesis. Evidence supporting a tightly bound
omplex has been provided by Tissiers et al [3]. These work-
report that during protein synthesis the most active ribosome
ion (70 S fraction) from E. Coli forms an undisassociated com-
with the newly formed protein. They postulate that the pro-
is released by dissociation of the 70 s ribosome fraction to
and 30 s fractions which do not irreversibly bind the protein.
er and Lingel [4] also found it necessary to postulate the
for a special release factor in the case of protein synthesis
t liver ribosomes. It appears from such results as these that
ease step is required at the termination of the synthesis. If
resents the total number of amino acids in the complete pro-
then the terminal reactions may be presented by

$$M + E\,T\,M_{N-1} \underset{k_{N2}}{\overset{k_{N1}}{\rightleftharpoons}} M\,E\,T\,M_{N-1} \quad ,$$

$$M\,E\,T\,M_{N-1} \xrightarrow{k_{N3}} M_N + E\,T \quad ,$$

release occurs concomitant with the addition of the last amino
Alternatively, it may be that the addition of the amino acid
ue occurs first and that this is followed by release:

$$M\ E\ T\ M_{N-1} \xrightarrow{k_{N3}} E\ T\ M_N \quad ,$$

$$E\ T\ M_N \xrightarrow{k_r} M_N + E\ T \quad ,$$

k_r is the rate constant associated with the release of bound
in.

sis

It is convenient to summarize the mechanism discussed above.

= Concentration of nucleic acid template.

= Concentration of enzyme.

= Concentration of monomer.

= Concentration of polymer consisting of i monomers.

Then the system of reactions is given by the set of equations:

$$E + T \underset{k_{02}}{\overset{k_{01}}{\rightleftharpoons}} E.\ T \quad ,$$

$$M.\ E.\ T \underset{k_{12}}{\overset{k_{11}}{\rightleftharpoons}} M.\ E.\ T \quad ,$$

$$M.\ E.\ T. \xrightarrow{k_{13}} E.\ T.\ M \quad ,$$

$$M + E.\ T.\ M \underset{k_{22}}{\overset{k_{21}}{\rightleftharpoons}} M.\ E.\ T.\ M \quad ,$$

$$M.\ E.\ T.\ M \xrightarrow{k_{23}} E.\ T.\ M_2 \quad ,$$

$$M + E.\ T.\ M_2 \underset{k_{32}}{\overset{k_{31}}{\rightleftharpoons}} M.\ E.\ T.\ M_2 \quad ,$$

$$M.\ E.\ T.\ M_2 \xrightarrow{k_{33}} E.\ T.\ M_3 \quad ,$$

. . . . . .

In general, after the first equation in the above we have
quence of the following pairs of reactions:

$$M + \text{E. T. } M_i \underset{k_{(i+1)2}}{\overset{k_{(i+1)1}}{\rightleftharpoons}} \text{M. E. T. } M_i \quad ,$$

$$\text{M. E. T. } M_i \xrightarrow{k_{(i+1)3}} \text{E. T. } M_{(i+1)} \quad ,$$

e i = o, 1, ..., N - 1, and M_o is to be interpreted as the null
mer (a chain having no monomers).

If N is the total number of monomers in the chain, then the last
reactions are

$$M + \text{E. T. } M_{N-1} \underset{k_{N2}}{\overset{k_{N1}}{\rightleftharpoons}} \text{M. E. T. } M_{N-1} \quad ,$$

$$\text{M. E. T. } M_{N-1} \xrightarrow{k_{N3}} M_N + \text{E. T} \quad .$$

Here M_N is the complete protein.

Write $x_i = \text{M. E. T. } M_{(i-1)} \qquad (i \geq 1)$,

$y_i = \text{E. T. } M_i \qquad (i \geq o)$.

Then the system of O.D.E's corresponding to the reactions (1)
(2) is

$$\frac{dE}{dt} = -k_{o1}(E)(T) + k_{o2}\, y_o \quad ,$$

$$\frac{dT}{dt} = -k_{o1}(E)(T) + k_{o2}\, y_o \quad ,$$

$$\frac{dy_0}{dt} = k_{01}(E)(T) - k_{02}y_0 - k_{11}My_0 + k_{12}x_1 + k_{N3}x_N \quad ,$$

. . . . . .

$$\frac{dx_i}{dt} = k_{i1}My_{i-1} - (k_{i2} + k_{i3})x_i \qquad (i = 1, 2, \cdots, N-1),$$

$$\frac{dy_i}{dt} = k_{i3}x_i - k_{(i+1)1}My_i + k_{(i+1)2}x_{i+1} \quad ,$$

. . . . . .

$$\frac{dx_N}{dt} = k_{N1}My_{N-1} - (k_{N2} + k_{N3})x_N \quad ,$$

$$\frac{dM_N}{dt} = k_{N3}x_N \quad .$$

This system will be considered under the assumptions that M is
ɔant and the k_{i1}, k_{i2}, k_{i3} are all non-negative numbers. Note
the last equation may be considered apart from the rest of the
ɜm.

The system (3) has certain first integrals which are now de-
i. These may be used to eliminate some of the variables and thus
ɔe the order of the system.

If all the equations in (3) except the second and the last are
l together, we get

$$\frac{dE}{dt} + \frac{dy_o}{dt} + \sum_{i=1}^{N-1}\left(\frac{dx_i}{dt} + \frac{dy_i}{dt}\right) + \frac{dx_N}{dt} = -k_{11} M y_o + k_{12} x_1 + k_{N3} x_N$$

$$+ \sum_{i=1}^{N-1}\left(k_{i1} M y_{i-1} - k_{(i+1)1} M y_i - k_{i2} x_i + k_{(i+1)2} x_{i+1}\right)$$

$$+ k_{N1} M y_{N-1} - (k_{N2} + k_{N3}) x_N = 0 \quad .$$

Thus

$$E + y_o + \sum_{i=1}^{N-1}(x_i + y_i) + x_N = c_1 \quad , \tag{4}$$

where c_1 is a constant of integration.

Similarly, if we add all the equations in (3) except the first and last, then

$$\frac{dT}{dt} + \frac{dy_o}{dt} + \sum_{i=1}^{N-1}\left(\frac{dx_i}{dt} + \frac{dy_i}{dt}\right) + \frac{dx_N}{dt} = 0 \quad . \tag{5}$$

Thus we have the integral

$$T + y_o + \sum_{i=1}^{N-1}(x_i + y_i) + x_N = c_2 \quad , \tag{6}$$

where c_2 is another constant of integration.

Note: If we assume that all the x_i and y_i are zero initially, then $c_1 = E(o) = E_o$ and $c_2 = T(o) = T_o$, the initial amounts of enzyme and template, respectively.

The system (3) does not appear to have any other simple first integrals besides (4) and (6) and furthermore, in general, no closed-form solution seems to exist. However, in the special case where $k_{o2} = o$, a closed form can be derived and this will be given in the next section.

2. Protein Synthesis in the Case $k_{02} = o$

Let $$u = y_o + \sum_{i=1}^{N-1}(x_i + y_i) + x_N \quad .$$

Then the two integrals (4) and (6) become

$$E + u = c_1 \quad , \qquad T + u = c_2 \quad .$$

In (3), with $k_{02} = o$, add all the equations except the first two and the last. Then

$$\frac{dy_o}{dt} + \sum_{i=1}^{N-1}\left(\frac{dx_i}{dt} + \frac{dy_i}{dt}\right) + \frac{dx_N}{dt} = k_{01}(E)(T) \quad .$$

Writing this in terms of u by means of (7), we get

$$\frac{du}{dt} = k_{01}(c_1 - u)(c_2 - u) \quad ,$$

an equation which is immediately integrable since the variables are separated.

Carrying through the integration, we find that

$$u = \frac{c_1 - c_2 c_3 e^{2\beta k_{01} t}}{1 - c_3 e^{2\beta k_{01} t}}$$

if $c_1 \neq c_2$. Here $\beta = \frac{c_1 - c_2}{2}$ and c_3 is an arbitrary constant of integration.

Note: When $c_1 = c_2$,

$$u = \frac{(c_1 c_3 - 1) + c_1 k_{01} t}{c_3 + k_{01} t} \quad .$$

For the initial conditions $x_i(o) = y_j(o) = o$, $E(o) = E_o$, $T(o) = T_o$, (8) yields

$$u = \frac{E_o T_o (1 - e^{2\beta k_{01} t})}{T_o - E_o e^{2\beta k_{01} t}} \quad ,$$

with $\beta = (E_o - T_o)/2$.

Then by (7),

$$E = E_o (T_o - E_o) e^{2\beta k_{01} t} / (T_o - E_o e^{2\beta k_{01} t}) \quad ,$$

$$T_o = T_o (T_o - E_o) / (T_o - E_o e^{2\beta k_{01} t}) \quad .$$

When the solutions for E and T given by (10) are substituted in the third equation of (3), the system (3), starting with third equation, becomes a non-homogeneous linear system of O.D.E's having constant coefficients. Since the initial conditions are all zero, the solution may be worked out easily using Laplace Transforms.

Let

$$\mathcal{L}[x_i(t)] = X_i(s) \quad , \quad \mathcal{L}[y_i(t)] = Y_i(s) \quad , \quad \mathcal{L}[M_N(t)] = m(s) \quad .$$

Then transforming (3) leads to following system of linear algebraic equations for the transforms:

$$s Y_o = -k_{11} M Y_o + k_{12} X_1 + k_{N3} X_N + k_{01} \mathcal{L}[(E)(T)] \quad ,$$

. . . . . .

$$s X_i = k_{i1} M Y_{i-1} - (k_{i2} + k_{i3}) X_i \quad , \qquad (i = 1, 2, \cdots, N-1)$$

$$s Y_i = k_{i3} X_i - k_{(i+1)1} M Y_i + k_{(i+1)2} X_{i+1} \quad ,$$

. . . . . .

$$sX_N = k_{N1} M Y_{N-1} - (k_{N2} + k_{N3}) X_N \quad ,$$

$$s\,m(s) = k_{N3} X_N \quad ,$$

or

$$(s + k_{11}M) Y_0 = k_{12} X_1 + k_{N3} X_N + k_{01} \mathcal{L}[(E)(T)] \quad ,$$

. . . . . .

$$(s + k_{i2} + k_{i3}) X_i = k_{i1} M Y_{i-1} \quad ,$$

12) $(i = 1, 2, \cdots, N-1)$

$$(s + k_{(i+1)1} M) Y_i = k_{i3} X_i + k_{(i+1)2} X_{i+1} \quad ,$$

. . . . . .

$$(s + k_{N2} + k_{N3}) X_N = k_{N1} M Y_{N-1} \quad ,$$

$$s\,m(s) = k_{N3} X_N \quad .$$

Omitting the last equation in (12) for the moment, and pairing other equations successively, we have as a typical pair (except for the first pair)

$$(s + k_{(i+1)1} M) Y_i - k_{(i+1)2} X_{i+1} = k_{i3} X_i \quad ,$$

$(i = 1, 2, \cdots, N-1)$

$$-k_{(i+1)1} Y_i + (s + k_{(i+1)2} + k_{(i+1)3}) X_{i+1} = 0 \quad .$$

Then solving this set in terms of X_i ,

13) $$Y_i = \frac{k_{i3} (s + k_{(i+1)2} + k_{(i+1)3})}{f_{i+1}(s)} X_i \quad ,$$

14) $$X_{i+1} = \frac{k_{(i+1)1} k_{i3} M}{f_{i+1}(s)} X_i \quad ,$$

where $f_{i+1}(s) = (s + k_{(i+1)1} M)(s + k_{(i+1)2} + k_{(i+1)3}) - k_{(i+1)1} k_{(i+1)2}$.

The first pair of equations in (12) yields

$$Y_o = \frac{\{k_{N3} X_N + k_{01} \mathcal{L}[(E)(T)]\}\{s + k_{12} + k_{13}\}}{f_1(s)} \quad , \tag{15}$$

$$X_1 = \frac{k_{11} M\{k_{N3} X_N + k_{01} \mathcal{L}[(E)(T)]\}}{f_1(s)} \quad . \tag{16}$$

Equation (14) is now iterated starting with i = N - 1, and the value of X_1 is substituted from (16). This gives

$$X_N = \frac{(k_{N1} k_{(N-1)1} \cdots k_{11})(k_{(N-1)3} \cdots k_{13}) M^N\{k_{N3} X_N + k_{01} \mathcal{L}[(E)(T)]\}}{\prod_{i=1}^{N} f_i(s)}$$

which when solved for X_N results in

$$X_N = \frac{k_{01} ABM^N \mathcal{L}[(E)(T)]}{k_{N3}\left[\prod_{i=1}^{N} f_i(s) - ABM^N\right]} \quad . \tag{17}$$

Here $A = \prod_{i=1}^{N} k_{i1}$ and $B = \prod_{i=1}^{N} k_{i3}$.

In view of the last equation of (12), we have, finally

$$m(s) = \frac{k_{01} ABM^N \mathcal{L}[(E)(T)]}{s\left[\prod_{i=1}^{N} f_i(s) - ABM^N\right]} \quad . \tag{18}$$

Eq. (18) may be inverted using the convolution theorem.

If

$$F(t) = \mathcal{L}^{-1}\left\{\frac{k_{01} ABM^N}{s\left[\prod_{i=1}^{N} f_i(s) - ABM^N\right]}\right\} \quad , \tag{19}$$

then

$$M_N(t) = F(t) * (E)(T) \quad , \tag{20}$$

where E and T are given explicitly by (10). By actual substitution, we can check that the polynomial multiplier of s in the denominator of eq. (19) has s = o as a simple zero. It can be shown, using a continuity argument, that all other zeros of this polynomial are in the left half-plane. Thus, assuming these zeros are simple, F(t) is of the form

$$F(t) = at + b + \sum (a_i e^{-\alpha_i t} \cos \beta_i t + b_i e^{-\alpha_i t} \sin \beta_i t), \ (\alpha_i > o).$$

The explicit "working out" of the convolution in (20), either numerically or in terms of known functions, is left for a subsequent investigation.

3. Perturbation Solution for Small k_{o2} (Protein Synthesis)

By assuming that k_{o2} is small in the system of O.D.E's given by (3), that system may be solved by using the standard perturbation procedure, and the solution given as power series in k_{o2} with coefficients which are functions of time. Because of the special structure of the system (3), it turns out that these coefficients may be represented in comparatively simple fashion by means of quadratures.

For simplicity write $\lambda = k_{o2}$. If we add all the equations in (3) except the first two and the last, we get

$$\frac{dy_o}{dt} + \sum_{i=1}^{N-1} \left(\frac{dx_i}{dt} + \frac{dy_i}{dt} \right) + \frac{dx_N}{dt} = k_{o1}(E)(T) + \lambda y_o \quad ,$$

or in terms of the variable u introduced in Section 2,

$$\frac{du}{dt} = k_{01}(c_1 - u)(c_2 - u) + \lambda y_0 \quad . \tag{21}$$

Denote $k_{01}\ (c_1 - u)\ (c_2 - u)$ by v . Proceeding with the perturbation technique, write

$$u = u_0 + u_1\lambda + \cdots + u_n\lambda^n + \cdots \quad , \tag{22}$$

$$y_0 = y_{00} + y_{10}\lambda + \cdots + y_{n0}\lambda^n + \cdots \quad , \tag{23}$$

where the u_n , and y_{no} are functions of time. Then we may write

$$v = k_{01}(c_1 - u)(c_2 - u) = \sum_{n=0}^{\infty} v_n \lambda^n \quad , \tag{24}$$

where, by virtue of (22) the v_n may be expressed in terms of the u_n as follows:

$$\begin{aligned} v_0 &= k_{01}(c_1 - u_0)(c_2 - u_0) \\ v_n &= k_{01}\left\{-(c_1 + c_2)u_n + (u_0 u_n + u_1 u_{n-1} + \cdots + u_n u_0)\right\}, \quad n \geq 1 \end{aligned} \tag{25}$$

If (22), (23) and (24) are substituted in (21) and coefficients of like powers of λ are equated, the following system of D.E's results

$$\begin{aligned} u_0' &= v_0 \quad , \\ u_1' &= v_1 + y_{00} \quad , \\ &\cdots\cdots\cdots \\ u_n' &= v_n + y_{(n-1)0} \quad . \\ &\cdots\cdots\cdots \end{aligned} \tag{26}$$

In (26), the ν_n are to be replaced by their expressions in terms of the u_n given by (25). We now find similar expressions for the y_{no} in terms of the u_n. These will also be substituted in (26) so that the latter system will be written completely in terms of the u_n.

For the case $\lambda = k_{02} \neq 0$, the system of transform equations (11) remains the same except that in the first equation the coefficient of Y_o becomes $- k_{11}M - k_{02}$ in place of just $- k_{11}M$. Thus the solution of the system (12) will now yield the following expressions for Y_o and X_n

$$Y_o = \frac{(s + k_{12} + k_{13})(k_{N3} X_N + V)}{g_1(s)} \quad , \tag{27}$$

$$X_N = \frac{ABM^N V}{k_{N3}\left[g_1(s) \prod_{i=2}^{N} f_i(s) - ABM^N\right]} \quad . \tag{28}$$

Here $V = \mathcal{L}[v(t)] = \mathcal{L}[k_{01}(E)(T)]$,

$g_1(s) = (s + k_{02} + k_{11}M)(s + k_{12} + k_{13}) - k_{11}k_{12}M$,

and A, B, $f_i(s)$ have the meaning assigned to them in Section 2.

Replacing X_N in (27) by using (28), we get

$$Y_o = \frac{(s + k_{12} + k_{13}) \prod_{i=2}^{N} f_i(s) \cdot V}{g_1(s) \prod_{i=2}^{N} f_i(s) - ABM^N} \quad ; \tag{29}$$

or, since $g_1(s) = (s + k_{11}M)(s + k_{12} + k_{13}) - k_{11}k_{12}M + \lambda(s + k_{12} + k_{13})$,

$= f_1(s) + \lambda(s + k_{12} + k_{13})$,

we may write Y_o (using the geometric series expansion) as a power series in λ in the following way:

$$(30) \qquad Y_o = V \sum_{n=0}^{\infty} [(-1)^n (s + k_{12} + k_{13})^{n+1} h_1^{n+1}(s) \lambda^n] / h^{n+1}(s)$$

where $h(s) = \prod_{i=1}^{N} f_i(s) - ABM^N$, $h_1(s) = \prod_{i=2}^{N} f_i(s)$.

Let $G(t) = \mathcal{L}^{-1}\left[\dfrac{(s + k_{12} + k_{13}) h_1(s)}{h(s)}\right]$

and $G_n(t) = \underbrace{G(t) * G(t) * \cdots * G(t)}_{(n+1)}$ $\qquad (* = \text{convolution})$,

so that $G_o(t) = G(t)$. Then inverting eq. (30) yields

$$y_o = v * \sum_{n=0}^{\infty} (-1)^n G_n(t) \lambda^n$$

$$= \sum_{n=0}^{\infty} v_n \lambda^n * \sum_{n=0}^{\infty} (-1)^n G_n(t) \lambda^n$$

Thus

$$(31) \qquad y_o = \sum_{n=0}^{\infty} (-1)^n [v_o * G_n(t) - v_1 * G_{n-1}(t) + \cdots + (-1)^n v_n G_o(t)] \lambda^n .$$

Equating the coefficients of λ^n in (31) to the like coefficients in (23), we get the relations

$$(32) \qquad y_{no} = (-1)^n [v_o * G_n(t) - v_1 * G_{n-1}(t) + \cdots] , \quad n = 0, 1 \cdots .$$

Since the $G_i(t)$ are "known functions" and the v_i are given in terms of the u_i by (25), eq. (32) represents the y_{no} in terms of the u_i. Note that the functions $u_o, u_1, \ldots, u_n$, only, are involved in the expressions for y_{no}.

Coming back to the system of D.E's (26), the general equation of this system reads

$$u_n' = v_n + y_{n-1,o} \quad ,$$

or, in view of (25) and (32),

$$u_n' = k_{01}\left[-(c_1+c_2) + 2u_o\right]u_n + \text{terms involving } u_o, \ldots, u_{n-1} \, . \tag{33}$$

Thus assuming $u_o, \ldots, u_{n-1}$ are known, (33) determines u_n. Since (33) is a linear equation of the first order in u_n, its solution may be given in closed form in terms of quadratures. In particular, note that the coefficient of u_n is the same for all these equations. Calling this coefficient A and the terms free of u_n, B_n, and taking account of the initial conditions $u_n(o) = o$, the solution of (33) may be written in the form

$$u_n = e^{\int_o^t A\,dt} \int_o^t e^{-\int_o^t A\,dt} \cdot B_n\,dt \, . \tag{34}$$

Since the first equation of (33), giving u_o is immediately integrable, (cf. Section 2), the induction may be carried through and all succeeding terms u_n represented in closed form by means of quadratures.

For example, the second equation of (33) would be

$$u_1' = k_{01}\left[-(c_1+c_2) + 2u_o\right]u_1 + k_{01}\left[(c_1-u_o)(c_2-u_o)\right] * G_o(t) \quad ,$$

since $y_{oo} = v_o * G_o(t)$ from (32) and $v_o = k_{o1}(c_1 - u_o)(c_2 - u_o)$ from (25). Here u_o is to be replaced by the u_o found in Section 2 (the unperturbed solution).

There remains the development of an expression for the amount of protein, $M_N(t)$, in the perturbed solution. From (28) and (29), we have

$$X_N = \frac{ABM^N}{k_{N3}} \cdot \frac{1}{(s+k_{12}+k_{13})h_1(s)} \cdot Y_o \quad ;$$

and since

$$m(s) = \mathcal{L}[M_N(t)] = k_{N3} X_N / s \quad \text{by (12), this}$$

gives

(35) $$m(s) = ABM^N \cdot \frac{1}{s(s+k_{12}+k_{13})h_1(s)} \cdot Y_o \quad .$$

If now,

(36) $$H(t) = \mathcal{L}^{-1}\left[\frac{1}{s(s+k_{12}+k_{13})h_1(s)}\right] \quad ,$$

then

(37) $$M_N(t) = ABM^N \sum_{n=0}^{\infty} [y_{no} * H(t)] \lambda^n \quad .$$

Since

$$\mathcal{L}[H(t) * G_o(t)] = \frac{1}{s(s+k_{12}+k_{13})h_1(s)} \cdot \frac{(s+k_{12}+k_{13})h_1(s)}{h(s)} = \frac{1}{sh(s)} \quad ,$$

eq. (37) can be rewritten, in view of (32), as

(38) $$M_N(t) = ABM^N \sum_{n=0}^{\infty} m_n(t) \lambda^n \quad ,$$

where $$m_n(t) = H_1(t) * (-1)^n [v_n - v_{n-1} * G_2(t) + \cdots], \quad n \geq 0$$

$$H_1(t) = \mathcal{L}^{-1}\left[\frac{1}{sh(s)}\right]$$

and v_n are to be expressed in terms of the u_n by (25).

4. Specialization and Evaluation of Results (Protein Synthesis, $k_{02} = 0$)

In this section, the results obtained previously will be worked out in some detail, and certain end formulas will be developed which may be used to connect theory with experiment. In order not to complicate the end relations unnecessarily, certain simplifying assumptions will be made at this point. We suppose that all reaction constants of the same kind are equal to each other. Thus, let

$$k_{11} = k_{21} = \cdots = k_{N1} = k_1$$

$$k_{12} = k_{22} = \cdots k_{N2} = k_2$$

$$k_{13} = k_{23} = \cdots k_{N-1,3} = k_3$$

For the present k_{N3} is not taken equal to k_3 in order to allow for the possibility of a slower reaction time in the last reaction when the protein is separated from the enzyme-template complex. These assumptions seem reasonable in the light of existing knowledge concerning the synthesis process. As a result of this specialization, the polynomials $f_i(s)$, $(i = 1,2,\ldots,N-1)$ become identical and we can drop the subscript and denote all of them by $f(s)$ where

$$f(s) = (s + k_1 M)(s + k_2 + k_3) - k_1 k_2 M = s^2 + \delta s + \gamma ,$$

and $\delta = k_1 M + k_2 + k_3$, $\gamma = k_1 k_3 M$.

For $f_N(s)$ we have

$$f_N(s) = (s + k_1 M)(s + k_2 + k_{N3}) - k_1 k_2 M = s^2 + \delta' s + \gamma' ,$$

where $\delta' = k_1 M + k_2 + k_{N3}$ and $\gamma' = k_1 k_{N3} M$.

In this notation, equation (18) becomes

$$m(s) = \frac{k_{o1} k_{N3} \gamma^N}{k_3 s[f_N(s)[f(s)]^{N-1} - k_{N3} \gamma^N / k_3]} \cdot \mathcal{L}[(E)(T)] . \qquad (18')$$

The solution for $M_N(t)$ may then be obtained as usual, by expanding the rational function in (18') into partial fractions, inverting each term and convoluting the inverse transforms with (E). (T).

(a) Asymptotic Behavior for Large t.

As noted in Section 2, the rational function of s represented by the fraction in eq. (18') has a double pole at s = o; all other poles are in the left-hand plane. More explicit information regarding the location of the poles can be given if it is assumed that $k_{N3} = k_3$. This will be done later to get an indication of the transient behavior of the system for intermediate values of time. For the present, we are interested only in obtaining the asymptotic behavior of the system for large time and we can ignore the poles in the left-half plane since it can be shown that the convolution of terms corresponding to these with (E). (T) approaches zero as $t \longrightarrow \infty$.

Thus, we are concerned solely with the principal part of $k(s) = k_{o1} k_{N3} \gamma^N / s(f(s)^{N-1} f_N(s) - \frac{k_{N3} \gamma^N}{k_3})$ at s = o .

We may write

$$k(s) = \frac{A_{-2}}{s^2} + \frac{A_{-1}}{s} + \cdots \quad ,$$

and find by standard techniques that

$$A_{-2} = \frac{k_{01} k_{N3} \gamma}{k_3 \delta' + (N-1) k_{N3} \delta} \quad ,$$

$$A_{-1} = - \frac{k_{01} k_{N3} [2\gamma (k_3 + (N-1) k_{N3}) + (N-1)\delta (2 k_3 \delta' + (N-2) k_{N3} \delta)]}{2[k_3 \delta' + (N-1) k_{N3} \delta]^2} \quad .$$

Thus the dominant terms in $M_N(t)$ for large time are given by

$$M_N(t) = A_{-2} \cdot t * (E)(T) + A_{-1} \cdot 1 * (E)(T) + o(1) \quad .$$

Evaluating the convolutions, we find

$$t * (E)(T) = \frac{1}{k_{01}} \left\{ \frac{(E_o + T_o)}{2} t + \frac{1}{k_{01}} \log \left(\frac{E_o - T_o}{E_o e^{k_{01} \beta t} - T_o e^{k_{01} \beta t}} \right) \right\}$$

$$1 * (E)(T) = \frac{E_o T_o}{k_{01}} \left(\frac{e^{k_{01} \beta t} - e^{-k_{01} \beta t}}{E_o e^{k_{01} \beta t} - T_o e^{-k_{01} \beta t}} \right) \quad .$$

Here, as before, $\beta = (E_o - T_o)/2$.

These expressions may be simplified further if we subdivide the discussion into two cases depending upon whether $E_o > T_o$ or $E_o < T_o$. Thus if $E_o > T_o$ then $\beta > o$ and we find for large t that

$$t * (E)(T) = \frac{1}{k_{01}} \left\{ T_o t + \frac{1}{k_{01}} \log \left(\frac{E_o - T_o}{E_o} \right) \right\} + o(1) \quad ,$$

$$1 * (E)(T) = \frac{T_o}{k_{01}} + o(1) \quad .$$

In this case, then, we have

$$M_N(t) = \frac{A_{-2}}{k_{01}}\left\{T_o t + \frac{1}{k_{01}}\log\left(\frac{E_o - T_o}{E_o}\right)\right\} + \frac{A_{-1}T_o}{k_{01}} + O(1) \quad . \tag{39}$$

The result for the case $E_o < T_o$ may be obtained from this one by interchanging E_o and T_o in eq. (39).

We may perform a similar asymptotic analysis for each of the $x_i (i = 1, \ldots, N)$ and $y_i (i = o, \ldots, N-1)$. Thus, for our specialization, eq. (17) becomes

$$X_N = \frac{k_{01}\gamma^N}{k_3\left[f_N(s)[f(s)]^{N-1} - k_{N3}\gamma^N/k_3\right]} \cdot \mathcal{L}[(E)(T)] \quad . \tag{17'}$$

Using eq. (14) to express X_{N-1} in terms of X_N, then X_{N-2} in terms of X_{N-1}, etc. we find by induction that

$$X_i = \frac{k_{01}\gamma^i f_N(s)[f(s)]^{N-i-1}}{k_3\left[f_N(s)[f(s)]^{N-1} - k_{N3}\gamma^N/k_3\right]} \cdot \mathcal{L}[(E)(T)] \quad , \tag{40}$$

$(i = 1, 2, \cdots, N)$.

Equation (13) then gives

$$Y_{N-1} = \frac{k_{01}\gamma^{N-1}(s + k_2 + k_{N3})}{\left[f_N(s)[f(s)]^{N-1} - k_{N3}\gamma^N/k_3\right]} \cdot \mathcal{L}[(E)(T)] \quad ; \tag{41}$$

$$Y_i = \frac{k_{01}\gamma^i(s + k_2 + k_3)f_N(s)[f(s)]^{N-i-2}}{\left[f_N(s)[f(s)]^{N-1} - k_{N3}\gamma^N/k_3\right]} \cdot \mathcal{L}[(E)(T)] \quad . \tag{42}$$

The dominant term in x_i and y_i corresponds to the simple pole of X_i and Y_i at s = o. Writing

$$X_i = \left[\frac{A_{-1i}}{s} + \cdots\right] \cdot \mathcal{L}[(E)(T)] \quad , \quad (i = 1, \cdots, N) \quad , \tag{43}$$

$$Y_i = \left[\frac{B_{-1i}}{s} + \cdots\right] \cdot \mathcal{L}[(E)(T)] \quad , \quad (i = 0, \cdots, N-1) \quad , \tag{44}$$

we find

$$A_{-1N} = k_{01}\gamma/\Delta \quad ,$$

$$A_{-1i} = k_{01}\gamma'/\Delta \quad , \quad (i = 1, 2, \cdots N-1) \quad ,$$

$$B_{-1(N-1)} = k_{01}(k_2 + k_{N3})/\Delta \quad , \tag{45}$$

$$B_{-1i} = k_{01}\gamma'(k_2 + k_3)/\gamma\Delta \quad , \quad (i = 0, 1, \cdots, N-2) \quad ,$$

where $$\Delta = [k_3\delta' + (N-1)k_{N3}\delta] \quad .$$

Note that both A_{-1i}, (i = 1,2,...,N-1) and B_{-1i}, (i = o,1,...,N-2) are independent of i.

Equations (43) and (44) in conjunction with the previously evaluated convolution 1 $\ast$(E)(T) imply that as $t \to \infty$,

$$x_i(t) = A_{-1i}\frac{T_o}{k_{c1}} + o(1) \quad , \quad (i = 1, \ldots, N) \quad , \tag{46}$$

$$y_i(t) = B_{-1i}\frac{T_o}{k_{c1}} + o(1) \quad , \quad (i = o, \ldots, N-1) \quad ,$$

if $E_o > T_o$. Replace T_o by E_o in these formulas if $E_o < T_o$.

(b) Asymptotic Behavior for Small Time.

Equation (18'), written as $m(s) = k(s) \cdot \mathcal{L}[(E)(T)]$, leads to the time domain equation

$$M_N(t) = \int_o^t K(t - \tau) \cdot (E)(T) d\tau \tag{47}$$

where $K(t)$ is the inverse of $k(s)$. Since $\lim_{s \to \infty} [s^{2N} k(s)] = o$ and $\lim_{s \to \infty} [s^{2N+1} k(s)] = k_{o1} k_{N3} \gamma^N / k_3$,

it follows from the Initial Value Theorem that $D^i K(o) = o$ for $i = o, 1, \ldots, 2N - 1$ while $D^{2N} K(o) = k_{o1} k_{N3} \gamma^N / k_3$, $(D = d/dt)$.

Then differentiating (47) with respect to t yields $D^i M_N(o) = o$, $i = o, 1, \ldots, 2N$, $D^{2N-1} M_N(o) = E_o T_o \cdot k_{o1} k_{N3} \gamma^N / k_3$.

Thus, for t small,

$$M_N(t) = \frac{E_o T_o k_{o1} K_{N3} \gamma^N}{k_3 (2N + 1)!} t^{2N+1} + o(1) . \tag{48}$$

A similar analysis of eqs. (17'), (40), (41) and (42) yields asymptotic relations for each of the x_i and y_i, viz

$$y_i(t) = \frac{E_o T_o k_{o1} \gamma^i}{(2i + 1)!} t^{2i+1} + o(1), \quad (i = o, 1, \ldots, N - 1) , \tag{49}$$

$$x_i(t) = \frac{E_o T_o k_{o1} \gamma^i}{k_3 \cdot (2i)!} t^{2i} + o(1) , \quad (i = 1, \ldots, N) . \tag{50}$$

(c) Intermediate Values of Time.

The question as to whether the asymptotic results obtained in (a) and (b) above will suffice to delineate the behavior of $M_N(t)$ or whether additional formulas are necessary for intermediate values of

time is still open. It depends upon the actual values of the characteristic roots, i.e. the poles of k(s), and these in turn depend upon the numerical values of the reaction constants. In order to get some indication of the location of the characteristic roots in the complex plane, the case in which $k_{N3} = k_3$ will now be considered. Here we can solve the characteristic equation explicitly in terms of the reaction constants.

With $k_{N3} = k_3$, and $f_N(s) \equiv f(s)$ the denominator in (18') becomes $k_3 s[f(s)^N - \gamma^N]$. Thus, we must solve the equation

$$[f(s)]^N - \gamma^N = 0 \tag{51}$$

to get the characteristic roots other than the one provided by the factor s, i.e., s = o. First, we note that (51) can have multiple roots only if $N[f(s)]^{N-1} f'(s) = o$ for s a root of (51). This leads to the conclusion that the only possible multiple root corresponds to $s = -\delta/2$, and this value of s will be a double root if and only if $\delta^2 = 8\gamma$ and N is even. Thus for all practical purposes, the roots of (51) will be simple.

Now let $\rho = \exp \frac{2\pi i}{N}$ so that ρ is a primitive Nth root of unity. Then

$$f(s) = \gamma \rho^j \qquad (j = o, 1, \ldots, N - 1) ,$$

and solving this quadratic equation we find the 2N roots of (51) given by

$$s_j = -\frac{\delta}{2} \pm \frac{1}{2} \sqrt{\delta^2 - 4 \gamma (1 - \rho^j)} \quad (j = o, 1, \ldots, N - 1) \tag{52}$$

Note that for j = o, we get the roots o and $-\delta$. Thus o is a double pole of k(s). If N is even and j = N/2, then $\rho^{N/2} = -1$ and it can be shown that we again have two real roots. Except for these cases

the roots s_j are all complex.

Equation (52) expresses the s_k in terms of the square root of a complex number. As is well-known, this square root may be written in the form a + bi where a and b involve only real square roots. For possible future computational use, we list this alternate form of (52).

$$s_j = -\frac{\delta}{2} \pm \frac{1}{2}(a + bi) ,$$

(53)
$$a = \sqrt{\frac{a_1^2 + \sqrt{a_1^2 + b_1^2}}{2}} , \qquad b = \operatorname{sgn} b_1 \sqrt{\frac{\sqrt{a_1^2 + b_1^2} - a_1}{2}} ,$$

$$a_1 = \delta^2 + \gamma\left(\cos\frac{2\pi j}{N} - 1\right) \qquad b_1 = 4\gamma \sin\frac{2\pi j}{N}$$

$$(j = o, 1, \ldots, N - 1) .$$

5. Conclusions

The results in Section 4 indicate that the synthesis of protein (M_N) in the model under consideration can be regarded as consisting of three stages: (a) an initial stage in which the concentration of protein is given by $M_N \sim C_1 t^{2N+1}$, and therefore representing an exceedingly slow buildup of the protein complex quite indistinguishable for awhile from a zero protein concentration; (b) an intermediate state in which M_N is determined by an exponential polynomial; (c) a final state in which $M_N \sim C_2 t + C_3$, i.e. linear growth but with an offset C_3. The constants C_1, C_2 and C_3 are given in Section 4 in terms of the various reaction constants.

Nirenberg [5] has reported the results of certain experimental studies of protein reaction kinetics in vitro. Comparing his actual findings with our theoretical prediction, we see that there is substantial agreement between the two. Nirenberg also shows a fourth stage following the linear one in which the protein concentration approaches an asymptotic value. The latter can be interpreted in the light of our model to reflect the situation resulting from a decay in messenger RNA.

As a first consequence of this agreement between Nirenberg's experimental results and our formulas, it becomes possible to use the former (or the results of experiments similar to them) to determine values for certain of the fundamental reaction constants which underlies the protein synthesis process. For example, the slope (C_2) of the straight line in the third stage of synthesis can easily be determined from the experimental data. On the other hand, the formulas in Section 4 (cf. eq. (39)) show that

$$C_2 = C_2 (E_o, E_o, M, N, k_{i1}, k_{i2}, k_{i3}).$$

If we conduct the experiment several times with different concentrations of monomer $M^{(j)}$, but fixed E_o and T_o, and denote the measured slopes by $C_2M^{(j)}$, we get a set of algebraic equations

$$C_2(E_o, T_o, M^{(j)}, k_{i1} \ldots k_{i3}) = C_2M^{(j)}$$

which serve to determine some of the reaction constants.

To illustrate this procedure, let us assume, for simplicity, that the specialization of the constants assumed in Section 4 applies

and that, furthermore, $k_{N3} = k_3$ and $E_o < T_o$. Then we have $\gamma = \gamma' = k_1 k_3 M$, $\delta = \delta' = k_1 M + k_2 + k_3$, and eq. (59), with these values inserted, yields

$$C_2 = \frac{k_1 k_3 T_o M}{N(k_1 M + k_2 + k_3)} . \tag{54}$$

Using two different values of M, designated as $M^{(1)}$ and $M^{(2)}$, in this relation, we readily find that

$$k_3 = \frac{M^{(1)} - M^{(2)}}{T_o} \frac{1}{N} \cdot \left[\frac{M^{(1)}}{C_2 M^{(1)}} - \frac{M^{(2)}}{C_2 M^{(2)}} \right]^{-1} . \tag{55}$$

Thus it becomes possible to determine the important polymerization constant k_3. By making assumptions regarding the relative magnitude of the other constants and also by using the M_N intercept, represented by C_3 in the linear asymptotic form, further reaction constants may be evaluated.

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