DEREPRESSION KINETICS OF ORNITHINE TRANSCARBAMYLASE IN *ESCHERICHIA COLI*

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ABSTRACT A mathematical model for the derepression of ornithine transcarbamylase (OTC) in *Escherichia coli* strain W was derived from a set of 14 assumptions concerning the arginine regulon. The model assumes that active repressor for the arginine regulon is unstable and is only formed when the level of arginyl-tRNA is in excess of the level necessary to maintain protein synthesis for a given cell doubling time. The presence of active repressor was assumed to inhibit the synthesis of messenger RNA coding for the synthesis of the enzymes of the arginine biosynthetic pathway. Numerical estimates of the model's parameters were made and, by simulation on a digital computer, the model was shown to fit kinetic data for derepression of OTC in *E. coli* W cells in minimal medium growing in flask culture with a doubling time of 60 min and growing in a chemostat with a generation time of 460 min for an assumed OTC-specific mRNA half-life ($t_{1/2}$) of 9 min. The model was also shown to predict the increase in the size of bursts of OTC synthesis elicited by addition of arginine to cultures of derepressing *E. coli* cells with the increase in the delay time before arginine addition. Approximate analytical solutions to the model were obtained for the early phase of derepression and for repression of OTC. These were used to derive graphical methods for determining $t_{1/2}$ from repression and derepression transient changes in the OTC level.

INTRODUCTION

Experimental studies of the kinetics of enzyme repression and induction have proven very valuable. For example, kinetic studies by Jacob and Monod (1961) led to the postulation of the existence of a short-lived messenger RNA serving as a template for the synthesis of β-galactosidase in *E. coli*. Sadler and Novick (1965) have presented kinetic evidence that the repressor for the lactose operon in the same organism is composed of subunits and that the rate of enzyme synthesis varies inversely with the first power of repressor concentration. With the accumulation of such experimental information it becomes meaningful and worthwhile to attempt the formulation of a quantitative theory for the regulation of biochemical pathways.

In the following development a single regulatory device is considered: the end-

product repression of the enzyme OTC by arginine in *E. coli*. As shown in Fig. 1, OTC is the sixth of a sequence of eight enzymes involved in the synthesis of arginine from glutamate in *E. coli*. Arginine both represses the synthesis of all eight enzymes (Maas, 1961; Gorini, Gunderson, and Burger, 1961; Vogel, Bacon, and Baich, 1963) and inhibits the activity of the branch-point enzyme *N*-acetylglutamate synthetase as well (Ennis and Gorini, 1961; Vyas and Maas, 1963).

The arginine regulon was chosen for a detailed theoretical study because much experimental work has been carried out on this system in *E. coli* in a chemostat (Novick and Szilard, 1950), an instrument that allows the conditions of culture to be maintained in a steady state. This wealth of experimental information makes possible the estimation of the parameters of a mathematical model for the arginine regulon, which would not be possible yet for most other biochemical control systems.

Another reason for attempting to model the arginine regulon is that its derepression kinetics are relatively complex. If a single system is changing from one level of repression to another, the approach to the new steady state ordinarily follows the equation

$$E_t/M_t = E_0/M_0[\alpha + e^{-kt}(1 - \alpha)], \tag{1}$$

where E/M is the ratio of the enzyme activity to the total cell mass (M), the zero subscripts denote initial values, and α is the factor by which this ratio will have changed when the system has reached the steady state (Maaloe and Kjeldgaard, 1966, p. 58). This formulation describes repression for values of α less than 1 and describes derepression for values of α greater than 1. The enzymes of the arginine regulon do not obey equation 1. When *E. coli* cells are grown in an arginine medium, synthesis of OTC is repressed. When the cells are transferred to minimal medium, the OTC is initially synthesized at an excessively high rate and the enzyme level approaches its steady state from above as shown by the data displayed in Fig. 4 and 5 (Gorini and Maas, 1957). This kinetic behavior will be termed an "overshoot" here since the enzyme level at first overshoots its steady-state level.

THE ARGININE REGULON IN *E. COLI*

Figure 1 is a flow chart for the arginine biosynthetic pathway showing the assumed interdependencies of the synthesis of mRNA, protein, and arginine. Protein synthesis yields, among other things, the eight arginine biosynthetic enzymes as well as the arginyl-tRNA synthetases necessary for the synthesis of arginyl-tRNA. The arginyl-tRNA, in turn, serves as an input to protein synthesis by delivering up arginine during messenger RNA translation. Arginyl-tRNA may also interact with aporepressor in some way to yield an active repressor that specifically inhibits the synthesis of messenger RNA coding for any of the eight enzymes of the arginine biosynthetic pathway. Whether arginyl-tRNA actually is involved in the activation of repressor and

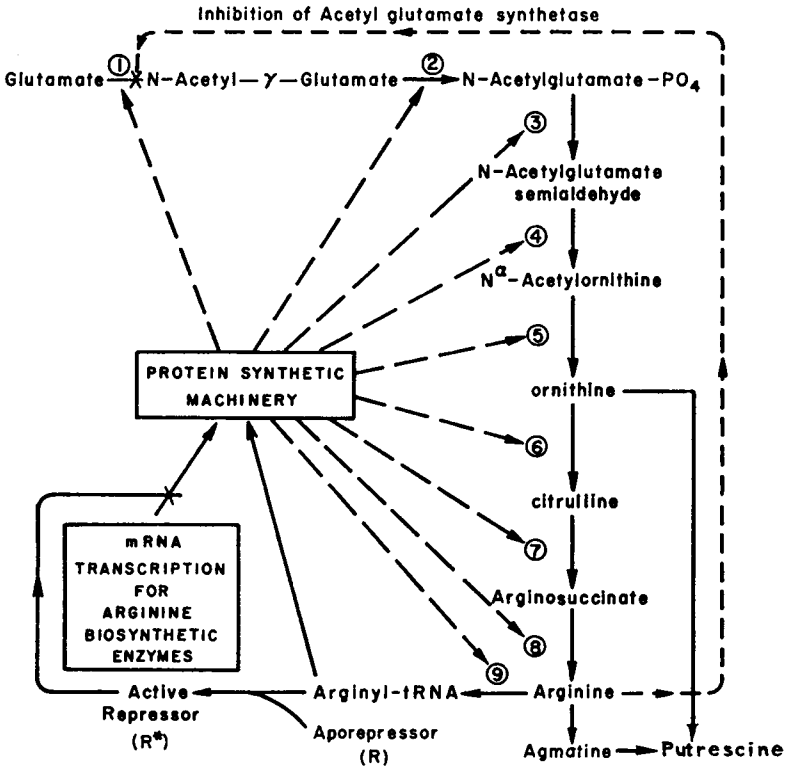

FIGURE 1 The arginine regulon. The circled numbers 1–8 denote the arginine biosynthetic enzymes as follows: 1. *N*-acetylglutamate synthetase; 2. *N*-acetyl-γ-glutamokinase; 3. *N*-acetylglutamic-γ-semialdehyde dehydrogenase; 4. acetylornithine-δ-transaminase; 5. acetylornithinase; 6. ornithine transcarbamylase; 7. arginosuccinate synthetase; 8. arginosuccinase. The circled 9 denotes arginyl-tRNA synthetase.

whether repressor specifically inhibits mRNA transcription or mRNA translation remain unsettled questions. Besides serving in some unknown way in the activation of repressor, arginine inhibits the activity of enzyme 1, *N*-acetylglutamate synthetase (Vyas and Maas, 1963). Arginine is also metabolized to agmatine and both ornithine and agmatine are converted to putrescine in *E. coli* (Morris and Pardee, 1966).

Derepression of OTC: the Overshoot Phenomenon

Gorini and Maas (1957) found that when cells of the W strain of *E. coli* are harvested from minimal medium and then grown in the presence of arginine (20 μg/ml), the synthesis of OTC is repressed to a level approximately $\frac{1}{100}$ the initial concentration of the enzyme. When these cells are washed and transferred to a growth medium without arginine, the cells derepress OTC as shown in Figs. 4 and 5. The derepression kinetics is characterized by about a 10 min delay during which there is very little apparent increase in OTC followed by a burst of synthesis which reaches a peak after

50 min at which time the enzyme level is 5.3 units/mg bacteria dry weight; the level then slowly subsides to 1.6 units/mg.[1] One enzyme unit is the amount of enzyme that synthesizes 1 μmole of citrulline per hour.

DEVELOPMENT AND COMPUTER SIMULATION OF A MODEL OF DEREPRESSION OF OTC IN *E. COLI*

Assumptions

(1) *Active repressor R* inhibits the synthesis of messenger RNA coding for the arginine biosynthetic enzymes rather than inhibiting the translation of these messages.*

There is no direct evidence for or against this assumption but experiments performed by Faanes and Rogers (1968) suggest that this is the case. They found that addition of arginine after 5, 10, or 15 min to a derepressing culture of *E. coli* W resulted in a burst of synthesis of OTC (Fig. 2), the burst height increasing the longer the arginine addition was delayed up to about 15 min. The fact that the burst height obtained upon addition of arginine increases for about the first 15 min shows that the potentiality for the synthesis of OTC was increasing during the first 15 min following the shift from arginine-rich medium to medium lacking arginine. The simplest explanation seems to be that the low internal arginine concentration in the cells due to the low enzyme levels imposed by pregrowth in the arginine-rich medium is not sufficient to keep the aporepressor activated, and the transcription of messenger RNA coding for all the arginine biosynthetic enzymes is allowed to proceed at a rapid rate. The addition of arginine allows the cells to translate this accumulating messenger RNA resulting in a burst of synthesis of enzyme. The longer one waits up to about 15 min, the more message has accumulated and the bigger the burst.

Faanes and Rogers also measured the rate of decay of the capacity to synthesize OTC during arginine repression using a tryptophan auxotroph of *E. coli* W. The cells were grown in a medium rich in arginine and then starved for both arginine and tryptophan for 20 min. Arginine was then added and samples were withdrawn and added to flasks containing tryptophan after additional delays of 2, 5, 10, 15, 20, and 30 min and the yield of OTC measured. It was found that the OTC burst height decreased exponentially as the delay before addition of tryptophan increased. A plot of the logarithm of the burst height against the delay time before tryptophan addition showed that under arginine repression the capacity to synthesize OTC falls off with a half-life of about 3 min. Assuming that "the capacity to synthesize OTC" is OTC-specific mRNA, the half-life of OTC-specific mRNA was estimated at about 3 min.

(2) *The rate of synthesis of new OTC-specific messenger RNA is* $1/(A + mR^*)$ *where A and m are constants and active repressor R* is metabolically unstable.*

The relationship between the rate of synthesis of enzyme and the repressor level has been determined for the case of the enzyme β-galactosidase in *E. coli* using

[1]For convenience, "mg bacteria dry weight" will be denoted simply by mg.

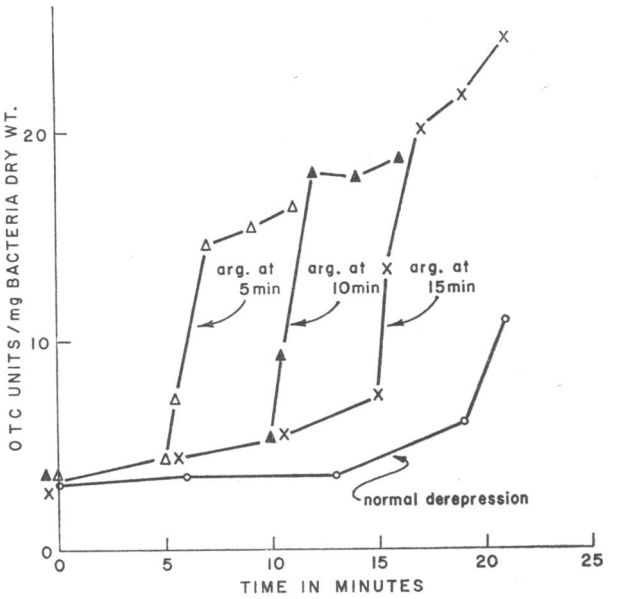

FIGURE 2 Development of the capacity to synthesize OTC during derepression. *E. coli* W wild stock was grown in AF medium which contains 1% glucose and defined supplements of amino acid, vitamins, purines, and pyrimidines and supplemented with L-arginine (generation time = 40–45 min). Washed cells were incubated in AF medium lacking arginine at time zero in 5 separate flasks. Arginine (200 μg/ml) was added to the cultures as follows: at 5 min (△); at 10 min (▲); at 15 min (×); no arginine added (○). Samples were withdrawn at the times indicated and OTC activity was determined. Data are from Faanes and Rogers (1968).

emperature-sensitive mutants (Novick, Lennox, and Jacob, 1963; Sadler and Novick, 1965) the relationship postulated being

$$\frac{dz}{dB} = \frac{K}{K + R^*}, \tag{2}$$

where z is the concentration of the enzyme β-galactosidase, $dz/dB = (dz/dt)/(dB/dt)$ is the differential rate of synthesis of the enzyme, B is the bacterial mass, R^* is the active repressor concentration, and K is a constant. Sadler and Novick (1965) concluded that the repressor for the *lac* operon is growth unstable with a mean life of $\frac{1}{10}$–$\frac{1}{5}$ of a generation. Gallant and Stapleton (1964) reported that the repressor for the synthesis of alkaline phosphatase in a mutant of *E. coli* is metabolically unstable. Bowne and Rogers (1963) concluded that active repressor for the arginine regulon is also unstable. We assume here that equation 2 applies to the enzymes of the arginine regulon. The factor $K/(K + R^*)$ in equation 2 is a special case of the more general expression $1/(A + mR^*)$ where the constants have been specialized to the case $A = 1, m = 1/K$.

(3) *The level of aporepressor is always sufficiently above the saturation level necessary to bind corepressor as to be considered constant.*

Although no direct proof of this exists it is a reasonable assumption since addition of arginine to a culture of *E. coli* W wild stock in sufficient concentration will always immediately repress the synthesis of the arginine biosynthetic enzymes.

(4) *Active repressor, whose concentration is designated by R*, is formed by some interaction between aporepressor and some product of the arginine biosynthetic pathway (possibly arginyl-tRNA but not necessarily). The kinetics of the formation of active repressor from aporepressor follows Michaelis-Menten kinetics.*

This is assumed for the sake of simplicity.

(5) *For definiteness, it is assumed that the corepressor is arginyl-tRNA denoted by M*.*

For a discussion of this point see Hirshfield, DeDeken, Horn, Hopwood, and Maas (1968).

(6) *There exists a threshold level T_r of arginyl-tRNA; the only arginyl-tRNA available for formation of active repressor is that in excess of T_r.*

T_r is the concentration of arginine in the form arginyl-tRNA necessary to maintain protein synthesis for a given cell doubling time. Gorini (1958) found that the bacterial density attained by arginine auxotrophs of *E. coli* W grown in flasks in minimal medium supplemented with arginine was strictly proportional to the concentration of the arginine in the medium. When *E. coli* W wild stock was cultivated anaerobically in a chemostat in the presence of a limiting concentration of glucose and the steady-state level of OTC measured as a function of the concentration of arginine in the medium, the enzyme level was found to remain constant up to a concentration of arginine of 8–9 μg/ml and above that it decreased abruptly (Fig. 3, curve A). The bacterial density was maintained at 0.217 mg/ml. To maintain the same bacterial density with an arginine auxotroph required that the medium be supplemented with 8 μg/ml arginine. This implies that repression sets in only when the concentration of arginine in the medium is in excess of that necessary to maintain a given bacterial density, or equivalently, a given doubling time.

When the same experiment was carried out on *E. coli* strain C (Fig. 3, curve B) and on a phenylalanine auxotroph of *E. coli* W (Fig. 3, curve C), similar results were obtained, the repression threshold in the former being 11.5 μg/ml for a bacterial density of 0.310 mg/ml and 5.5 μg/ml for a bacterial density of 0.171 mg/ml in the latter. The existence of a repression threshold level for exogenously derived arginine is therefore clearly established. If endogenously produced and exogenously derived arginine serve equally well as a source of corepressor, then the existence of a repression threshold for endogenously produced arginine may be inferred (Sercarz and Gorini, 1964).

(7) *When the supply of arginine is rate limiting in protein synthesis, the over-all rate of protein synthesis is proportional to the arginyl-tRNA level when this level is below threshold ($M^* < T_r$).*

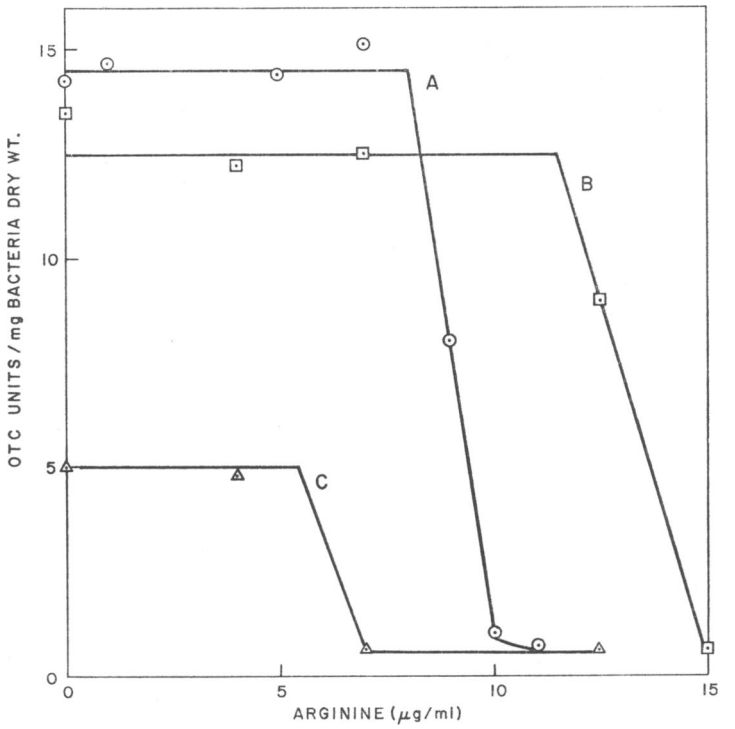

FIGURE 3 The influence of arginine on the level of enzyme. The influence of arginine on the OTC level in cells cultivated in a chemostat with a generation time of 2 hr ± 15 min was determined by varying the arginine concentration in the culture medium. Curve A: *E. coli* W wild stock cultivated anaerobically with glucose growth limiting. Bacterial density = 0.217 ± 0.010 mg dry weight/ml. Curve B: *E. coli* C wild stock cultivated aerobically with glucose rate limiting. Bacterial density = 0.310 ± 0.010 mg dry weight/ml. Curve C: *E. coli* W phenylalanine auxotroph cultivated anaerobically with phenylalanine (2 μg/ml) growth limiting. Bacterial density = 0.171 ± 0.010 mg dry weight/ml. The curves are plotted such that repression sets in at the level of arginine in the medium found necessary to maintain arg^- cells at the given bacterial density. Data are from Gorini (1958).

The dependence of the growth rate on the percentage of the arginine-specific tRNA that is charged was measured on an arginine auxotroph (MA 1010) of *E. coli* in a chemostat with arginine limiting the growth rate (Hirshfield et al., 1968). The growth rate was found to vary proportionally with the per cent of charged $tRNA^{arg}$.

(8) *The rate of translation of mRNA coding for the arginine biosynthetic enzymes is proportional to the arginyl-tRNA level when this level is below threshold; i.e.,*

$$\text{rate of translation} = \begin{cases} b, & M^* \geq T_r \\ bM^*/T_r, & M^* < T_r \end{cases} \qquad (3)$$

per unit of mRNA where b is the appropriate proportionality constant. When the arginyl-tRNA level is at or above threshold, the translation rate is at its maximum level b.

Given assumption 7, this says that when arginine is limiting the over-all rate of protein synthesis, it is equally limiting for the synthesis of OTC and the other arginine biosynthetic enzymes. That is, for $M^* < T_r$, both the over-all rate of protein synthesis and the rate of translation of OTC-specific mRNA are proportional to M^*/T_r.

(9) *The rate of synthesis of new arginyl-tRNA is proportional to the OTC level.*

(10) *All molecular interactions and processes are much more rapid than the translation of messenger RNA.*

Since the rate of messenger RNA translation is proportional to the arginyl-tRNA level for levels of arginyl-tRNA less than the repression threshold and since the arginyl-tRNA level must be especially low following a "shift-down" (i.e., withdrawal of arginine), the possibility of a significant time delay in messenger translation following a shift-down must be considered. Even when no nutrients are limiting the rate of messenger RNA translation, the time necessary to add a single peptide bond to a growing polypeptide chain in *Salmonella typhimurium* is estimated to be 60 msec at 37° (Maaloe and Kjeldgaard, 1966, p. 91).

(11) *The attachment of arginyl-tRNA to the arginine codons on the messenger RNA is a Poisson process specified by the parameter* λ. *Thus, the expected value of the waiting time until n such arginyl-tRNA's have been placed into position on the growing polypeptide chain is*

$$E(W_n) = \frac{n}{\lambda},$$

(Parzen, 1962, pp. 133–4).

(12) *First-order decay constants can be defined for the rates of loss of OTC-specific mRNA, OTC, and arginyl-tRNA due to molecular instability and to dilution by growth.*

(13) *OTC and arginyl-tRNA are considered stable molecules so that their decay constants for loss due to molecular instability are equal to zero.*

There are no direct data bearing on this. In the case of OTC, studies on repression after a phase of derepression show little significant drop in the OTC level (P. Rogers, personal communication).

(14) *The rate of synthesis of new OTC is proportional to the OTC-specific mRNA level.*

MATHEMATICAL MODEL

Let x denote the level of mRNA specific for OTC in micrograms per milligram bacteria dry weight. Let y be the OTC level in units per mg dry weight, and let $\dot{x}(t)$ and $\dot{y}(t)$ denote their respective time derivatives at time t. By assumption 2, the rate of synthesis of OTC-specific mRNA is

$$\text{rate of synthesis of mRNA}_{\text{OTC}} = \frac{1}{A + mR^*(t)}.$$

The concentration of $mRNA_{OTC}$ in each milligram dry weight is decreased both by molecular breakdown and by increase in total protein[2] due to growth. Thus, for $M^* \geq T_\tau$,

$$\text{rate of loss of } mRNA_{OTC} = ax(t) + cx(t),$$

where the term $ax(t)$ is the loss due to molecular breakdown and a is the decay constant (assumption 12), the second term is the loss due to increase in total protein, and c is a decay constant (assumption 12). Then $\dot{x}(t)$ is just the rate of synthesis minus the rate of loss of $mRNA_{OTC}$:

$$\dot{x}(t) = \frac{1}{A + mR^*(t)} - ax(t) - cx(t), \qquad M^* \geq T_\tau. \tag{4}$$

Since the rate of translation of OTC-specific mRNA is proportional to M^*/T_τ (assumption 8) for $M^* < T_\tau$, and since the rate of increase of total protein is assumed proportional to this rate (assumption 8), we have

$$\dot{x}(t) = \frac{1}{A + mR^*(t)} - ax(t) - c\frac{M^*(t)x(t)}{T_\tau}, \qquad M^* < T_\tau. \tag{5}$$

Since no active repressor is formed when $M^* < T_\tau$ (assumption 6) and since active repressor is assumed to be very unstable so that any present is soon destroyed (assumption 2), we can combine equations 4 and 5 as

$$\dot{x}(t) = \begin{cases} \dfrac{1}{A} - ax(t) - c\dfrac{M^*(t)x(t)}{T_\tau}, & M^* < T_\tau \\ \dfrac{1}{A + mR^*(t)} - ax(t) - cx(t), & M^* \geq T_\tau. \end{cases} \tag{6}$$

The rate of translation of OTC-specific mRNA as given by equation 3 combined with the assumption that the rate of synthesis of OTC is proportional to the value of x at a time τ min preceding (assumptions 10 and 14) gives

$$\text{rate of OTC synthesis} = \begin{cases} \dfrac{bM^*(t)x(t-\tau)e^{-(a+c)\tau}}{T_\tau}, & M^* < T_\tau \\ bx(t-\tau)e^{-(a+c)\tau}, & M^* \geq T_\tau. \end{cases} \tag{7}$$

The factor $e^{-(a+c)\tau}$ appears in equations 7 because messenger decays exponentially for τ min before it yields active enzyme. By assumptions 7, 8, 12, and 13, the rate of

[2] The rates of increase of dry weight and total protein are equal for any given growth rate.

loss of OTC is

$$\text{rate of loss of OTC} = \begin{cases} \dfrac{cM^*(t)y(t)}{T_r}, & M^* < T_r \\ cy(t), & M^* \geq T_r. \end{cases} \quad (8)$$

$\dot{y}(t)$ is the rate of synthesis minus the rate of loss of OTC. From equations 7 and 8,

$$\dot{y}(t) = \begin{cases} \dfrac{bM^*(t)x(t-\tau)e^{-(a+c)\tau}}{T_r} - \dfrac{cM^*(t)y(t)}{T_r}, & M^* < T_r \\ bx(t-\tau)e^{-(a+c)\tau} - cy(t), & M^* \geq T_r. \end{cases} \quad (9)$$

The time derivative of the arginyl-tRNA concentration is

$$\dot{M}^*(t) = K_1 y(t) + K_2 - K_3 M^*(t). \quad (10)$$

The first term is the rate of synthesis of new arginyl-tRNA which is proportional to the OTC level, y (assumption 9). K_2 is the contribution to the arginyl-tRNA pool from protein turnover during arginine starvation. The term, $-K_3M^*(t)$, is the rate of loss of arginyl-tRNA due to incorporation into protein. K_1, K_2, and K_3 are constants. Arginyl-tRNA is assumed stable and its rate of loss due to dilution by growth is negligible compared to K_3M^*.

Using assumptions 3, 4, 5, and 6, we can write for active repressor R^*:

$$\dot{R}^*(t) = \begin{cases} -(q+c)R^*, & M^* < T_r \\ \dfrac{p(M^*(t) - T_r)R}{G + (M^*(t) - T_r)R} - (q+c)R^*, & M^* \geq T_r, \end{cases}$$

where R is the level of aporepressor in micrograms per milligram dry weight, G is the Michaelis constant, and p and q are constants. If we assume active repressor to be so unstable (assumption 2) that R^* always appears to be in a steady state relative to M^*, then $I^* = 0$ and we may solve for R^*:

$$R^*(t) = \begin{cases} 0, & M^* < T_r \\ \dfrac{p}{(q+c)} \dfrac{(M^*(t) - T_r)R}{G + (M^*(t) - T_r)R}, & M^* \geq T_r. \end{cases} \quad (11)$$

Since the units for R^* are arbitrary, take $p/(q+c)$ equal to 1 and divide equation 11 through by R:

$$R^*(t) = \begin{cases} 0, & M^* < T_r \\ \dfrac{(M^*(t) - T_r)}{H + (M^*(t) - T_r)}, & M^* \geq T_r. \end{cases} \quad (12)$$

Then the constant $H = G/R$ and $0 \leq R^* \leq 1$.

Likewise, since we have no direct measurements of the amount of OTC-specific mRNA involved in the derepression kinetics we might as well take $b = 1$. Then, when arginine is not rate limiting in protein synthesis ($M^* \geq T_r$), equation 9 yields the steady-state solution

$$y^0 = \frac{x^0}{c} e^{-(a+c)\tau} \tag{13}$$

(steady-state values are denoted by superscript zeroes). One unit of OTC-specific mRNA is then the amount that yields $e^{-(a+c)\tau}/c$ units of OTC in the steady state in minimal medium.

Let τ_0 be the time necessary to translate an OTC-specific mRNA molecule when arginine is not rate limiting in protein synthesis ($M^* \geq T_r$). Let the number of arginine residues in OTC be n and let θ be the fraction of *E. coli* protein that is arginine. The expected waiting time to insert n arginine residues is, by assumption 11, n/λ. When arginyl-tRNA is rate limiting, λ should be proportional to the arginyl-tRNA level; i.e.,

$$\lambda = C_1 \frac{M^*}{T_r}, \qquad M^* < T_r,$$

where C_1 is a proportionality constant. When M^* rises above the threshold T_r,

$$\lambda = C_1, \qquad M^* \geq T_r.$$

Accordingly, the expected waiting time to insert n arginine residues is

$$E(W_n) = \begin{cases} \dfrac{nT_r}{M^* C_1}, & M^* < T_r \\ \dfrac{n}{C_1}, & M^* \geq T_r. \end{cases}$$

But, for $M^* \geq T_r$, $E(W_n) = \theta\tau_0$ since in this case the insertion of arginine is not rate limiting in protein synthesis. Hence, $n/C_1 = \theta\tau_0$ when $M^* \geq T_r$. The time necessary to translate all nonarginine codons of mRNA_{OTC} is always $\tau_0(1 - \theta)$. The total translation time delay under all conditions is therefore

$$\tau(t) = \begin{cases} \tau_0 \left[(1 - \theta) + \theta \dfrac{T_r}{M^*(t)} \right], & M^* < T_r \\ \tau_0, & M^* \geq T_r. \end{cases} \tag{14}$$

Computer Simulation Studies

The mathematical model represented by equations 6, 9, 10, 12, and 14 was solved on an IBM 7094 digital computer using a Runge-Kutta integration procedure, employ-

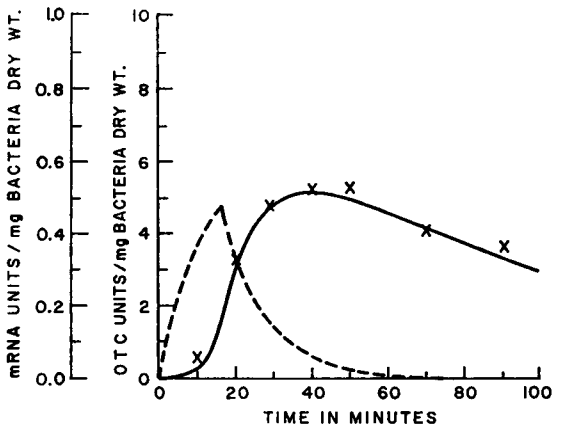

FIGURE 4 Derepression of OTC in flask culture: $t_{1/2}$ = 9 min. *E. coli* cells taken from exponentially growing cultures maintained on arginine were washed, inoculated into minimal medium A (Davis and Mingioli, 1950) + lactate, 0.5%, and incubated at 37° with shaking. Cell division time was 60 min. Data points (×) are taken from Gorini and Maas (1957). The solid line represents the theoretical course of derepression under the assumption $t_{1/2}$ = 9 min; the dashed line represents OTC-specific mRNA. Time scale extends from 0 to 100 min. Parameter estimates: $a = 0.0772$; $A = 18.9$; $m = 65{,}700$; $K_1 = 0.335$; $K_2 = 0.048$; $K_3 = 53.0$; $c = 0.0115$; $\tau_0 = 0.400$; $T_r = 0.01094$; $H = 0.01825$; $\theta = 0.083$. Initial conditions: $x(0) = 0.00019$; $y(0) = 0.016$; $M^*(0) = 0.001$; $\tau(0) = 0.73$; $R^*(0) = 0$.

ing an integration step size of 0.02 min. The variable $x(t - \tau)$ was obtained from the function $x(t)$ by creating two vectors, ZZ and Z. Each time the Runge-Kutta subroutine generated a set of values for the variables, a counting variable NCOMP was incremented by 1 and the value of x was stored in $ZZ(N\text{COMP})$. The variable $N = (N\text{COMP} - N\text{TAU})$, where $N\text{TAU} = \tau/\text{STEP}$ and STEP is the step size, was defined and for all values of N greater than or equal to 1, $Z(N\text{COMP})$ took on the value $ZZ(N)$. In this way, the vector Z represented the function $x(t - \tau)$.

The equations of the model include 13 constants, 5 time-dependent variables, and their initial conditions. Obviously, if we are free to choose the values of the constants and initial conditions, the model could easily be made to fit a wide range of data without justifying the model in the least. But, as shown in the Appendix, if a value for one constant is assumed, it is possible to estimate the other 12 constants as well as the initial conditions and we can proceed to ask meaningful questions of the model. Given this one degree of freedom, the first question asked was: what must the half-life ($t_{1/2}$) of OTC-specific mRNA be in order to obtain the overshoot kinetics of Gorini and Maas (Fig. 4 and 5)? Using the parameter estimates derived in the Appendix, we find that the computed curve $y(t)$ in Fig. (4) is in excellent agreement with the data points for the first 100 min when it is assumed that $t_{1/2}$ is 9 min. Fig. 5 shows this computation extended to 500 min.

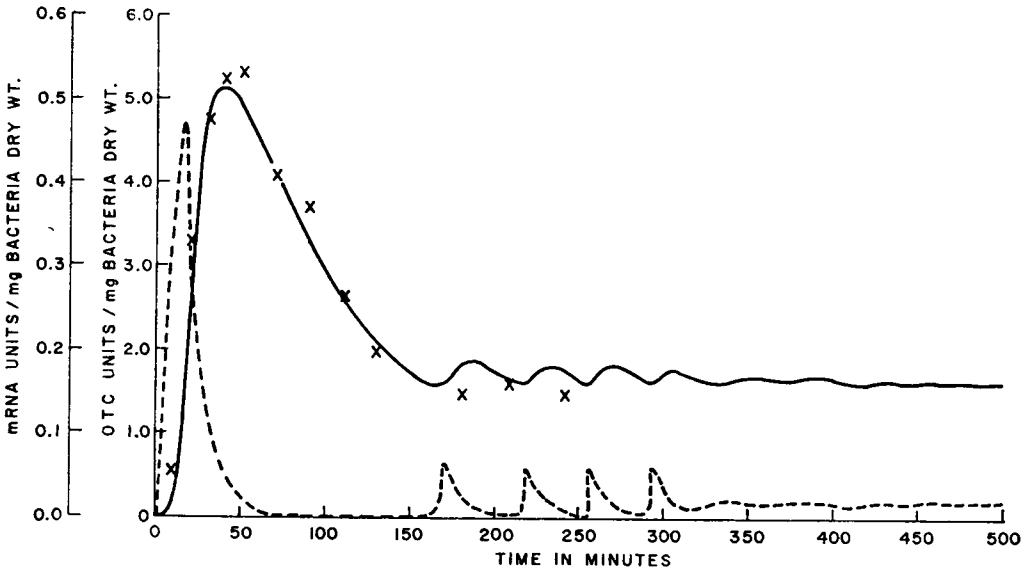

FIGURE 5 Derepression of OTC in flask culture: $t_{1/2}$ = 9 min. Same as Fig. 4 but time scale extended to 500 min.

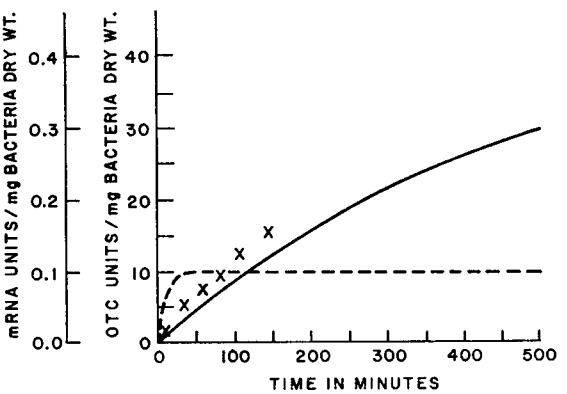

FIGURE 6 Derepression of OTC in the chemostat with arginine growth limiting. A histidine$^-$ arginine$^-$ auxotroph of *E. coli* W pregrown in the presence of arginine to produce a low level of OTC was washed and inoculated into minimal medium + lactate (0.5%) supplemented with histidine (10 μg/ml) and arginine (5 μg/ml). With arginine thus limiting growth, OTC concentration reaches 45 units/mg dry weight bacteria after four cell divisions. The cell generation time was 460 min. Data points (×) taken from Gorini and Maas (1957). The solid line represents the theoretical course of derepression; the dashed line represents OTC-specific mRNA. Parameter estimates: a = 0.0772; c = 0.00217; A = 118.0; K_1 = 0.335; K_2 = 0.048; K_3 = 53.0; τ_0 = 0.400; T_r = 0.01094; H = 0.01825; θ = 0.083; M^* = T_r; R^* = 0. Initial conditions: $x(0)$ = 0.00019; $y(0)$ = 0.016.

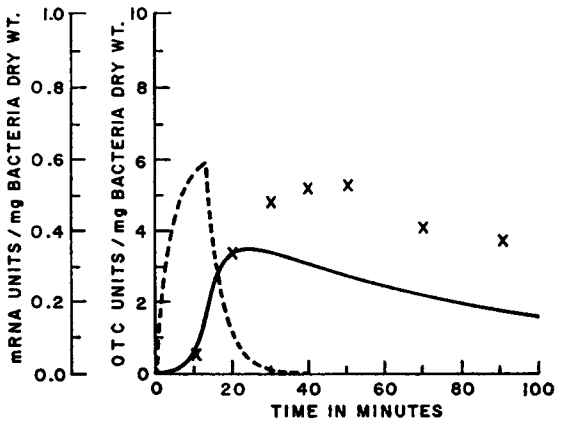

FIGURE 7 Derepression of OTC in flask culture: $t_{1/2} = 3$ min. The solid line represents the theoretical course of derepression; the dashed line represents OTC-specific mRNA. Data points (×) are taken from Fig. 4. Parameter estimates: $a = 0.231$; $A = 6.85$; $m = 18{,}800$; $K_1 = 0.335$; $K_2 = 0.048$; $K_3 = 53.0$; $c = 0.0115$; $\tau_0 = 0.400$; $T_r = 0.01094$; $H = 0.01825$; $\theta = 0.083$. Initial conditions: $x(0) = 0.00022$; $y(0) = 0.016$; $M^*(0) = 0.001$; $\tau(0) = 0.73$.

To test the importance of the variable time delay, τ was assumed constant at 0.4 min. The model was recomputed using the same parameter values as used in Fig. 4 and virtually identical results were obtained, the value of the OTC level always being within 5% of the value obtained with the variable time delay. The possibility of a variable time delay therefore seems relatively unimportant based on the present analysis.

Again using the same parameter values and assuming a constant time delay, Fig. 6 shows how well the theory accounts for the derepression of arg⁻his⁻ *E. coli* W cells growing in a chemostat with a generation time of 460 min. The data points are taken from Gorini and Maas (1957). Here $\dot{M}^*$ was taken equal to zero and M^* was made identically equal to T_r to give total derepression. Since the generation time of the cells in the chemostat was 460 min, the value $c = \frac{1}{460}$ min^{-1} was assumed. Therefore, $A = 118.0$ by equation 44.

Faanes and Rogers have estimated the half-life of OTC-specific mRNA to be about 3 min for *E. coli* W cells growing in AF medium as discussed earlier. Assuming $t_{1/2} = 3$ min, then $a = 0.231$ min^{-1}, $A = 6.85$ min/mRNA unit, and $m = 18{,}800$. Using these parameter estimates, the function $y(t)$ is found to pass through the first two data points but fails to rise to the experimentally determined peak value of 5.3 units/mg (Fig. 7). Instead, it peaks at 3.48 units/mg at 24 min although the rate of decay of the enzyme after repression sets in appears about right. This discrepancy might be due to underestimating the value of T_r. T_r was calculated (see Appendix) for the steady-state condition and assumed constant during the derepression but it need not be. During the initial period of arginine starvation, the levels of the other amino acids may have risen so that a correspondingly higher level of arginyl-tRNA

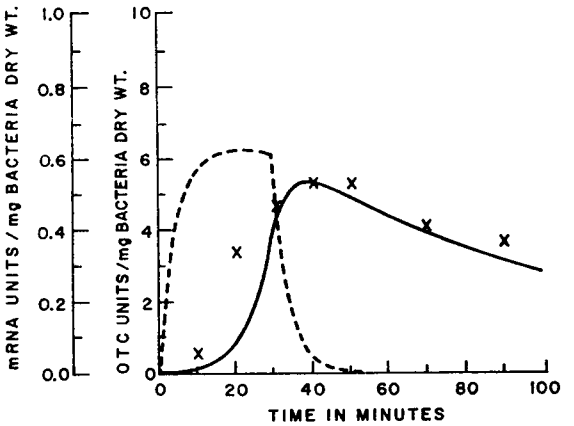

FIGURE 8 Derepression of OTC in flask culture: $t_{1/2} = 3$ min. $T_r = 0.024$. The solid line represents the theoretical course of derepression; the dashed line represents OTC-specific mRNA. Data points (X) are taken from Gorini and Maas (1957). Parameter estimates: $a = 0.231$; $A = 6.85$; $m = 18{,}800$; $K_1 = 0.335$; $K_2 = 0.048$; $K_3 = 53.0$; $c = 0.015$; $\tau_0 = 0.400$; $T_r = 0.024$; $H = 0.01825$; $\theta = 0.083$. Initial conditions: $x(0) = 0.00022$; $y(0) = 0.016$; $M^*(0) = 0.001$; $\tau(0) = 0.73$.

would be necessary before there would be any excess of it beyond the demands of protein synthesis; that is, T_r may be higher during the initial arginine starvation period than in the steady-state condition. In that case, repression would be delayed until the OTC level rose high enough to provide an arginyl-tRNA level in excess of T_r. This would allow a longer time to synthesize the OTC-specific messenger RNA. The level of this mRNA would thus be higher when repression finally did set in and this should raise the peak value of OTC. With this working hypothesis, it was found that a constant repression threshold of 0.024 would give about the right peak height as Fig. 8 shows. In this computation, it is assumed that after repression sets in, the value of T_r slips back down to its steady-state level. It is seen that in this case the initial rate of synthesis of OTC is too slow and the peak occurs a bit too soon.

AN APPROXIMATE ANALYTICAL SOLUTION

The fact that certain of the model's parameters are large in relation to others can be used to simplify the model so that an analytical solution can be obtained.

During the initial phase of derepression, $M^* < T_r$ and, from equations 6 and 9,

$$\dot{x} = \frac{1}{A} - ax - c\frac{M^*y}{T_r}, \tag{15}$$

$$\dot{y} = \frac{M^*x}{T_r} - c\frac{M^*y}{T_r}, \tag{16}$$

if we can neglect the translation time delay τ. But, by equation 45 (see Appendix),

the approximate equality holds

$$M^* \approx \frac{K_1 y + K_2}{K_3}. \tag{45}$$

Substituting $(K_1y + K_2)/K_3$ for M^* into equations 15 and 16 gives

$$\dot{x} = \frac{1}{A} - ax - c\frac{(K_1 y + K_2)x}{T_r K_3}, \tag{17}$$

$$\dot{y} = \frac{(K_1 y + K_2)x}{T_r K_3} - c\frac{(K_1 y + K_2)y}{T_r K_3}. \tag{18}$$

Since c and K_2 are small compared to a and K_1, we can neglect all terms in equation 17 containing these constants as factors. This will always be true as long as the doubling time is long compared to the mRNA half-life and as long as the rate of yield of arginyl-tRNA from protein turnover is small compared to the rate of yield from the arginine biosynthetic pathway. These remarks apply also to equation 18 except that K_2 should be retained in the first term since K_2 will be large compared to K_1y during the early stage of derepression. Equations 17 and 18 simplify to

$$\dot{x} = \frac{1}{A} - ax, \tag{19}$$

$$\dot{y} = Pxy + qx, \tag{20}$$

where

$$P = \frac{K_1}{T_r K_3}, \tag{21}$$

$$Q = \frac{K_2}{T_r K_3}. \tag{22}$$

Equation 19 has solution

$$x(t) = \frac{1}{Aa}(1 - e^{-at}) + x(0)\, e^{-at}. \tag{23}$$

Substitution of the right-hand side of equation 23 for x into equation 20 gives

$$\dot{y}(t) = (Py + Q)(1 - e^{-at})/Aa, \tag{24}$$

where the term $x(0)e^{-at}$ is neglected.

Equation 24 has solution

$$y(t) = \left[y(0) + \frac{K_2}{K_1}\right] \exp\left\{\frac{K_1}{T_r K_3 Aa}\left[t - \frac{1}{a}(1 - e^{-at}\right]\right\} - \frac{K_2}{K_1} (M^* < T_r). \tag{25}$$

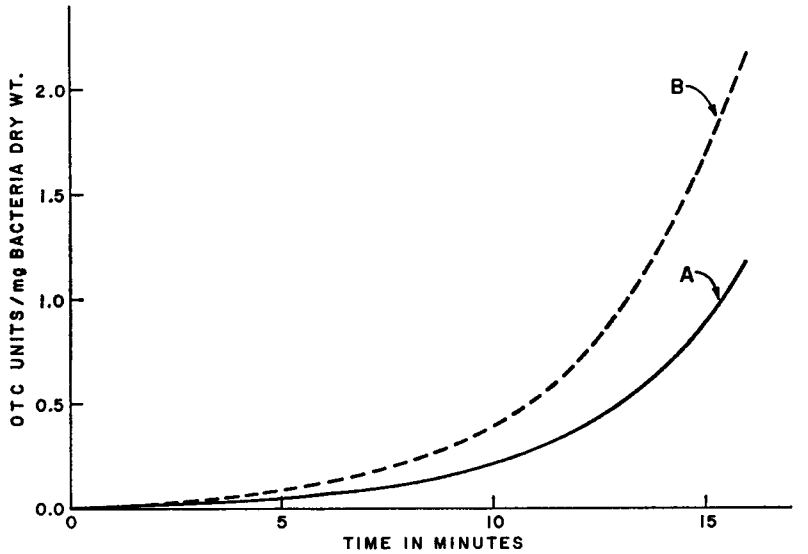

FIGURE 9 Comparison of computed and approximate solutions for derepression of OTC in flask culture. Curve A is the computed solution of Fig. 4. Curve B is the approximate solution according to equation 25 for $M^* < T_r$.

That this is a good approximate solution can be seen by comparing a plot of y vs. time in Fig. 9 using the computed solution (curve A) of Fig. 4 and the approximate solution (curve B) before repression sets in.

The case of $M^* \geq T_r$ can be treated similarly. Suppose the cells are growing in minimal medium in a steady state and arginine is added to the medium at time zero. If we can assume that this instantaneously reduces the rate of synthesis of OTC-specific mRNA to zero, then

$$\dot{x} = -(a + c)x, \tag{26}$$

$$\dot{y} = x - cy, \tag{27}$$

where again the messenger RNA translation time delay is neglected and $x(0) = x^0$ $y(0) = y^0$ (i.e. steady-state levels in minimal medium). Hence,

$$x = x^0 e^{-(a+c)t}, \tag{28}$$

$$y = e^{-ct}\left(-\frac{x^0 e^{-ct}}{a} + C\right), \tag{29}$$

where C is a constant of integration. At $t = 0$, $y = y^0$ so that

$$C = y^0 + x^0/a = y^0(1 + c/a). \tag{30}$$

Hence,

$$y(t) = y^0 e^{-ct}\left(1 + \frac{c}{a} - \frac{c}{a} e^{-at}\right). \quad (31)$$

DISCUSSION

The theoretical model for the derepression kinetics of OTC in *E. coli* presented here is based on the key assumption that active repressor for the arginine regulon is present only when the arginyl-tRNA level is in excess of the level necessary to maintain protein synthesis at a given cell doubling time. Thus, the overshoot observed in derepression of OTC in flask culture is due to overproduction of OTC-specific mRNA in the early phase of derepression when there is no active repressor present. This excess mRNA continues to be translated into OTC even after the arginyl-tRNA level is sufficient for the demands of protein synthesis resulting in the overshoot. When the derepression proceeds with the arginyl-tRNA level always rate limiting in protein synthesis (Fig. 6) however, no overshoot is observed since no active repressor is ever formed and the arginine regulon becomes fully derepressed. The regulation of arginine biosynthesis therefore depends on the growth rate since the repression threshold T_r is a function of the over-all rate of protein synthesis. The parameters of the model were considered constant here for a *single growth rate*. In general, these parameters are all functions of the growth rate.

While the above interpretation of the derepression kinetics is reasonable in itself it requires certain qualifications. It has never been shown directly that the level of OTC-specific mRNA changes at all during repression or derepression of this enzyme. Since the function of mRNA in the derepression kinetics is still uncertain, the variable x should be understood to denote whatever molecular species that may perform the function here attributed to mRNA. Also, the possible inhibition of messenger RNA synthesis due to arginine starvation in the early phase of derepression has been ignored. Amino acids affect RNA synthesis catalytically (Pardee and Prestidge, 1959; Gros and Gros, 1958) but whether mRNA synthesis is affected has not been determined conclusively (Maaloe and Kjeldgaard, 1966, pp. 139–152). Finally, the model does not require that arginyl-tRNA be involved directly in the formation of active repressor. Any molecular species whose level remains proportional to the arginyl-tRNA level during derepression may be assumed to act as the corepressor without any change in the model.

Predictions

It has been shown that the mathematical model presented here can account for the overshoot kinetics of OTC derepression in flask culture (Fig. 4 and 5), the derepression of OTC in the chemostat for arginine growth limiting to the fully derepressed level (Fig. 6) and the increase in the size of bursts of OTC synthesis elicited by addi-

tion of arginine to cultures of derepressing cells with the increase in the delay time before the arginine addition. This last effect is the obvious consequence of the rate of synthesis of $mRNA_{OTC}$ decreasing from $1/A$ to $1/(A + mR^*)$ upon the addition of arginine to minimal medium causing premature inhibition of the synthesis of $mRNA_{OTC}$.

Some further predictions can be obtained from the approximate analytical solution. Equation 25 can be rewritten as

$$\frac{y + \frac{K_2}{K_1}}{y(0) + \frac{K_2}{K_1}} = \exp\left\{\frac{K_1}{T_r K_3 A a}\left[t - \frac{1}{a}(1 - e^{-at})\right]\right\},$$

and, taking logarithms of both sides of the equation,

$$\log_{10}\frac{y + \frac{K_2}{K_1}}{y(0) + \frac{K_2}{K_1}} = \frac{K_1}{T_r K_3 A a(2.303)}\left[t - \frac{1}{a}(1 - e^{-at})\right]. \tag{32}$$

Thus, if *E. coli* W cells are grown in a chemostat at a long doubling time and derepression of OTC is followed, a plot of $\log_{10}(y + K_2/K_1)/(y(0) + K_2/K_1)$ vs. time should, after an initial transient due to the changing mRNA level, yield a straight line with slope and intercept given by

$$\text{slope} = \frac{K_1}{T_r K_3 A a(2.303)}, \tag{33}$$

$$\text{intercept} = \frac{-K_1}{T_r K_3 A a^2(2.303)}. \tag{34}$$

Accordingly, an estimate of the parameter a can be obtained from the ratio of the slope to the intercept:

$$a = \frac{\text{slope}}{\text{intercept}}. \tag{35}$$

This method is illustrated in Fig. 10, curve B, using $y(0) = y_r = 0.016$, $K_1 = 0.335$, $K_2 = 0.048$ and for y the values obtained from the computed solution shown in Fig. 4; the values of y obtained from the approximate analytical solution for the same parameter estimates and shown in Fig. 9 were also used (curve A). Curve A yields the estimate 0.134 for a which corresponds to $t_{1/2} = (\ln 2)/a = 5.18$ min. The computation from which these data are drawn assumed that $a = 0.0772$ for which $t_{1/2} = 9$ min. The discrepancy is due to the rather short doubling time which allows de-

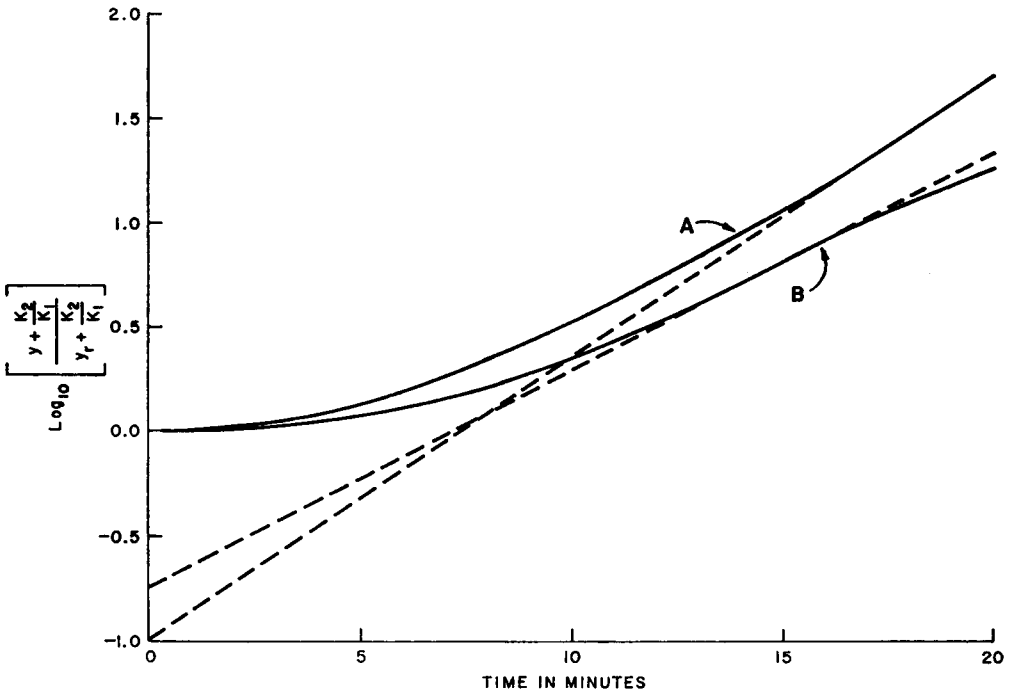

FIGURE 10 Illustration of a method for determining the half-life of OTC-specific mRNA based on an approximate analytical solution. Log_{10} of $[y + K_2/K_1]/[y(0) + K_2/K_1]$ is plotted vs. time. The estimates $K_1 = 0.335$, $K_2 = 0.048$, and $y(0) = y_r = 0.016$ are used. For curve A the computed values of y for the first 20 min of derepression of OTC in Fig. 4 were used. For curve B the corresponding values of y calculated according to equation 25 and plotted in Fig. 9, curve B, were used. Straight line tangents are drawn through curves A and B as dashed lines.

repression to occur sufficiently rapidly that the term e^{-at}/a in equation 32 remains large over the time range considered.

If the cells are grown in minimal medium in a steady state and arginine is added to the medium at time zero in sufficient quantity to repress the cells fully, then the approximate solution of equation 31 applies for $M^* > T_r$:

$$y = y^0 e^{-ct}\left(1 + \frac{c}{a} - \frac{c}{a} e^{-at}\right). \tag{31}$$

Again, if the initial transient due to the term ce^{-at}/a is ignored, then

$$\log_{10}(y/y^0) = \log_{10}(1 + a/c) - ct/2.303. \tag{36}$$

Hence, a plot of $\log_{10}(y/y^0)$ vs. time should, after an initial transient, yield a straight line with slope and intercept given by

$$\text{slope} = -\frac{c}{2.303} \tag{37}$$

$$\text{intercept} = \log_{10}(1 + a/c). \qquad (38)$$

With the value of c known, the parameter a and thus $t_{1/2}$ can be calculated using equation 38.

ABBREVIATIONS

DT	cell doubling time
g	gram
hr	hour
mg	milligrams bacteria dry weight
min	minute
ml	milliliter
mRNA	messenger ribonucleic acid
OTC	ornithine transcarbamylase (carbamoylphosphate L-ornithine carbamoyltransferase, E.C. 2.1.3.3)
sec	second
tRNA	transfer ribonucleic acid
$tRNA^{arg}$	arginine-specific transfer ribonucleic acid

SYMBOLS

Variables

t	time in minutes
x	messenger RNA units per milligram bacteria dry weight
y	OTC units per milligram bacteria dry weight
M^*	arginyl-tRNA in micrograms per milligram bacteria dry weight
R^*	active repressor in units per milligram bacteria dry weight
τ	time necessary to translate OTC-specific mRNA

Constants

A	reciprocal of the maximum possible rate of OTC-specific mRNA synthesis
m	a measure of the sensitivity of the mRNA transcribing machinery to active repressor
τ_0	time necessary to translate OTC-specific mRNA when no nutrients are rate limiting
c	rate of loss of OTC (units per milligram) due to increase in total protein per unit of OTC per milligram bacteria dry weight per minute when arginyl-tRNA is not rate limiting in protein synthesis
G	Michaelis constant for the formation of active repressor from aporepressor and arginyl-tRNA when active repressor is measured in micrograms per milligram bacteria dry weight
R	level of aporepressor in micrograms per milligram bacteria dry weight
H	G/R
T_τ	threshold level of arginyl-tRNA necessary to maintain a given rate of protein synthesis; when M^* is less than T_τ, arginyl-tRNA is rate limiting
K_1	proportionality constant relating the level of OTC to the rate of synthesis of new arginyl-tRNA

K_2	rate at which protein turnover yields arginyl-tRNA
K_3	proportionality constant relating the rate of loss of arginyl-tRNA by uptake into protein to the arginyl-tRNA level
y_r	level of OTC under total repression
y_d	level of OTC under total derepression
$t_{1/2}$	half-life of OTC-specific mRNA
θ	fraction of OTC that is arginine
a	decay constant for $mRNA_{OTC}$

It is a pleasure to thank Dr. Richard Levins, Dr. David Kirk, Dr. John Westley, Dr. Palmer Rogers, and Dr. Werner Maas for their encouragement and helpful discussion.

The results presented here form part of the author's doctoral dissertation presented to the University of Chicago.

This work was supported by the United States Public Health Service Training Grant No. 5T1 GM 833 from the National Institute of General Medical Sciences.

Received for publication 17 July 1969 and in revised form 5 January 1970 and 29 May 1970.

APPENDIX

Estimation of Parameters

Let $t_{1/2}$ denote the half-life of OTC-specific mRNA. It will be shown that the model can be made to describe the data of Figs. 4–6 if $t_{1/2}$ is 9 min. Then

$$a = \frac{\ln 2}{t_{1/2}}, \tag{39}$$

$$= 0.0772.$$

Similarly, denoting the cell doubling time by DT,

$$c = \frac{\ln 2}{DT},$$

and for a 60 min doubling time $c = 0.0115\ \text{min}^{-1}$.

When the cells are totally derepressed, $R^* = 0$ and the derepressed level of OTC (y_d) is, in the steady state,

$$y_d^0 = \frac{x^0}{c} e^{-(a+c)\tau_0} \tag{40}$$

from equation 9. In the case of an arginine auxotroph grown on minimal medium supplemented with a limiting concentration of arginine, equation 6 becomes

$$\dot{x}(t) = \frac{1}{A} - ax(t) - cx(t), \tag{41}$$

since R^* is zero in this case and $M^* = T_r$. Thus,

$$x^0 = \frac{1}{A(a + c)}. \tag{42}$$

Using equation 42 in equation 40 yields

$$y_d^0 = \frac{1}{A(a + c)c} e^{-(a+c)\tau_0}. \tag{43}$$

Hence,

$$A = \frac{e^{-(a+c)\tau_0}}{y_d(a + c)c}. \tag{44}$$

Since the steady-state level of OTC in *E. coli* W arginine-histidine double auxotrophic cells grown in the chemostat in minimal medium supplemented with histidine and growth-limiting arginine was 47.4 units/mg,[3] y_d is 47.4 units/mg when the generation time is 460 min.[4] Using these values in equation 44 gives $A = 18.9$ min/mRNA unit for $\tau_0 = 0.4$ min. The latter estimate assumes that the molecular weight of OTC is 60,000 daltons (Rogers and Novelli, 1962), the average weight of an amino acid is 150 daltons, and the time necessary to add one amino acid to a growing polypeptide chain during $mRNA_{OTC}$ translation is 60 msec (Maaloe and Kjeldgaard, 1966, p. 91):

$$\frac{60{,}000 \text{ daltons}}{150 \text{ daltons}/1 \text{ amino acid}} \times \frac{0.06 \text{ sec}}{\text{amino acid}} \times \frac{1 \text{ min}}{60 \text{ sec}} = 0.40 \text{ min}.$$

E. coli protein is about 8.3% arginine (Goldstein, Goldstein, and Lowney, 1964); hence, $\theta = 0.083$. The rate of protein turnover is 7%/hr in amino-acid–starved cells (Pine, 1966) and since 50% of the bacterial dry weight is protein[5] (Luria, 1960), the yield of arginine from protein turnover is

$$\frac{0.07 \text{ mg protein}}{(\text{hr})\ (\text{mg protein})} \times \frac{0.083 \text{ mg arginine}}{\text{mg protein}} \times \frac{1 \text{ mg protein}}{2 \text{ mg bacteria dry weight}}$$

$$\times \frac{1 \text{ hr}}{60 \text{ min}} = 0.048 \ \mu\text{g arginine/min per mg dry weight}.$$

When the cells reach a steady state in minimal medium this rate will decrease by about one-third (Pine, 1966) but we will assume that this rate stays constant throughout the process of

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[3] The data obey the equation of constant rate kinetics: $\%\ E_{max} = 100(1 - e^{-at})$ where the E_{max} is the maximum final level of enzyme, a is the rate constant of the equation for exponential growth: $dN/dt = aN$ and N is the bacterial density. Since the enzyme level was 45 units/mg dry weight after four generation times, which corresponds to 95% of E_{max}, the maximum final level of enzyme was 47.4 units/mg (Gorini and Maas, 1957).

[4] This experiment was repeated by this author using a doubling time of 60 min and y_d was found to be 51.0 units/mg (Coyne, unpublished results).

[5] By determining the protein content of known dilutions of a suspension of 1.0 mg dry weight *E. coli* W per ml, it was found that 56% of the bacterial dry weight is protein (Coyne, unpublished results).

derepression for the sake of simplicity. Since the major supply of arginine is from the arginine biosynthetic pathway, this should cause no great error. It is assumed that the arginine is rapidly and essentially quantitatively converted into arginyl-tRNA so that we can take $K_2 = 0.048$. This should be especially true during the temporary arginine starvation when the cells are first transferred to minimal medium.

The rate of synthesis of arginine in *E. coli* K-12 is 55 μmoles/g wet weight per hr for cells growing at a doubling time of 60 min. Of this, 5 μmoles/g per hr is metabolized to agmatine and 50 μmoles/g per hr is incorporated into protein (W. Maas, personal communication). Assuming that these figures apply also to *E. coli* strain W and that 25% of *E. coli* wet weight is dry weight (Luria, 1960), then arginyl-tRNA is incorporated into protein at the rate

$$\frac{50\ \mu\text{moles}}{(\text{hr})\ (\text{g wet weight})} \times \frac{175\ \mu\text{g}}{\text{mole}} \times \frac{1\ \text{hr}}{60\ \text{min}} \times \frac{1\ \text{g wet weight}}{0.25\ \text{g dry weight}} \times \frac{1\ \text{g dry weight}}{1000\ \text{mg dry weight}} = 0.583\ \mu\text{g/min per mg}$$

dry weight when the rate of protein synthesis corresponds to a cell doubling time of 60 min.

In the steady state, equation 10 is

$$\dot{M}^* = K_1 y^0 + K_2 - K_3 M^{*0} = 0.$$

But $K_3 M^* = 0.583$ μg/min per mg and $K_2 = 0.048$ μg/min per mg as computed above. Hence, $K_1 = (0.583 - 0.048)/1.6 = 0.335$ μg arginine in the form of arginyl-tRNA per unit of OTC per minute.

Maaloe and Kjeldgaard (1966, p. 91) estimate the number of tRNA molecules per genome in *Salmonella typhimurium* to be about 2.5×10^5. Assuming a similar population size in *E. coli* and assuming an average of two copies of the genome per cell when the doubling time is 60 min, the total of tRNA molecules per cell would be 5.0×10^5. Of these, about every 1 in 20 might be specific for arginine so that the population of arginine-specific tRNA would be about 2.5×10^4. Morris and DeMoss (1965) found that $tRNA^{arg}$ is about 30% charged in *E. coli* cells growing in exponential culture. Accepting this estimate we have for the concentration of arginine in the form arginyl-tRNA

$$2.5 \times 10^4 \frac{\text{molecules}}{\text{cells}} \times \frac{175\ \mu\text{g}}{6 \times 10^{23} \times 10^{-6}\ \text{molecules}} \times \frac{5 \times 10^9\ \text{cells}}{\text{mg cells dry weight}} \times 30\% = 0.0111\ \mu\text{g/mg}.$$

As noted above, $K_3 M^{*0} = 0.583$ μg/min per mg so that $K_3 = (0.583\ \mu\text{g/min per mg})/(0.011\ \mu\text{g/mg}) = 53.0\ \text{min}^{-1}$. This corresponds to a half-life for arginyl-tRNA of $(\ln 2)/(53.0\ \text{min}^{-1}) = 0.013$ min. K_3 is so large that as y varies M^* will appear to be in a steady state relative to it. From equation 10,

$$M^* \approx \frac{K_1 y + K_2}{K_3}. \tag{45}$$

This means that M^* could be replaced by $(K_1 y + K_2)/K_3$ in equations 6 and 9 and the model would have the same behavior. Hence, provided M^* is a small number in the neighborhood of

the estimated value 0.011, the exact value need not be known. Whether we estimate that $tRNA^{arg}$ is 30% charged or 60% charged will have no detectable effect on the model's behavior.

The initial rate of formation of M^* after the cells are first transferred to minimal medium will be

$$K_1 y(0) + K_2 = 0.053 \ \mu g/min \text{ per mg}$$

since $y(0)$ is $\frac{1}{100}$ of the steady-state value in minimal medium or 0.016 units/mg.[6]

Since K_3 is so large, the concentration of arginyl-tRNA will, upon transferring the cells from arginine-rich to minimal medium, very rapidly assume the value

$$M^*(0) = \frac{0.053 \ \mu g/min \text{ per mg}}{53.0 \ min^{-1}},$$

$$= 0.001 \ \mu g/mg.$$

When the cells are fully repressed (the condition at time zero in Fig. 4), $R^* = 1$ and, from equations 6 and 9,

$$y_r^0 = \frac{e^{-(a+c)\tau(0)}}{ac(A + m)}, \tag{46}$$

where y_r is the fully repressed level of OTC. Solving for m and using $y_r = 0.016$, $\tau = \tau_0$, we obtain the rough estimate of 68,000 for m.

When the cells are grown in minimal medium, the OTC level is 1.6 units/mg as shown in Fig. 5. Hence

$$y^0 = \frac{e^{-(a+c)\tau_0}}{(A + mR^*)(a + c)c} = 1.6$$

so that $R = 0.00967$.

The parameter m measures the sensitivity of the genome to inhibition by the active repressor. Since m is so large, even rather low levels of R^* suffice to drastically reduce the rate of synthesis of OTC-specific mRNA. Accordingly, the parameter H can vary over a fairly wide range and not make much difference in the observed kinetics. For definiteness, assume $H = 0.5 \ M^*_{max}$ where M^*_{max} is the arginyl-tRNA pool size when $tRNA^{arg}$ is 100% charged. Then, $M^*_{max} = 0.365 \ \mu g/mg$ and

$$H = 0.01825 \ \mu g/mg.$$

Then, for cells in the steady state in minimal medium,

$$\frac{(M^{*0} - T_r)}{H + (M^{*0} - T_r)} = R^* = 0.00967.$$

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[6] This estimate assumes that repression by arginine reduces the OTC level by a factor of 100 from the steady-state level (Gorini and Maas, 1957). An independent determination of y_r yielded 0.044 units/mg (Coyne, unpublished results).

Taking $M^{40} = 0.0111$ μg/mg and $H = 0.01825$ μg/mg gives

$$T_r = 0.01094 \text{ μg/mg.}$$

From equation 14, the initial value of the time delay in translation i

$$\tau(0) = 0.4\left[0.917 + 0.083\,\frac{0.01094}{0.001}\right] \text{min}$$

$$= 0.73 \text{ min}$$

Accordingly,

$$x(0) = \frac{y(0)c}{e^{-(a+c)\tau(0)}} = 0.00019 \text{ units/n g}$$

and

$$m = 65{,}700.$$

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