

## GLOBAL ASYMPTOTIC STABILITY OF CERTAIN MODELS FOR PROTEIN SYNTHESIS AND REPRESSION\*

By

H. T. BANKS AND J. M. MAHAFFY

*Brown University*

**Abstract.** We consider a class of mathematical models involving nonlinear differential equations with hereditary terms. Included as special cases are a number of models that have been proposed as qualitative models for protein synthesis in eukaryotic cells. We establish global stability for these models and discuss the implications of our results.

**1. Introduction.** Our discussions and analysis in this paper deal with a class of mathematical models for the regulatory pathways involved in the synthesis of proteins. These models are based on the Jacob-Monod hypothesis [11, 15] for gene regulation in prokaryotic cells, which since it was first proposed has been the subject of a large amount of research, both theoretical and experimental. While the basic tenets of the Jacob-Monod theory for prokaryotes are now widely accepted, much less is understood about regulation of protein synthesis in eukaryotes and higher organisms [15]. This is, therefore, an area where theoretical models could play a significant role in the near future in attempting to further our understanding.

In discussing the Jacob-Monod theory, one must distinguish between two basic types of systems often referred to as *positive* and *negative feedback* systems respectively. The positive feedback or *inducible* systems involve an enzyme that is normally present in the cell only in trace amounts and whose synthesis is induced by the presence of a metabolite (e.g., the substrate for the enzyme) through positive feedback mechanisms (when the metabolite is presented to the bacterium, repression of the operon is blocked, thereby allowing structural genes for the enzyme to be transcribed). The classical example of an inducible enzyme is  $\beta$ -galactosidase in the bacterium *Escherichia coli*. Negative feedback or *repressible* systems are involved in the synthesis of enzymes in the biosynthetic pathways leading to essential metabolites such as nucleic and amino acids. Since our emphasis here will be on repressible or end-product repression systems, we shall discuss the pertinent mechanisms in some detail in a subsequent section of this paper. The biosynthesis of histidine [15, 33] (one of ten basic amino acids which man, as well as many microorganisms, can synthesize) is an example of a synthetic pathway involving end-product repression. This pathway, which has been studied extensively in the bacteria *Escherichia*

---

\* Received December 30, 1977. This research was supported by the National Science Foundation under NSF-MCS76-07247.

*coli* and *Salmonella typhimurium*, is a gene-regulated sequence of enzymatic reactions (nine structural genes code for ten enzymes involved in the pathway) which is controlled at the level of the first enzyme through end-product repression of synthesis of this enzyme and the succeeding ones in the pathway.

After a brief description of the Jacob-Monod theory for repressible systems in the next section, we shall discuss a class of mathematical models based on these principles (B. Goodwin was an early proponent of such models [6, 7] and certain of these are often referred to as "Goodwin's models") and present answers to qualitative questions related to these mathematical models. Specifically, we give rigorous arguments that, for a large class of models (involving discrete and distributed delays), the equilibrium state is globally asymptotically stable for all choices of kinetic parameters. We conclude the paper with a discussion of the interpretation of our results.

**2. The Jacob-Monod model for end-product repression in prokaryotes.** We briefly summarize the general principles underlying the so-called Jacob-Monod model for enzyme repression. For further specifics, the reader may see the more detailed developments in [15]. Proteins, and in particular enzymes, are synthesized in prokaryotic cells under regulation of a set of genes—segments of deoxyribonucleic acid (DNA)—called *operons*. Each operon contains structural genes for each of the enzymes whose synthesis it regulates, as well as an operator gene and sometimes a regulator gene. The unique sequence of amino acids in the polypeptide chains of each type of protein is specified or coded for by a sequence of nucleotide residues in DNA. However, the structural genes themselves do not serve directly as coding templates during the biosynthesis of proteins which takes place at the ribosomes. Rather the genetic message or code in the structural gene is first enzymatically *transcribed* to form a ribonucleic acid called messenger RNA (mRNA) and this mRNA acts as the coding template in the biosynthesis at the ribosomes.

The operator loci control the structural genes where mRNA's are synthesized by transcription of the genes. At the ribosomes, mRNA (in complex reactions with ribosome and aminoacyl-tRNA's) is *translated* and enzymes (i.e. polypeptide chains) are produced. These enzymes catalyze a specific (usually sequential) pathway which results in formation of a particular end-product (e.g., histidine, tryptophan, etc.). This end-product is itself involved in repression of its associated operator gene. It may combine with one the enzymes of the sequence (often the first; e.g., as in the biosynthesis of histidine) to form an active repressor complex. Other known pathways (e.g., biosynthesis of tryptophan, another amino acid) involve a regulator gene in the operon which codes for an aporepressor which plays the role of the inactive repressor, combining with the end-product to form the activated repressor complex. Transcription (of DNA), translation (of mRNA), as well, of course, as the enzyme sequences themselves, are all enzyme-regulated processes, so that any dynamic mathematical model must ultimately be based on velocity approximations for the pertinent reactions.

In Fig. 1 we present a schematic for the Jacob-Monod model described above. In this figure, we have used dashed lines (typified by tryptophan biosynthesis) and dotted lines (typified by histidine biosynthesis) to illustrate two different modifications which are known to occur in prokaryotes.

**3. Mathematical models based on the Jacob-Monod hypotheses.** There have been numerous investigations of mathematical models [1, 5–9, 13, 14, 16–19, 21, 22, 24, 26–29, 31, 32, 34] for protein synthesis and many of these have been based on the efforts of B.

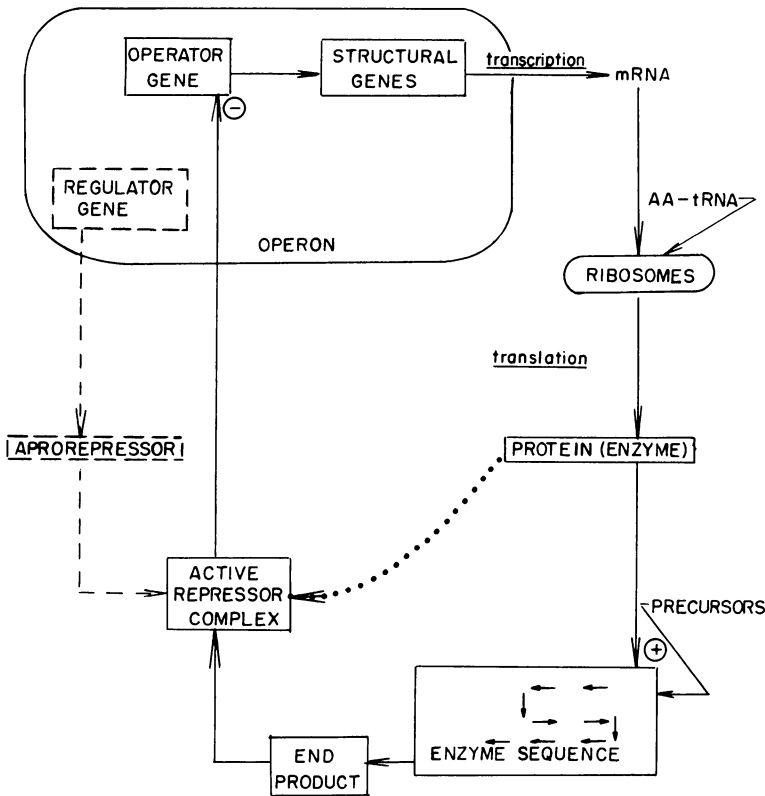


Fig. 1

Goodwin in the early 1960s. Goodwin, employing nonlinear models in an attempt to explain organizational aspects of biochemical processes in cells [6, 7], was one of the first to give serious attention to certain aspects of these models. One can find many variations of the early Goodwin models in the literature and it has become customary to speak of the “Goodwin models” or “Goodwin-type models” for protein synthesis. One such model (and perhaps the most extensively studied) which can be derived either from kinetic velocity approximations [19, 34] or from simple probabilistic notions [24] is based on the schematic in Fig. 2. This clearly can be interpreted as an approximation to the Jacob-Monod scheme outlined above.

The simple nonlinear mathematical model (the three-dimensional Goodwin ODE model [7]) consists of equations

$$\begin{aligned}
 \dot{x}_1(t) &= \frac{a}{K + kx_3(t)} - bx_1(t), \\
 \dot{x}_2(t) &= \alpha x_1(t) - \beta x_2(t), \\
 \dot{x}_3(t) &= \gamma x_2(t) - \delta x_3(t),
 \end{aligned}
 \tag{3.1}$$

where  $x_1, x_2, x_3$  are concentrations of mRNA, protein, and repressor complex respectively. (All of the models discussed in this paper—and most of those found in the literature—are for a large aggregate cell population. In prokaryotic cells, there is usually only one specific

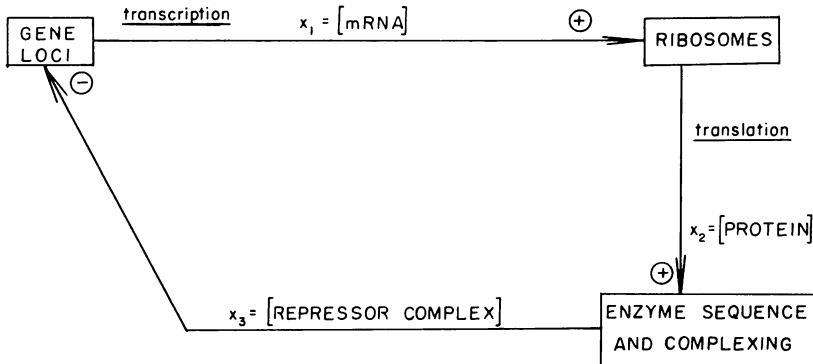


Fig. 2

operon per cell for a particular pathway and the discrete number of protein or repressor molecules for this pathway may be small. These discrete quantities in a single cell or small finite colony of cells are replaced by a continuum described by differential equations in most models in the literature. Thus, models based on concentrations of mRNA, genes, repressors, etc., should be interpreted so that the numbers and parameters involved represent “averaged” values over an aggregate.)

Often in the literature [7, 8, 9, 24] one finds a (perhaps only implicit) simplifying assumption that  $x_3 = \lambda x_2$ , so that in place of (3.1) one considers a two-dimensional version (the two-dimensional Goodwin model)

$$\begin{aligned}\dot{x}_1(t) &= \frac{a}{K + kx_2(t)} - bx_1(t), \\ \dot{x}_2(t) &= \alpha x_1(t) - \beta x_2(t)\end{aligned}\quad (3.2)$$

In the other direction, one may specify more of the intermediate variables (presumably some of those in the enzyme sequence and complexing reactions) to obtain the  $n$ -dimensional Goodwin-type model [18, 34]

$$\begin{aligned}\dot{x}_1(t) &= \frac{a}{K + kx_n(t)} - bx_1(t), \\ \dot{x}_i(t) &= \alpha_i x_{i-1}(t) - \beta_i x_i(t), \quad i = 2, 3, \dots, n.\end{aligned}\quad (3.3)$$

Finally, another often-studied modification [8, 18, 34] involves a model in which cooperative inhibition of transcription is postulated. In this case one essentially assumes that  $\rho$  repressor molecules combine to effect the repression of the operator gene and modifies the mathematical models by replacing the nonlinearity in the first equation by  $a/[K + kx_n(t)^\rho]$ . While there is evidence [30, 35] to suggest that  $\rho > 1$  is a realistic assumption for certain pathways (especially in inducible systems), experimental findings [32, 33, 35] indicate that the case  $\rho = 1$  is of paramount importance for end-product repression models for prokaryotes. We shall, therefore, in this paper restrict our attention to this latter case, with our findings for models with  $\rho > 1$  to be detailed in a future paper.

Our considerations here in actuality do not deal just with the model embodied in (3.3); rather, we investigate an extension which allows for both discrete and distributed time delays in the biosynthetic pathways. To see the importance of such models, we must turn to a discussion of some fundamental differences between prokaryotic and eukaryotic cells when one is considering the synthesis of proteins.

If one reads carefully the many papers on Goodwin-type models for protein synthesis (including Goodwin's early work), it becomes clear that almost all of the authors are treating the Goodwin models as approximations for protein synthesis in eukaryotes. (We indicated above how the Goodwin type models can properly be viewed as approximations to the well-accepted Jacob-Monod theory in prokaryotes. Unfortunately, no such well-accepted theory exists for eukaryotes.) One fundamental difference (there appear to be *significant* others [15]) between prokaryotes and eukaryotes that is important in biosynthesis is that in eukaryotes the DNA (in the nucleus) is physically separated from the ribosomes (found in the cytoplasm where some adhere to the endoplasmic reticulum) so that translation does not begin until some time after transcription is completed and the resulting mRNA is transported across the nuclear membrane and to the ribosomes. Furthermore, the repressor complex molecules involve an end-product—produced in the cytoplasm—which must be transported to the DNA in the nucleus before transcription can be repressed (all of this, of course, is assuming that the basic tenets involving transcription, translation, repression, etc. of the Jacob-Monod theory can be extrapolated to hold for eukaryotes). Experimental evidence [27] does support the idea (suggested by Goodwin in [6] and many others subsequently) that there are substantial time delays between transcription and translation and perhaps inhibition (repression) in eukaryotes. This may be due to a number of phenomena, including diffusion, packaging and transport across membranes, etc. Some authors [1, 26, 27, 32] have used partial differential equation models to investigate this aspect of protein synthesis in eukaryotes while others [5, 9, 16, 17, 24, 28, 29] have tried to modify the models of Goodwin to include some type of time delay directly. Our interest in this paper is in the latter alternative.

In any case, if one takes the viewpoint (as do Goodwin and a number of authors [26, 27, 32]) that the essential difference between protein synthesis in eukaryotes and that in prokaryotes is related to spatial localization due to the nuclear membrane, then it is quite natural to investigate the qualitative behavior of Goodwin-type models which have been extended or modified to account for the delays between transcription, translation, and repression. In the literature one unfortunately finds misconceptions of how one should extend the Goodwin models to include time delays (this, in our opinion, does not involve merely adding more intermediate variables with dynamics simultaneously coupled to the original Goodwin model variables). The most direct way to account for delays (and that employed by a number of investigators of extended Goodwin-type models) involves model equations with discrete delays of the form:

$$\begin{aligned}\dot{x}_1(t) &= \frac{a}{K + kx_n(t - \tau_n)} - bx_1(t), \\ \dot{x}_j(t) &= \alpha_j x_{j-1}(t - \tau_{j-1}) - \beta_j x_j(t), \quad j = 2, 3, \dots, n.\end{aligned}\tag{3.4}$$

However, since there is evidence that distributed delays are more appropriate approximations to reality (this can be argued based on results of fitting other types of delay models to experimental data for enzyme-regulated processes in microorganisms [3] and/or from modeling principles that assert that the discrete delays in (3.4) are really approximations to distributed delays that must be present in any "averaged" or aggregate model), one really should perhaps consider models

$$\begin{aligned}\dot{x}_1(t) &= a \left/ \left( K + k \int_{-\tau}^0 x_n(t + \theta) \zeta_n(\theta) d\theta \right) \right. - bx_1(t), \\ \dot{x}_j(t) &= \alpha_j \int_{-\tau}^0 x_{j-1}(t + \theta) \zeta_{j-1}(\theta) d\theta - \beta_j x_j(t), \quad j = 2, 3, \dots, n.\end{aligned}\tag{3.5}$$

Various special cases of (3.4) or (3.5) have been partially analyzed in the literature [5, 9, 16, 17, 24, 28, 29] (as Goodwin [6, p. 8] correctly observes, rigorous analysis of delay equations poses nontrivial difficulties; it is not surprising that much of the analysis of delay system protein models has involved linearization, approximation, numerical simulation, or a combination of these). In view of a number of incorrect conclusions and claims based on non-rigorous analysis and/or computer simulations of other protein synthesis models (e.g., some authors carry out analysis of a linearized system, obtain local stability results and incorrectly conclude that this rules out oscillations; for remarks on other erroneous claims see [31]), it is exceedingly important in trying to argue validity or lack thereof of these models that one have completely rigorous discussions to support any claims for a certain qualitative behavior that a particular model might or might not possess. We offer below rigorous analysis of a class of models that include as special cases the models represented by (3.3), (3.4) or (3.5).

The model we analyze, in detail, in a subsequent section is given by

$$\begin{aligned} \dot{x}_1(t) &= a \left/ \left( K + k \int_{-r}^0 x_n(t + \theta) d\mu_n(\theta) \right) - bx_1(t) \right. \\ \dot{x}_j(t) &= \alpha_j \int_{-r}^0 x_{j-1}(t + \theta) d\mu_{j-1}(\theta) - \beta_j x_j(t), \quad j = 2, 3, \dots, n, \end{aligned} \quad (3.6)$$

for  $t > 0$ , with initial data  $x_j(\theta) = \phi_j(\theta)$ ,  $-r \leq \theta \leq 0$ , where the  $\phi_j$  are given nonnegative functions. The measures  $\mu_i$  each consist of a finite number of saltus functions plus an absolutely continuous part. That is, there are finite constants  $c_{ij}, r_k, i = 1, \dots, n, j = 0, 1, \dots, \nu, k = 0, 1, \dots, \nu$ , and scalar functions  $\zeta_i \in L_2(-r, 0), i = 1, \dots, n$ , such that

$$\int_{-r}^0 \psi(\theta) d\mu_i(\theta) = \sum_{j=0}^{\nu} c_{ij} \psi(-r_j) + \int_{-r}^0 \psi(\theta) \zeta_i(\theta) d\theta \quad (3.7)$$

where  $0 = r_0 < r_1 < r_2 < \dots < r_\nu = r$ . Our analysis will reveal that (3.6) has a unique equilibrium which is globally asymptotically stable in the sense that any solution corresponding to non-negative initial functions will remain non-negative (component-wise) for all  $t > 0$  and will approach the equilibrium solution as  $t \rightarrow \infty$ . Before turning to our detailed analysis of (3.6), we indicate briefly the motivation behind our efforts.

A fundamental question that has occupied a central role in many investigations of protein synthesis models is that of whether sustained oscillations (i.e., periodic solutions) can exist or not. The inspiration for this query appears to be the early efforts of Goodwin [6] who sought to explain "fundamental periodicities occurring in dynamic organization of cellular processes". A review of the experimental and theoretical literature [10] reveals at least two (perhaps associated) types of oscillations that many investigators believe are intimately related to biosynthesis of proteins. First, there is some evidence [10, 13, 14, 19, 23, 32] that many enzymes are synthesized periodically rather than continuously within the cell. This produces oscillations (epigenetic oscillations) in concentration levels of enzymes, corresponding mRNA, repressor complex molecules, etc. Models for synthesis of proteins should reflect (at least qualitatively) features of these gene regulation-related oscillations.

A second oscillatory phenomenon, circadian rhythms, has been associated with protein synthesis in that many investigators feel that end-product repression of ribosomal activity may be of primary importance in producing these rhythms. While circadian rhythms as studied in [12, 20, 25] and elsewhere may well be related to epigenetic

oscillations, one must exercise some care in speaking of these as if they were the same. The two phenomena appear distinct in that circadian rhythms have been found only in eukaryotes [25, 27] while evidence of epigenetic oscillations has been claimed for both prokaryotic and eukaryotic cells [10, 13, 14].

Whatever the case may be, almost all of the literature on mathematical models for protein synthesis involves consideration of the possibility of sustained oscillations. Our analysis shows unequivocally that any mathematical model which is a special case of (3.6) cannot possess periodic solutions (of small or large amplitude).

**4. Stability results.** We turn now to a careful analysis of the system (3.6), our results being summarized in the following theorem. Our assumption throughout is that  $a, b, K, k, \alpha_j, \beta_j$  are positive and finite, but otherwise unrestricted.

**THEOREM.** The system (3.6) has a unique equilibrium solution in the positive orthant  $P = \{x \in \mathbb{R}^n \mid x_i \geq 0\}$ . Corresponding to any continuous initial data  $\phi$  with  $\phi_i(\theta) \geq 0, -r \leq \theta \leq 0, i = 1, 2, \dots, n$ , a unique solution of (3.6) exists for all  $t > 0$  where its trajectory remains in the positive orthant  $P$  and as  $t \rightarrow \infty$ , the solution approaches the equilibrium solution.

Since each of the measures  $\mu_i$  in (3.6) are of finite total variation on  $[-r, 0]$  we may, without loss of generality, assume that each is a probability measure. That is, denoting by  $L_i(\psi)$  the quantity defined in (3.7), we assume  $L_i(1) = 1$  for each  $i$ . Furthermore, our application already involves the implicit assumption that  $\psi^1 \geq \psi^2 \Rightarrow L_i(\psi^1) \geq L_i(\psi^2)$ . Further rescaling of the nonlinear term in (3.6) leads to equivalent equations

$$\begin{aligned} \dot{x}_1(t) &= \frac{\tilde{a}}{1 + \tilde{k}L_n(x_n^t)} - bx_1(t), \\ \dot{x}_j(t) &= \alpha_j L_{j-1}(x_{j-1}^t) - \beta_j x_j(t), \end{aligned}$$

where  $\tilde{a} = a/K, \tilde{k} = k/K$  and we have adopted the notation  $x^t$  for the function  $\theta \rightarrow x(t + \theta)$  on  $[-r, 0]$ . In the analysis below we shall, for convenience in exposition, drop the tildes on the parameters.

The existence statement (and positivity of solutions) of the theorem follows in the case of discrete lags alone (e.g. (3.4)) using the method of steps [4]. For the more general case of (3.6) the ideas again are standard as one can construct Picard iterates in the positive cone of continuous functions  $C([-r, 0], P)$  to obtain existence, uniqueness, and positivity of solutions. Details of these arguments may be found in [2] and will not be given again here since they involve only rather classical ideas along with the technical details necessary to carry them out.

We shall here concentrate on the statements regarding behavior of solutions as  $t \rightarrow \infty$ . We present detailed arguments for the case  $n = 2$ . This restriction is *only* for ease in exposition and notation. All of the arguments given below can be trivially extended to the case for general  $n$ . Absolutely no new ideas are involved, but the notation and details are not particularly pleasant (see [2]).

We are, therefore, considering the system

$$\begin{aligned} \dot{x}_1(t) &= \frac{a}{1 + kL_2(x_2^t)} - bx_1(t), \\ \dot{x}_2(t) &= \alpha L_1(x_1^t) - \beta x_2(t), \quad t \geq 0. \end{aligned} \tag{4.1}$$

Equilibrium solutions will be constants  $\bar{x}_1, \bar{x}_2$  which (since  $L_i(\bar{x}_i) = \bar{x}_i L_i(1) = \bar{x}_i$ ) must satisfy

$$a/(1 + k\bar{x}_2) = b\bar{x}_1, \quad \alpha\bar{x}_1 = \beta\bar{x}_2$$

or

$$kb\alpha\bar{x}_1^2 + \beta b\bar{x}_1 - \beta a = 0, \quad \bar{x}_2 = \frac{\alpha}{\beta} \bar{x}_1. \quad (4.2)$$

The one (the only positive) solution of interest to us is

$$\begin{aligned} \bar{x}_1 &= \frac{-\beta b + (\beta^2 b^2 + 4kb\alpha\beta a)^{1/2}}{2kb\alpha} \\ &= \frac{\beta b \{(1 + 4k\alpha a/\beta b)^{1/2} - 1\}}{2kb\alpha} \\ &= \frac{\beta}{2k\alpha} \{(1 + 4k\alpha a/\beta b)^{1/2} - 1\} \end{aligned} \quad (4.3)$$

with

$$\bar{x}_2 = \frac{\alpha}{\beta} \bar{x}_1 = \frac{1}{2k} \{(1 + 4k\alpha a/\beta b)^{1/2} - 1\}. \quad (4.4)$$

To complete the arguments regarding behavior of a given solution as  $t \rightarrow \infty$ , our method of argument is as follows: We shall construct monotonic sequences of numbers  $\{U_i^m\}_{m=1}^\infty, \{V_i^m\}_{m=1}^\infty, i = 1, 2$ , so that  $U_i^m \nearrow \bar{x}_i, V_i^m \searrow \bar{x}_i, i = 1, 2$ , as  $m \rightarrow \infty$ . We show that these sequences are such that for any fixed  $m_0$  there exists  $T_{m_0}$  such that the given solution satisfies

$$U_i^{m_0} \leq x_i(t) \leq V_i^{m_0} \quad \text{for all } t \geq T_{m_0}, \quad i = 1, 2. \quad (4.5)$$

This will establish the desired behavior for the solution.

The fundamental (and essentially only) tools we employ in these arguments are simple differential inequalities: if  $\dot{\omega}(t) \leq A - B\omega(t)$  for  $t \geq \tau$ , then  $\omega(t) \leq W(t)$  for  $t \geq \tau$  where  $W$  is the solution of  $\dot{W}(t) = A - BW(t), W(\tau) = \omega(\tau)$ . Also,  $\dot{\omega}(t) \geq A - B\omega(t)$  for  $t \geq \tau$  implies  $\omega(t) \geq W(t)$  for  $t \geq \tau$  where  $W$  is as just defined. We shall use these again and again along with the elementary result that solutions of  $\dot{W}(t) = A - BW(t), W(\tau) = W_0$  are given by  $W(t) = (A/B) + \{W_0 - (A/B)\} \exp(-B\{t - \tau\})$ .

To begin our arguments, we consider a fixed solution (denoted by  $x_1, x_2$  throughout) of (4.1) corresponding to fixed nonnegative initial functions. According to our previous comments, the solution will then exist for all  $t \geq 0$  where it will remain nonnegative. In our selection of the monotone sequence  $\{\epsilon_m\}$  below, we make the choice satisfy  $0 < \epsilon_m < 1/m$  at each step so that  $\epsilon_m \rightarrow 0$ .

Let  $v_1^1$  be the solution of  $\dot{z} = a - bz, z(0) = x_1(0)$ . Then since  $L_2(x_2^t) \geq 0$  for all  $t$ , we find that  $\dot{x}_1(t) \leq a - bx_1(t)$  for  $t > 0$ . Thus, our differential inequality implies

$$x_1(t) \leq v_1^1(t) = \frac{a}{b} + \left\{ x_1(0) - \frac{a}{b} \right\} \exp(-bt),$$

or, choosing  $0 < \epsilon_1 < 1$ , there exists  $t_1 > 0$  such that

$$x_1(t) \leq V_1^1 \equiv \frac{a}{b} + \epsilon_1 \quad \text{for } t \geq t_1.$$



Next we let  $v_2^1$  be the solution for  $t > t_1 + r$  of  $\dot{z} = \alpha V_1^1 - \beta z$ ,  $z(t_1 + r) = x_2(t_1 + r)$ ; hence

$$v_2^1(t) = \frac{\alpha}{\beta} V_1^1 + \left\{ x_2(t_1 + r) - \frac{\alpha}{\beta} V_1^1 \right\} \exp(-\beta\{t - (t_1 + r)\}),$$

and choosing  $\epsilon_2 < \epsilon_1$ , there is a  $t_2$  such that

$$v_2^1(t) \leq \frac{\alpha}{\beta} V_1^1 + \epsilon_2 \equiv V_2^1 \quad \text{for } t \geq t_2 > t_1 + r.$$

Since  $x_1(t + \theta) \leq V_1^1$  for  $t + \theta \geq t_1$  we find that  $L_1(x_1^t) \leq V_1^1$  for  $t \geq t_1 + r$  so that

$$\dot{x}_2(t) \leq \alpha V_1^1 - \beta x_2(t) \quad \text{for } t \geq t_1 + r.$$

It thus follows that

$$x_2(t) \leq V_2^1 \quad \text{for } t \geq t_2 > t_1 + r.$$

We define  $u_1^1$  to be the solution of

$$\dot{z}(t) = \frac{a}{1 + kV_2^1} - bz(t), \quad t \geq t_2 + r, \quad z(t_2 + r) = x_1(t_2 + r)$$

so that

$$\begin{aligned} u_1^1(t) &= \frac{a}{b[1 + kV_2^1]} + \left\{ x_1(t_2 + r) - \frac{a}{b[1 + kV_2^1]} \right\} \exp(-b\{t - (t_2 + r)\}) \\ &\geq U_1^1 \equiv \frac{a}{b[1 + kV_2^1]} - \epsilon_3 \quad \text{for } t \geq t_3 > t_2 + r \end{aligned}$$

for some  $t_3 > t_2 + r$ , where  $\epsilon_3$  has been chosen so that  $\epsilon_3 < \epsilon_2$  and  $U_1^1 > 0$ . Since  $x_2(t + \theta) \leq V_2^1$  for  $t + \theta \geq t_2$  we have  $1 + kL_2(x_2^t) \leq 1 + kV_2^1$  for  $t \geq t_2 + r$  so that  $\dot{x}_1(t) \geq a/(1 + kV_2^1) - bx_1(t)$  for  $t \geq t_2 + r$ . Thus,  $x_1(t) \geq u_1^1(t)$  for  $t \geq t_2 + r$  and

$$x_1(t) \geq U_1^1 \quad \text{for } t \geq t_3 > t_2 + r.$$

Now let  $u_2^1$  be the solution for  $t > t_3 + r$  of  $\dot{z} = \alpha U_1^1 - \beta z$ ,  $z(t_3 + r) = x_2(t_3 + r)$  so that for  $\epsilon_4$  chosen with  $\epsilon_4 < \epsilon_3$  and  $(\alpha/\beta) U_1^1 - \epsilon_4 > 0$ ,

$$\begin{aligned} u_2^1(t) &= \frac{\alpha}{\beta} U_1^1 + \{x_2(t_3 + r) - \frac{\alpha}{\beta} U_1^1\} \exp(-\beta\{t - (t_3 + r)\}) \\ &\geq U_2^1 \equiv \frac{\alpha}{\beta} U_1^1 - \epsilon_4, \quad t \geq t_4 > t_3 + r \end{aligned}$$

for some  $t_4$  chosen sufficiently large. The inequality  $x_1(t + \theta) \geq U_1^1$  for  $t + \theta \geq t_3$  yields  $\dot{x}_2(t) \geq \alpha U_1^1 - \beta x_2(t)$  for  $t \geq t_3 + r$ , so that we obtain

$$x_2(t) \geq U_2^1 \quad \text{for } t \geq t_4 > t_3 + r.$$

We next let  $v_1^2$  be the solution of

$$\dot{z}(t) = \frac{a}{b[1 + kU_2^1]} - bz(t), \quad t \geq t_4 + r, \quad z(t_4 + r) = x_1(t_4 + r).$$

It follows in the usual manner that there exists  $t_5 > t_4 + r$  so that  $v_1^2(t) \leq V_1^2 \equiv a/(b[1 + kU_2^1]) + \epsilon_5$  for  $t \geq t_5$ , where  $\epsilon_5 < \epsilon_4$  has been chosen. Also, since  $x_2(t + \theta) \geq U_2^1$  for  $t + \theta$

$\geq t_4$ , we find  $1 + kL_2(x_2^t) \geq 1 + kU_2^1$  for  $t \geq t_4 + r$ . We thus conclude that

$$\dot{x}_1(t) \leq \frac{a}{b[1 + kU_2^1]} - bx_1(t), \quad t \geq t_4 + r$$

and hence

$$x_1(t) \leq V_1^2 \quad \text{for } t \geq t_5 > t_4 + r.$$

Continuing in this way one argues without difficulty that there exist  $\epsilon_6, \epsilon_7, \dots$ , and  $t_6, t_7, \dots$  such that

$$\begin{aligned} x_2(t) &\leq V_2^2 \equiv \frac{\alpha}{\beta} V_1^2 + \epsilon_6 && \text{for } t \geq t_6 > t_5 + r, \\ x_1(t) &\geq U_1^2 \equiv \frac{a}{b[1 + kV_2^2]} - \epsilon_7 > 0 && \text{for } t \geq t_7 > t_6 + r, \\ x_2(t) &\geq U_2^2 \equiv \frac{\alpha}{\beta} U_1^2 - \epsilon_8 > 0 && \text{for } t \geq t_8 > t_7 + r, \\ x_1(t) &\leq V_1^3 \equiv \frac{a}{b[1 + kU_2^2]} + \epsilon_9 && \text{for } t \geq t_9 > t_8 + r, \end{aligned} \tag{4.6}$$

etc. One thus obtains sequences  $\{V_i^m\}_{m=1}^\infty, \{U_i^m\}_{m=1}^\infty, i = 1, 2, \{t_j\}$  such that the  $V_i^m, U_i^m$  are positive,  $t_j < t_{j+1}$ , and

$$\begin{aligned} x_1(t) &\leq V_1^m \quad \text{for } t \geq t_{4m-3}, \\ x_2(t) &\leq V_2^m \quad \text{for } t \geq t_{4m-2}, \\ x_1(t) &\geq U_1^m \quad \text{for } t \geq t_{4m-1}, \\ x_2(t) &\geq U_2^m \quad \text{for } t \geq t_{4m}, \end{aligned} \tag{4.7}$$

for  $m = 1, 2, \dots$ . If one defines  $U_2^0 \equiv 0$ , the  $U$ s and  $V$ s are defined by the recurrence formulae

$$\begin{aligned} V_1^m &\equiv \frac{a}{b[1 + kU_2^{m-1}]} + \epsilon_{4m-3}, & V_2^m &\equiv \frac{\alpha}{\beta} V_1^m + \epsilon_{4m-2}, \\ U_1^m &\equiv \frac{a}{b[1 + kV_2^m]} - \epsilon_{4m-1}, & U_2^m &\equiv \frac{\alpha}{\beta} U_1^m - \epsilon_{4m}. \end{aligned} \tag{4.8}$$

It follows immediately from (4.7) and our constructive arguments that (4.5) obtains. To complete our arguments we need only demonstrate that  $V_i^m \searrow \bar{x}_i, U_i^m \nearrow \bar{x}_i, i = 1, 2$ . First, using the relationships (4.8) along with the monotonicity of  $\{\epsilon_j\}$ , an easy inductive argument shows that  $V_i^{m+1} < V_i^m, U_i^{m+1}, U_i^m, i = 1, 2, m = 1, 2, \dots$ . We also obtain boundedness for the monotone sequences  $U_i^m, V_i^m$ , whence

$$\bar{V}_i = \lim_{m \rightarrow \infty} V_i^m, \quad \bar{U}_i = \lim_{m \rightarrow \infty} U_i^m \tag{4.9}$$

exist for  $i = 1, 2$  and the limits must be finite and nonnegative. Taking the limit in (4.8) we see that the limit point must satisfy

$$\begin{aligned} \bar{V}_1 &= \frac{a}{b[1 + k\bar{U}_2]}, & \bar{V}_2 &= \frac{\alpha}{\beta} \bar{V}_1, \\ \bar{U}_1 &= \frac{a}{b[1 + k\bar{V}_2]}, & \bar{U}_2 &= \frac{\alpha}{\beta} \bar{U}_1. \end{aligned} \tag{4.10}$$

Simple algebraic arguments using (4.10) reveal that

$$\bar{V}_1 = \frac{a}{b[1 + k\bar{U}_2]} = \frac{a}{b\left[1 + k\frac{\alpha}{\beta}\bar{U}_1\right]} = \frac{a}{b\left\{1 + k\frac{\alpha a}{\beta b\left[1 + k\frac{\alpha}{\beta}\bar{V}_1\right]}\right\}}$$

or

$$\bar{V}_1\left\{\beta b\left[1 + k\frac{\alpha}{\beta}\bar{V}_1\right] + k\alpha a\right\} = a\beta\left[1 + k\frac{\alpha}{\beta}\bar{V}_1\right],$$

which can be simplified to yield

$$k\alpha b\bar{V}_1^2 + \beta b\bar{V}_1 - \beta a = 0. \tag{4.11}$$

Recalling (4.2), we see that  $\bar{V}_1$  must agree with  $\bar{x}_1$ , the only nonnegative solution of the quadratic equation (4.11), and that  $\bar{V}_2 = \alpha/\beta \bar{V}_1$  is the same as  $\bar{x}_2$ . The symmetry in (4.10) between the  $U$ s and  $V$ s allows one to argue in a like manner that  $\bar{U}_1 = \bar{x}_1$  and  $\bar{U}_2 = \bar{x}_2$ .

We thus obtain  $\bar{x}_i = \lim_{m \rightarrow \infty} V_i^m = \lim_{m \rightarrow \infty} U_i^m$  and the arguments to establish our claims are complete.

**5. Concluding remarks.** Early studies of Goodwin-type models for protein synthesis in eukaryotes involved use of ordinary differential equation models in which investigators sought to verify that these models describe adequately oscillatory phenomena that have been observed in living organisms. Early claims and incorrect conjectures based on partial analysis and/or computer simulations were eventually subsumed by more careful analyses which showed that many of these model systems could not possess periodic solutions for parameter values in a biologically meaningful range. A number of authors [12, 19, 20, 22, 24, 26, 27, 32], aware of features of eukaryotic cell physiology (e.g. transport across membranes as a possible etiological factor for oscillatory phenomena in cells [10, 25]) which might produce oscillation-sustaining delays in the biosynthetic pathways, have noted that perhaps the destabilizing effect of the introduction of delay terms in an ordinary differential equation model might be necessary to obtain approximations to reality that exhibit oscillatory behavior. (Goodwin [6, p. 8] himself suggested this in his early discussions.)

Our results in this paper show rather conclusively that for at least one class of models, the introduction of delays—in however sophisticated (discrete or distributed) a manner one attempts this—in a simple extrapolation of the Goodwin ordinary differential equation models will not produce a system with oscillations or even instabilities.

If one is studying the Goodwin models as an approximation for the Jacob-Monod theory in prokaryotes (it is not so easy to see the rationale for the introduction of delays in this case), experimental evidence for epigenetic oscillations is not unequivocal and one may or may not expect oscillatory behavior in repressible systems. (In asynchronously grown cell cultures only damped oscillations [13, 14] were observed, and these for an

inducible system, but conjectures favor the presence of sustained oscillations in synchronized populations.) For the modeling of eukaryotes our results, coupled with those of Thames, Elster and Aronson [1, 26, 27], suggest that simple extrapolation of the Jacob-Monod theory for repressible systems described by ordinary differential equation models to models which include delay or diffusion terms is not adequate to shed significant light on the basic mechanisms or phenomena involved. It remains to be seen whether a model employing diffusion terms *and* delays (partial differential equations with delays) might be of some usefulness in explaining eukaryotic protein synthesis as a straightforward extension of the Jacob-Monod theory. In view of the complexity of eukaryotic biosynthesis as currently hypothesized by many authors [15], our conjecture is that such an approach may not prove particularly fruitful.

*Note added in proof.* After this paper had been accepted for publication, the authors received a preprint of a paper by D. J. Allwright (*A global stability criterion for simple control loops*, J. Math. Biol., to appear) which also contains results for the discrete delay model (3.4) above. While Allwright's arguments differ from ours, his conclusions on stability for these models are the same.

## REFERENCES

- [1] D. G. Aronson and H. D. Thames, *Stability and oscillation in biochemical systems with localized enzymes*, J. Theor. Biol., to appear.
- [2] H. T. Banks and J. M. Mahaffy, *Mathematical models for protein biosynthesis*, LCDS Technical Report 77-2, Division of Applied Mathematics, Brown University, December, 1977
- [3] J. Caperon, *Time lag in population growth response of Isochrysis Galbana to a variable nitrate environment*, Ecology **50**, 188-192 (1969)
- [4] L. E. El'sgol'ts, *Introduction to the theory of differential equations with deviating arguments*, Holden-Day, Inc., San Francisco, 1966
- [5] A. Fraser and J. Tiwari, *Genetical feedback repression: II. cyclic genetic systems*, J. Theor. Biol. **47**, 397-412 (1974)
- [6] B. C. Goodwin, *Temporal organization in cells*, Academic Press, New York, 1963
- [7] B. C. Goodwin, *Oscillatory behavior in enzymatic control processes*, Adv. Enzyme Reg. **3**, 425-439 (1965)
- [8] J. S. Griffith, *Mathematics of cellular control processes*, I, II, J. Theor. Biol. **20**, 202-208, 209-216 (1968)
- [9] Z. Grossman and I. Gumowski, *Self-sustained oscillations in the Jacob-Monod mode of gene regulation*, 7th IFIP Conf. on Optimization Techniques, September, 1975, Nice, France
- [10] B. Hess and A. Boiteux, *Oscillatory phenomena in biochemistry*, Ann. Rev. Biochemistry **40**, 237-258 (1971)
- [11] F. Jacob and J. Monod, *On the regulation of gene activity*, Cold Spring Harbor Symp. Quant. Biol. **26**, 193-211, 389-401 (1961)
- [12] A. Johnsson and H. G. Karlsson, *A feedback model for biological rhythms: I. Mathematical description and basic properties of the model*, J. Theor. Biol. **36**, 153-174 (1972)
- [13] W. A. Knorre, *Oscillations of the rate of synthesis of  $\beta$ -galactosidase in E. Coli ML 30 and ML 308*, Biochem. Biophys. Res. Comm. **31**, 812-817 (1968)
- [14] W. A. Knorre, *Oscillations in the epigenetic system: biophysical model of the  $\beta$ -galactosidase control system*, in *Biological and biochemical oscillators* (B. Chance et al., eds.), Academic Press, New York, 1973, 449-457
- [15] A. L. Lehninger, *Biochemistry*, 2nd ed., Worth Publishers, New York, 1975
- [16] N. MacDonald, *Bifurcation theory applied to a simple model of a biochemical oscillator*, J. Theor. Biol. **65**, 727-734.
- [17] N. MacDonald, *Time lag in a model of a biochemical reaction sequence with end-product inhibition*, J. Theor. Biol. **67**, 549-556 (1977)
- [18] M. Morales and D. McKay, *Biochemical oscillations in "controlled" systems*, Biophys. J. **7**, 621-625 (1967)
- [19] H. G. Othmer, *The qualitative dynamics of a class of biochemical control circuits*, J. Math. Biol. **3**, 53-78 (1976)
- [20] T. Pavlidis, *Populations of biochemical oscillators as circadian clocks*, J. Theor. Biol. **33**, 319-338 (1971)

- [21] P. E. Rapp, *Biochemical oscillators—a search procedure*, *Math. Biosci.* **23**, 289–303 (1975)
- [22] P. E. Rapp, *A theoretical investigation of a large class of biochemical oscillators*, *Math. Biosci.* **25**, 165–188 (1975)
- [23] B. Sikyta and J. Slezák, *A periodic phenomenon in regulation of pyruvate biosynthesis in E. Coli B*, *Biochem. Biophys. Acta* **100**, 311–313 (1965)
- [24] J. M. Smith, *Mathematical ideas in biology*, Cambridge University Press, 1968
- [25] B. M. Sweeney, *A physiological model for circadian rhythms derived from the Acetabularia rhythm paradoxes*, *Intl. J. Chronobiology* **2**, 25–33 (1974)
- [26] H. D. Thames and D. Aronson, *Oscillation in a non-linear parabolic model of separated, cooperatively coupled enzymes*, in *Nonlinear systems and applications* (V. Lakshmikantham, ed.), Academic Press, New York, 1977, 687–693
- [27] H. D. Thames and A. D. Elster, *Equilibrium states and oscillations for localized two-enzyme kinetics: a model for circadian rhythms*, *J. Theor. Biol.* **59**, 415–427 (1976)
- [28] J. Tiwari and A. Fraser, *Genetic regulation by feedback repression*, *J. Theor. Biol.* **39**, 679–681 (1973)
- [29] J. Tiwari, A. Fraser, and R. Beckman, *Genetical feedback repression: I. Single locus models*, *J. Theor. Biol.* **45**, 311–326 (1976)
- [30] A. Travers, *Bacterial transcription*, in *MTP international review of science: biochemistry of nucleic acids* (K. Burton, ed.), Butterworth and Co., London, Vol. 6, 1974, 191–218
- [31] J. J. Tyson, *On the existence of oscillatory solutions in negative feedback cellular control processes*, *J. Math. Biol.* **1**, 311–315 (1975)
- [32] J. J. Tyson and H. G. Othmer, *The dynamics of feedback control circuits in biochemical pathways*, 1977, submitted to *Prog. in Theor. Biol.*
- [33] H. E. Umbarger, *Regulation of amino acid biosynthesis in microorganisms*, in *MTP international review of science: synthesis of amino acids and proteins* (H.R.V. Arnstein, ed.), Butterworth and Company, London, Vol. 7, 1975, 1–56
- [34] C. F. Walter, *Kinetic and thermodynamic aspects of biological and biochemical control mechanisms*, in *Biochemical regulatory mechanisms in eukaryotic cells* (E. Kun and S. Grisolia, eds.) Wiley, New York, 1972, 355–489
- [35] G. Yagil and E. Yagil, *On the relation between effector concentration and the rate of induced enzyme synthesis*, *Biophysical J.* **11**, 11–27 (1971).