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Model for the epigenetic mechanism during embryogenesis: a study of oscillations in a multiple-loop biochemical control network

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A model is constructed for transcription and translation during embryonic development, based on the principle of multiple-loop feedback inhibition in which the end product can inhibit some of the intermediate reactions. The dimension of the system is reduced by applying the singular perturbation technique. By explicit construction of a Lyapunov function for the reduced system, it is shown that the system is globally stable. Using the Hopf bifurcation theorem and the Nyquist diagram, it is also shown that for some range of values in the parametric space the system has a limit cycle oscillatory solution around the unstable local equilibrium. The stability of the limit cycle is then studied analytically using the fundamental Brujno theorem.

1. Introduction

Oscillations in biological control systems are well-recognized phenomenon which have drawn increasing interest, both in the experimental and the mathematical fields of investigation. Examples of periodic variations in physiological and biochemical systems are numerous (see Brahmachary 1967, Bunning 1973). Heart beats, mitosis, cell divisions, and menstrual cycles are some of the obvious examples of biological oscillations. Blood cell formations (Glass and Mackey 1979), synthesis of cyclic AMP (Brooker 1973, 1975, Shaffer 1975, Roos *et al.* 1977), glycolytic mechanisms (Ghosh and Chance 1964, Hess and Boiteux 1971, Pye 1973, Boiteux and Hess 1973, Gerisch and Wick 1975), variations of leukocyte count in leukemia (Kennedy 1970, Chikkappa *et al.* 1976) and of reticulocyte count in dogs (Morley and Stohlman 1969, Morley *et al.* 1970), membrane potential of neurone in aplysia (Junge and Stephens 1973, Eckert and Lux 1976) and many enzyme catalysed reactions, are some of the examples of periodic physiological and biochemical processes. Mathematical analyses of such periodic processes have been published by Chance *et al.* (1967), Walter (1970), Hess and Boiteux (1971), Goldbeter and Lefever (1972), Cooke and Goodwin (1972), Higgins *et al.* (1973), Boiteux *et al.* (1975), Hess *et al.* (1975), Hess (1976), Hammes and Rodbell (1976), Heiden (1976), Othmer (1976), Sell (1976), Cronin (1977 a, 1977 b), Goldbeter and Segel (1977), Heinrich *et al.* (1977), Murray (1977), Mees and Rapp (1978), Glass and Mackey (1979), Rapp (1979) and many others. A vast literature and elegant discussions on all these biological and biochemical oscillations can be found in Chance *et al.* (1973), Pavlidis (1974), Murray (1977) and Rapp (1979).

This paper deals with the epigenetic control network at a sub-cellular level. A realistic mathematical model has been constructed on the basis of the recognized control mechanism for transcription and translation during embryogenesis, and oscillations have been studied. Transcription and translation are oscillatory processes that have been experimentally observed by Mano (1960), Mazia (1961), Cummins and Rusch (1968), Brahmachary *et al.* (1971), Chance *et al.* (1973), Brodsky (1975) and

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others. Mathematical analysis of the oscillatory epigenetic control system can be performed by constructing suitable non-linear mathematical models containing single or multiple loops based on the Jacob–Monod operon concept of gene regulation (Jacob and Monod 1961). Studies of oscillations in single-loop negative feedback biochemical control networks have been made by several researchers, such as Goodwin (1963, 1965), Willems (1966), Griffith (1968), Knorre (1968), Walter (1969), Viniegra-Gonzalez (1973), Walter (1970), Knorre (1973), Lighthill and Mees (1973), Tyson (1973), Chowdhury and Atherton (1974), Hunding (1974), Poore (1975), Tyson (1975), Heiden (1976), MacDonald (1976 a, 1976 b), Othmer (1976), Allwright (1977 a, b), Cronin (1977 a, b), Hastings *et al.* (1977), Murray (1977), Rapp (1979), Tapaswi and Saha (1986), Tapaswi *et al.* (1987) and others. An extension to multiple-loop systems has been published by Mees and Rapp (1978) and Tapaswi and Bhattacharya (1981). In this work we have constructed a multi-loop negative feedback control network model which represents the entire epigenetic mechanism, from gene activation to protein synthesis followed by end-product feedback control which regulates the genes, transcribing different size classes of RNA that take part in the template formations and protein synthesis.

2. Mathematical model

The model is based on the following well-known principles of transcription and translation:

(1) each of the *r*RNA, *m*RNA and *t*RNA syntheses is proportional to the activation of its respective gene, and inversely proportional to the concentration of the repressor (end-product negative feedback);

(2) formation of polysomes is directly proportional to the available amount of *r*RNA and *m*RNA;

(3) protein synthesis is directly proportional to the available amount of *t*RNA and polysomes;

(4) the synthesis of the repressor molecules is directly proportional to the amount of protein;

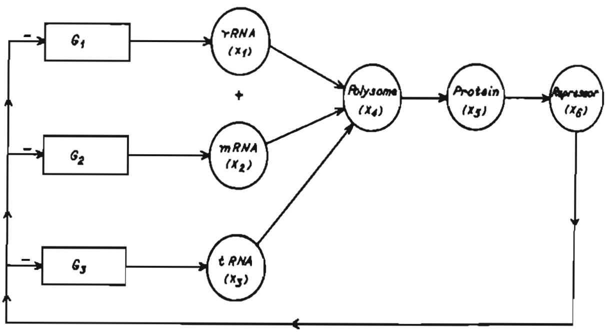

Figure 1. Schematic diagram of the epigenetic mechanism. G_1, G_2 and G_3 are the three genetic loci transcribing *r*RNA, *m*RNA and *t*RNA, respectively. For details see § 2 of the text.

(5) each of the components under investigation decays at a rate which is proportional to its own amount of accumulation.

A schematic representation of the above theories is given in Fig. 1. We denote the concentrations of *r*RNA, *m*RNA, *t*RNA, polysome, protein and repressor per cell by $X_1, X_2, X_3, \ldots, X_6$, respectively (throughout the whole investigation these variables will represent the respective concentrations per cell of the embryo).

Then, according to the principles (1)–(5), the rate of synthesis of the components $X_1, X_2, \ldots, X_6$ will be determined by the following set of simultaneous ordinary differential equations:

$$\left.\begin{aligned}
\frac{dX_1}{dt} &= \frac{\alpha_1}{1+h_1X_6^{m_1}} - \beta_1 X_1 \\
\frac{dX_2}{dt} &= \frac{\alpha_2}{1+h_2X_6^{m_2}} - \beta_2 X_2 \\
\frac{dX_3}{dt} &= \frac{\alpha_3}{1+h_3X_6^{m_3}} - \beta_3 X_3 \\
\frac{dX_4}{dt} &= \alpha_4 X_1 X_2 - \beta_4 X_4 \\
\frac{dX_5}{dt} &= \alpha_5 X_3 X_4 - \beta_5 X_5 \\
\frac{dX_6}{dt} &= \alpha_6 X_5 - \beta_6 X_6
\end{aligned}\right\} \qquad (2.1)$$

To obtain the dimensionless form we introduce the following dimensionless variables:

$$\left.\begin{aligned}
&\tau = [\alpha_2\alpha_3\alpha_5\alpha_6^4 h_i^{1/m_i}/(\alpha_1\alpha_4)^2]^{1/3} t \\
&x_1 = \frac{\alpha_4}{\alpha_6} X_1, \quad x_2 = \frac{\alpha_1\alpha_4}{\alpha_2\alpha_6} X_2, \quad x_3 = \frac{\alpha_1\alpha_4}{\alpha_3\alpha_6} X_3 \\
&x_4 = \left[\frac{\alpha_1\alpha_3\alpha_5}{\alpha_2^2\alpha_6^2} h_i^{1/m_i}\right]^{1/3} X_4 \\
&x_5 = \left[\frac{\alpha_1^2\alpha_2^4 h_i^{2/m_i}}{\alpha_2\alpha_3\alpha_5\alpha_6}\right]^{1/3} X_5 \\
&x_6 = (h_i)^{1/m_i} X_6 \\
&\gamma_i = \frac{\alpha_6\beta_i}{\alpha_1\alpha_4} \quad (i = 1, 2, 3) \\
&\gamma_i = [(\alpha_1\alpha_4)^2/(\alpha_2\alpha_3\alpha_5\alpha_6^4 h_i^{1/m_i})]^{1/3}\beta_i \quad (i = 4, 5, 6) \\
&\varepsilon = [\alpha_2\alpha_3\alpha_5\alpha_6^7 h_i^{1/m_i}/(\alpha_1\alpha_4)^5]^{1/3}\beta_i
\end{aligned}\right\} \qquad (2.2)$$

and

ρ = a real positive number (without dimension)

This leads to the following dimensionless form of the governing equations (2.1):

$$\left.\begin{aligned}
\varepsilon\frac{dx_1}{d\tau} &= \frac{1}{1+x_6^{m_1}} - \gamma_1 x_1 \\
\varepsilon\frac{dx_2}{d\tau} &= \frac{1}{1+x_6^{m_2}} - \gamma_2 x_2 \\
\varepsilon\frac{dx_3}{d\tau} &= \frac{1}{1+x_6^{m_3}} - \gamma_3 x_3 \\
\frac{dx_4}{d\tau} &= x_1 x_2 - \gamma_4 x_4 \\
\frac{dx_5}{d\tau} &= \rho x_3 x_4 - \gamma_5 x_5 \\
\frac{dx_6}{d\tau} &= x_5 - \gamma_6 x_6
\end{aligned}\right\} \tag{2.3}$$

3. Application of singular perturbation technique

The reduction in dimensions of the full system by singular perturbation is an efficacious method for the qualitative analysis of the model. Using Tikhonov's theorem (Tikhonov 1952) on singular perturbation, Othmer *et al.* (1985) have successfully analysed a fifteen-dimensional model system for signal relay adaptation in *Dictyostelium discoideum*. We shall follow the same method for the reduction of our six-dimensional system as given by (2.3).

First we assume that the parameter ε is very small. From (2.2) the condition for this is

$$\alpha_2\alpha_3\alpha_5\alpha_6^7 h_i^{1/m_i} \ll (\alpha_1\alpha_4)^5 \tag{3.1}$$

Now we write the system (2.3) in the following form:

$$\left.\begin{aligned}
\frac{dx}{d\tau} &= f(x, y) \\
\varepsilon\frac{dy}{d\tau} &= g(x, y)
\end{aligned}\right\} \tag{3.2}$$

where

$$\left.\begin{aligned}
x &= (x_4, x_5, x_6)^{\mathrm{T}} \in \mathbb{R}^3 \\
y &= (x_1, x_2, x_3)^{\mathrm{T}} \in \mathbb{R}^3 \\
f(x, y) &= \begin{bmatrix} x_1 x_2 - \gamma_4 x_4 \\ \rho x_3 x_4 - \gamma_5 x_5 \\ x_5 - \gamma_6 x_6 \end{bmatrix}
\end{aligned}\right\} \tag{3.3}$$

$$g(x, y) = \begin{bmatrix} \dfrac{1}{1+x_6^{m_1}} - \gamma_1 x_1 \\ \dfrac{1}{1+x_6^{m_2}} - \gamma_2 x_2 \\ \dfrac{1}{1+x_6^{m_3}} - \gamma_3 x_3 \end{bmatrix}$$

Tikhonov's theorem can be stated as follows.

Theorem 1

If the solution $y=\phi(x)$ of $g(x, y)=0$ is unique and positively stable in a closed bounded domain $D \subset R^n$, if the initial point $x(0)$, $y(0)$ belongs to the domain of influence† of this solution, and if the solution of the degenerate system given by

$$\frac{dx}{d\tau} = f(x, \phi(x))$$

belongs to D for $0 \leqslant t \leqslant T$, then the solution $(x(t, \varepsilon), y(t, \varepsilon))$ of the full system (3.2) tends to the solution of the above degenerate system as ε approaches 0, i.e.

$$\lim_{\varepsilon \to 0} y(t, \varepsilon) = \bar{y}(t) = \phi(\bar{x}, t) \quad (0 < t \leqslant T^0 < T)$$

and

$$\lim_{\varepsilon \to 0} x(t, \varepsilon) = \bar{x}(t) \quad (0 \leqslant t \leqslant T^0 < T)$$

Now we shall apply Tikhonov's Theorem to our system (2.3).

On setting $\varepsilon = 0$ in the second equation of (3.2), the unique solution is given by

$$x_i = \frac{1}{\gamma_i} \frac{1}{1+x_6^{m_i}} \quad (i = 1, 2, 3) \tag{3.4}$$

We write the second equation of (3.2) in the form

$$\varepsilon \frac{dy}{d\tau} = Ky + F \tag{3.5}$$

where

$$K = \begin{bmatrix} -\gamma_1 & 0 & 0 \\ 0 & -\gamma_2 & 0 \\ 0 & 0 & -\gamma_3 \end{bmatrix}, \quad y = (x_1, x_2, x_3)^T$$

and

$$F = \left[\frac{1}{1+x_6^{m_1}}, \frac{1}{1+x_6^{m_2}}, \frac{1}{1+x_6^{m_3}}\right]$$

† The domain of influence of a unique positively stable $y = \phi(x)$ is the set of points (x^*, y^*) such that the solution of the associated adjoined system $dy/ds = g(x^*, y^*)$ with initial condition $y(0) = y^*$ tends to $\phi(x^*)$ as $s \to \infty$ (see Othmer *et al.* 1985).

The eigenvalues of K can be found from the secular equation

$$(\gamma_1 + \lambda)(\gamma_2 + \lambda)(\gamma_3 + \lambda) = 0 \tag{3.6}$$

which shows that all the eigenvalues are negative. This implies that the manifold $y = \phi(x)$ is exponentially attracting and the solution $y^* = \phi(x^*)$ is unique and positively stable. Hence, according to Tikhonov's theorem for $0 < \varepsilon < 1$, the full system (2.3) can be approximated to order ε by the reduced form (replacing x_4, x_5, x_6 by y_1, y_2, y_3, respectively) given below:

$$\left.\begin{aligned}
\frac{dy_1}{d\tau} &= \frac{1}{\gamma_1\gamma_2(1 + y_3^{m_1})(1 + y_3^{m_2})} - \gamma_4 y_1 \\
\frac{dy_2}{d\tau} &= \frac{\rho y_1}{\gamma_3(1 + y_3^{m_3})} - \gamma_5 y_2 \\
\frac{dy_3}{d\tau} &= y_2 - \gamma_6 y_3
\end{aligned}\right\} \tag{3.7}$$

4. Steady state

The steady-state values of y_{10}, y_{20} and y_{30} of $y_1(t)$, $y_2(t)$, $y_3(t)$ as $t \to \infty$ are given by the following equations:

$$\left.\begin{aligned}
0 &= \frac{1}{\gamma_1\gamma_2(1 + y_{30}^{m_1})(1 + y_{30}^{m_2})} - \gamma_4 y_{10} \\
0 &= \frac{\rho y_{10}}{\gamma_3(1 + y_{30}^{m_3})} - \gamma_5 y_{20} \\
0 &= y_{20} - \gamma_6 y_{30}
\end{aligned}\right\} \tag{4.1}$$

Now we show that system (3.7) possesses a unique steady state. To do so we write system (3.7) in the following form:

$$\left.\begin{aligned}
\frac{dy_1}{d\tau} &= F_1(y_1, y_3) \\
\frac{dy_2}{d\tau} &= F_2(y_1, y_2, y_3) \\
\frac{dy_3}{d\tau} &= F_3(y_2, y_3)
\end{aligned}\right\} \tag{4.2}$$

where

$$F_1(y_1, y_3) = \frac{1}{\gamma_1\gamma_2(1 + y_3^{m_1})(1 + y_3^{m_2})} - \gamma_4 y_1$$

$$F_2(y_1, y_2, y_3) = \frac{\rho y_1}{\gamma_3(1 + y_3^{m_3})} - \gamma_5 y_2$$

$$F_3(y_2, y_3) = y_2 - \gamma_6 y_3$$

Lemma 1

The system (4.2) possesses a unique steady state in the positive quadrant.

Proof

The steady-state values y_{10}, y_{20}, y_{30} are given by

$$\left.\begin{aligned} \gamma_4 y_{10} &= \frac{1}{\gamma_1\gamma_2(1+y_{30}^{m_1})(1+y_{30}^{m_2})} \\ \gamma_5 y_{20} &= \frac{\rho y_{10}}{\gamma_3(1+y_{30}^{m_3})} \\ \gamma_6 y_{30} &= y_{20} \end{aligned}\right\} \tag{4.3}$$

Solving these equations we get

$$\left(\prod_{i=1}^{6}\gamma_i\right) y_{30} = \rho \Big/ \prod_{i=1}^{3}(1+y_{30}^{m_i}) = F(y_{30}) \tag{4.4}$$

Obviously $F(y_{30})$ is a decreasing function of y_{30} and so the graph of $\left(\prod_{i=1}^{6}\gamma_i\right) y_{30}$ and $F(y_{30})$ intersect exactly once in $(y_{30} > 0)$. Hence (4.4) has a unique solution giving

$$y_{30} = \frac{F(y_{30})}{\prod_{i=1}^{6}\gamma_i}$$

Then from (4.3) it follows that y_{10} and y_{20} also have a unique solution. Thus the system (4.2) possesses a unique steady state $\bar{y} = (y_{10}, y_{20}, y_{30})$. □

Now we prove that the system (4.2) is globally stable.

Lemma 2

The system (4.2) possesses a globally attracting set Γ containing the steady state $\bar{y}$.

Proof

Since $F_1(y_1, y_3)$ is monotone decreasing in y_3 and monotone increasing in y_1, we have

$$\dot{y}_1 \leqslant F_1(y_1, 0) = \frac{1}{\gamma_1\gamma_2} - \gamma_4 y_1$$

Define y_1^Γ by

$$\gamma_4 y_1^\Gamma = \frac{1}{\gamma_1\gamma_2}$$

which has a unique solution. Then

$$y_1 > y_1^\Gamma \Rightarrow \dot{y}_1 < 0$$

Define y_2^Γ and y_3^Γ similarly. Following a similar argument, it can be shown that $y_2 > y_2^\Gamma$ implies $\dot{y}_2 < 0$ and $y_3 > y_3^\Gamma$ implies $\dot{y}_3 < 0$. Hence Γ, defined as $\{y \mid y_i \leqslant y_i^\Gamma\}$, is globally attracting. From the above it is also obvious that y_i^Γ is a decreasing function of γ_j and the steady state $\bar{y} \in \Gamma$.

Now we show that for the system (4.2) $\bar{y}$ is a global attractor. To do so we use the following theorem due to Othmer (1976) and Mees and Rapp (1978).

Theorem 2

Let F_i in (4.2) be analytic functions and define

$$M = \sup_{y \in \Gamma} \|\gamma^{-1} J\|_2$$

where $\gamma = \operatorname{diag}(\gamma_j)$, $j = 4, 5, 6$ and J is the jacobian $(DF)_y$. Then $\bar{y}$ is globally attracting if $M < 1$. In fact, the values of the parameters γ_j ($j = 1, 2, \ldots, 6$) can be chosen so that there will always be $M < 1$. Consider a box Γ in R^3_+ with its sides as y_1^Γ, y_2^Γ and y_3^Γ (see Fig. 2).

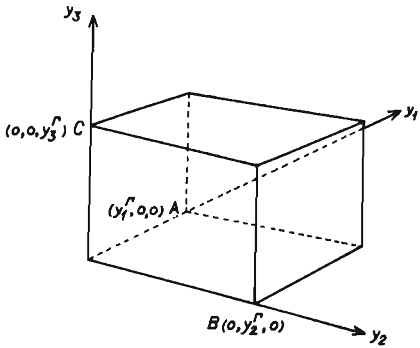

Figure 2. Box Γ in R^3_+ with sides y_1^Γ, y_2^Γ and y_3^Γ. The size of the box is inversely proportional to the values of the constants γ_i.

From Lemma 2 we have

$$y_1^\Gamma = \frac{1}{\gamma_1\gamma_2\gamma_4}, \quad y_2^\Gamma = \frac{\rho}{\prod_{i=1}^{5} \gamma_i}, \quad y_3^\Gamma = \frac{\rho}{\prod_{i=1}^{6} \gamma_i} \tag{4.5}$$

Evidently, if the γ_i can be increased, the box Γ gets smaller and $\sup_{y \in \Gamma} \|J\|_2 \leqslant \sup_{y \in \Gamma_0} \|J\|_2 = \alpha$ (say). Hence $M \leqslant \alpha/\gamma$ and so $M < 1$ for all γ sufficiently large. Hence $\bar{y} = (y_{10}, y_{20}, y_{30})$ is a global attractor. □

4.1. *Nyquist criterion, Hopf bifurcation and limit cycles*

Now we show that the system (4.2), and hence the full system (2.1), possesses a periodic solution. To do so we linearize the system (4.2) around the steady state $\bar{y} = (y_{10}, y_{20}, y_{30})$, applying the transformation $u_i = y_i - y_{i0}$ ($i = 1, 2, 3$) to obtain

$$\left.\begin{aligned} \frac{du_1}{d\tau} &= -a_1 u_3 - \gamma_4 u_1 \\ \frac{du_2}{d\tau} &= a_2 u_1 - a_3 u_3 - \gamma_5 u_2 \\ \frac{du_3}{d\tau} &= u_2 - \gamma_6 u_3 \end{aligned}\right\} \tag{4.6}$$

where

$$a_1 = -\frac{m_1 y_{30}^{m_1-1}(1+y_{30}^{m_2}) + m_2 y_{30}^{m_2-1}(1+y_{30}^{m_1})}{\gamma_1\gamma_2[(1+y_{30}^{m_1})(1+y_{30}^{m_2})]^2}$$

$$a_2 = \frac{\rho}{\gamma_3(1+y_{30}^{m_3})^2} \quad \text{and} \quad a_3 = \frac{\rho m_3 y_{10} y_{30}^{m_3-1}}{\gamma_3(1+y_{30}^{m_3})^2}$$

Linear stability analyses of single-loop systems have been performed by several authors (Walter 1969, Viniegra-Gonzalez 1973, Higgins *et al.* 1973, Hunding 1974, Othmer 1976, Rapp 1976 a, 1976 b and others). Mees and Rapp (1978) and Tapaswi and Bhattacharya (1981) have investigated linear stability properties and oscillations in multiple-loop systems. The former authors have applied the Nyquist criterion to investigate stability and oscillations in a single-loop system having a single open-loop transfer function. Here we employ the Nyquist criterion in a multi-loop feedback system with two open-loop transfer functions. Taking Laplace transformations $\xi_i(s) = \alpha\{y_i(t)\}$ $(i = 1, 2, 3)$, the linearized system (4.6) gives

$$\left.\begin{aligned} (S+\gamma_4)\xi_1(S) &= -a_1\xi_3(S) \\ (S+\gamma_5)\xi_2(S) &= a_2\xi_1(S) - a_3\xi_3(S) \\ (S+\gamma_6)\xi_3(S) &= \xi_2(S) \end{aligned}\right\} \tag{4.7}$$

Combining the three equations in (4.7) gives $\xi_3(S) = -G_1(S)a_1a_2\xi_3(S)$ where

$$\left.\begin{aligned} G_1(S) &= 1/[(S+\gamma_4)\{G_2^{-1}(S)+a_3\}] \\ G_2(S) &= 1/[(S+\gamma_5)(S+\gamma_6)] \end{aligned}\right\} \tag{4.8}$$

are two open-loop transfer functions.

The linearized system can then be represented by the control system

$$\xi_3(S) = \left[\frac{-G_1(S)a_1a_2}{1+G_1(S)a_1a_2}\right] I(S) \tag{4.9}$$

where $I(S)$ is the perturbing input. The closed-loop control system (4.9) is as shown in Fig. 3. The characteristic equation of (4.9) is $1 + G_1(S)a_1a_2 = 0$. Now for local stability analysis we are to examine the Nyquist loci of $G_1(S)$ and $G_2(S)$.

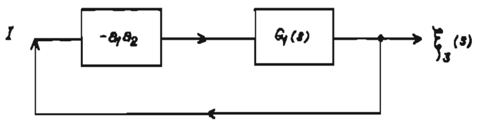

Figure 3. Block diagram of the closed-loop control (linearized) system.

We have

$$G_2(iw) = \frac{1}{(\gamma_5+iw)(\gamma_6+iw)}$$

Let

$$\left.\begin{aligned} \gamma_5 &= R_1\cos\psi_1, \quad w = R_1\sin\psi_1 \\ \gamma_6 &= R_2\cos\psi_2, \quad w = R_2\sin\psi_2 \end{aligned}\right\} \tag{4.10}$$

which implies

$$G_2(iw) = \frac{1}{R_1 R_2} \exp(-i(\psi_1 + \psi_2)) \tag{4.11}$$

Therefore

$$|G_2(iw)| = \frac{1}{R_1 R_2} \times \left[\frac{1}{(\gamma_5^2 + w^2)(\gamma_6^2 + w^2)}\right]^{1/2}$$

which tends to 0 as $w \to \infty$. Hence $|G_2(iw)|$ is monotone decreasing.

Again

$$\tan(\psi_1 + \psi_2) = \frac{w(\gamma_5 + \gamma_6)}{\gamma_5\gamma_6 - w^2}$$

Let $w_1 > w_2$, then

$$\frac{w_1(\gamma_5 + \gamma_6)}{\gamma_5\gamma_6 - w_1^2} - \frac{w_2(\gamma_5 + \gamma_6)}{\gamma_5\gamma_6 - w_2^2} > 0 \Rightarrow \gamma_5\gamma_6(w_1 - w_2) + w_1 w_2(w_1 - w_2) > 0$$

Hence, $\tan(\psi_1 + \psi_2)$ is monotone increasing. Therefore, $\arg G_2(iw)$ is also monotone decreasing. Following a similar procedure as above we can show that both $|G_1(iw)|$ and $\arg G_1(iw)$ are also monotone decreasing.

Now for stability analysis we use the Nyquist stability criterion which is stated as follows.

Theorem 3: *Nyquist stability criterion* (Nyquist 1932, Ogata 1970)

If the Nyquist path in the S-plane encircles Z zeros and P poles of $1 + G(S)F'(Y_n^0)$ and does not pass through any poles or zeros of $1 + G(S)F'(y_n^0)$ as a representative point S moves in the clockwise direction along the Nyquist path, then the corresponding contour in the $G(S)$ plane encircles the $-(1/F'(y_n^0)) + j0$ point $N = Z - P$ times in the clockwise direction (negative values of N imply counter-clockwise encirclements). If $N > 0$ in the clockwise direction then the system is unstable.

In our case when $G(S) = G_2(S) = (1/(S + \gamma_5)(S + \gamma_6))F'(y_n^0) = -a_3$ (<0), the number of zeros of $1 + G_2(S)a_3$ in the right half-plane is zero and the number of poles of $G_2(S)$ in the right half-plane is also zero.

Hence, according to this theorem the number of encirclements of $-(1/a_3)$ by the Nyquist contour in the $G_2(S)$ plane is zero. That is, the contour cannot encircle the point $-(1/a_3)$ as shown in Fig. 4.

Therefore, $G_2(S)$ acting alone cannot make the system unstable if $m_1 = 1$.

Next we show that $G_1(S) \supset G_2(S)$ can encircle $-1/a_1 a_2$.

Now the number of poles of $G_1(S) = 1/[(S + \gamma_4)\{G_2^{-1}(S) + a_3\}]$ in the right half S-plane is zero and the number of zeros of $1 + G_1(S)a_1 a_2$ in the right half S-plane is two. Hence, according to the Nyquist criterion, $N = Z - P$ the number of encirclements of the point $-1/a_1 a_2$ by the contour in the $G_1(S)$ plane is two in the clockwise direction (see Fig. 5). Therefore, although the single-loop problem cannot envisage a local instability, the multiple-loop negative feedback control can effect a local destabilization of the system even if $m_2 = 1$.

Next we find the critical value where local instability sets in. The open-loop transfer function is given by

$$G_1(iw) = \frac{1}{(iw + \gamma_4)\{(iw + \gamma_5)(iw + \gamma_6) + a_3\}}$$

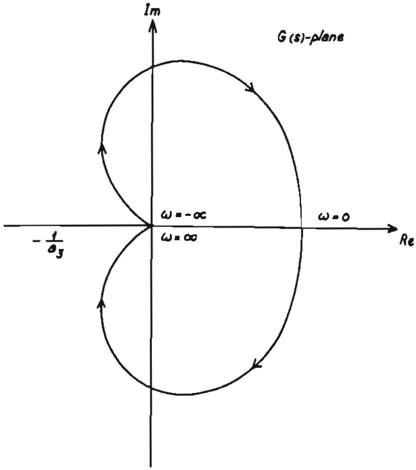

Figure 4. Nyquist contour for positive ω in the $G_2(s)$ plane. Arrows indicate the direction of increasing ω. The point $-1/a_3$ is not encircled by the contour.

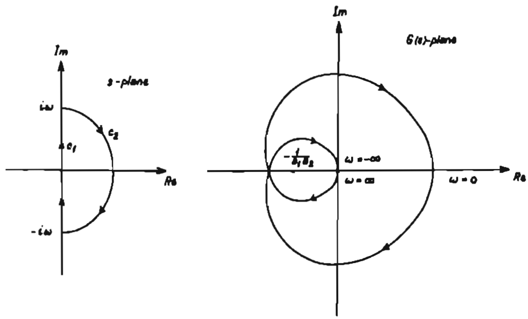

Figure 5. Closed contour in the s-plane and the corresponding Nyquist contour in the $G_1(s)$-plane. The Nyquist contour follows a clockwise direction as ω increases and the number of encirclements of the point $-1/(a_1a_2)$ by the contour is two.

Let us find the point where the Nyquist plot crosses the negative real axis. Let the imaginary part of $G_1(iw)$ be zero. This gives

$$w = \pm\left(\sum_{j \neq k} \gamma_j \gamma_k + a_3\right)^{1/2}, \quad j, k = 4, 5, 6$$

Substituting the positive value of w in $G_1(iw)$, we obtain

$$G_1\left(i\left(\sum_{j\neq k}\gamma_j\gamma_k+a_3\right)^{1/2}\right)=\frac{1}{-\Sigma\gamma_j\left(\sum_{j\neq k}\gamma_j\gamma_k+a_3\right)+\gamma_4(\gamma_5\gamma_6+a_3)}$$

The critical value of ρ (ρ_c) where instability sets in is obtained by equating this to $-1/a_1a_2$, i.e.

$$\frac{1}{-\Sigma\gamma_j\left(\sum_{j\neq k}\gamma_j\gamma_k+a_3\right)+\gamma_4(\gamma_5\gamma_6+a_3)}=-\frac{1}{a_1a_2}$$

or

$$\Sigma\gamma_j\left(\sum_{j\neq k}\gamma_j\gamma_k+a_3\right)-\gamma_4(\gamma_5\gamma_6+a_3)-a_1a_2=0$$

which implies

$$\rho_c=\frac{\gamma_3(1+y_{30}^{m_3})}{a_1}\left[\left(\Sigma\gamma_j\sum_{j\neq k}\gamma_j\gamma_k-\gamma_4\gamma_5\gamma_6\right)(1+y_{30}^{m_3})-\gamma_5\gamma_6m_3y_{30}^{m_3-1}(\gamma_5+\gamma_6)\right] \quad (4.12)$$

Hence the bifurcating value of ρ is given by (4.12). For a lower value of ρ than this the system is locally unstable and for a higher value of ρ than this the system is stable. Then, according to the Hopf bifurcation theorem, the system possesses a periodic solution (limit cycle) in the neighbourhood of the critical value of ρ as given in (4.12). The Nyquist contour encircling the point $-1/a_1a_2$ on the real axis is as shown in Fig. 5.

5. Stability of the limit cycle

Taking $m_1=m_2=m_3=1$, the dimensionless system (3.7) can be written, without loss of generalization, as

$$\left.\begin{aligned}\frac{dy_1}{d\tau}&=\frac{1}{y_1y_2(1+y_3)^2}-\gamma_4y_1\\ \frac{dy_2}{d\tau}&=\frac{\rho y_1}{y_3(1+y_3)}-\gamma_5y_2\\ \frac{dy_3}{d\tau}&=y_2-\gamma_6y_3\end{aligned}\right\} \quad (5.1)$$

Using the perturbation $y_i=y_{i0}+u_i$ ($i=1,2,3$) and expanding the right-hand side of (5.1) up to third order in u_i, we obtain

$$\left.\begin{aligned}\frac{du_1}{d\tau}&=-\gamma_4u_1-\frac{2u_3}{\gamma_1\gamma_2a_0^3}+\frac{3u_3^2}{\gamma_1\gamma_2a_0^4}-\frac{4u_3^3}{\gamma_1\gamma_2a_0^5}\\ \frac{du_2}{d\tau}&=\left(\frac{\rho u_1}{\gamma_3a_0}-\frac{\rho y_{10}u_3}{\gamma_3a_0^2}-\gamma_5u_2\right)+\left(\frac{\rho y_{10}u_3^2}{\gamma_3a_0^3}-\frac{u_1u_3}{a_0^2}\right)\\ &\quad+\left(\frac{\rho u_1u_3^2}{\gamma_3a_0^3}-\frac{y_{10}u_3^3}{\gamma_3a_0^4}\right)\\ \frac{du_3}{d\tau}&=u_2-\gamma_6u_3\end{aligned}\right\} \quad (5.2)$$

where $a_0=1+y_{30}$.

In matrix notation this can be written as

$$\frac{dU}{d\tau} = AU + F(U) + G(U) \tag{5.3}$$

where

$$U = \begin{bmatrix} u_1 \\ u_2 \\ u_3 \end{bmatrix}, \quad A = \begin{bmatrix} -\gamma_4 & 0 & -\dfrac{2}{\gamma_1\gamma_2 a_0^3} \\ \dfrac{\rho}{\gamma_3 a_0} & -\gamma_5 & -\dfrac{\rho y_{10}}{\gamma_3 a_0^2} \\ 0 & 1 & -\gamma_6 \end{bmatrix}$$

$$F(U) = \begin{bmatrix} \dfrac{3u_3^2}{\gamma_1\gamma_2 a_0^4} \\ \dfrac{\rho u_3}{\gamma_3 a_0^2}\left(\dfrac{y_{10}}{a_0}u_3 - u_1\right) \\ 0 \end{bmatrix} \quad \text{and} \quad G(U) = \begin{bmatrix} -\dfrac{4u_3^3}{\gamma_1\gamma_2 a_0^5} \\ \dfrac{\rho u_3^2}{\gamma_3 a_0^3}\left(u_1 - \dfrac{y_{10}}{a_0}u_3\right) \\ 0 \end{bmatrix}$$

The characteristic equation of the linear part of the system (5.1) with eigenvalues λ is

$$|A - \lambda I| = \lambda^3 + p_2\lambda^2 + p_1\lambda + p_0 = 0 \tag{5.4}$$

where

$$p_0 = \gamma_4\gamma_5\gamma_6 + \frac{\gamma_4\rho y_{10}}{\gamma_3 a_0^2} + \frac{2\rho}{\gamma_1\gamma_2\gamma_3 a_0^4}$$

$$p_1 = \gamma_4\gamma_5 + \gamma_5\gamma_6 + \gamma_6\gamma_4 + \frac{\rho y_{10}}{\gamma_3 a_0^2}$$

$$p_2 = \gamma_4 + \gamma_5 + \gamma_6$$

At the critical value of $\rho = \rho_c$ as given by (4.12) we have, by the Routh–Hurwitz criterion, $p_0 = p_1 p_2$ and (5.4) has a pair of purely imaginary eigenvalues $\pm i\sqrt{p_1}$ and a real negative root $-p_2$.

We assume, without loss of generalization, that $\gamma_4 = \gamma_5 = \gamma_6 = \gamma$. Then

$$\left.\begin{aligned} p_0 &= \gamma^3 + \frac{\rho_c}{\gamma_3 a_0^2}\left(\gamma y_{10} + \frac{2}{\gamma_1\gamma_2 a_0^2}\right) \\ p_1 &= 3\gamma^2 + \frac{\rho_c y_{10}}{\gamma_3 a_0^2} \\ p_2 &= 3\gamma \end{aligned}\right\} \tag{5.5}$$

Now we reduce the matrix A to Jordan canonical form given by

$$A = p\,\mathrm{diag}\,(-p_2, i\sqrt{p_1}, -i\sqrt{p_1})p^{-1}$$

where

$$p = \begin{bmatrix} \dfrac{1}{\gamma\gamma_1\gamma_2 a_0^3} & -\dfrac{2}{\gamma_1\gamma_2 a_0^3(\gamma + i\sqrt{p_1})} & -\dfrac{2}{\gamma_1\gamma_2 a_0^3(\gamma - i\sqrt{p_1})} \\ -2\gamma & \gamma + i\sqrt{p_1} & \gamma - i\sqrt{p_1} \\ 1 & 1 & 1 \end{bmatrix}$$

and

$$
p^{-1} = \begin{bmatrix} \gamma_1\gamma_2 a_0^3 \Delta & -\dfrac{2\Delta}{\gamma^2+p_1} & \dfrac{4\Delta\gamma}{\gamma^2+p_1} \\ \dfrac{\gamma_1\gamma_2 a_0^3\Delta(3\gamma - i\sqrt{p_1})}{2i\sqrt{p_1}} & +\dfrac{(3\gamma - i\sqrt{p_1})\Delta}{2i\sqrt{p_1}\,\gamma(\gamma - i\sqrt{p_1})} & -\dfrac{\Delta}{2i\sqrt{p_1}}\left[\dfrac{(\gamma - i)\sqrt{p_1})}{\gamma} - \dfrac{4\gamma}{\gamma - i\sqrt{p_1}}\right] \\ -\dfrac{\gamma_1\gamma_2 a_0^3\Delta(3\gamma + i\sqrt{p_1})}{2i\sqrt{p_1}} & -\dfrac{(3\gamma + i\sqrt{p_1})\Delta}{2i\sqrt{p_1}\,\gamma(\gamma + i\sqrt{p_1})} & \dfrac{\Delta}{2i\sqrt{p_1}}\left[\dfrac{(\gamma + i\sqrt{p_1})}{\gamma} - \dfrac{4\gamma}{\gamma + i\sqrt{p_1}}\right] \end{bmatrix} \tag{5.6}
$$

where

$$
\Delta = \frac{\gamma^2 + p_1}{9\gamma^2 + p_1}
$$

The substitution

$$
U = pZ, \quad Z = \begin{pmatrix} Z_1 \\ Z_2 \\ Z_3 \end{pmatrix} \tag{5.7}
$$

and $t' = \sqrt{p_1}\,t$, where $\pm i\sqrt{p_1}$ denote pure imaginary values of the matrix of the linear part, reduces the system (5.3) into diagonal form

$$
\frac{dZ}{dt'} = \operatorname{diag}(-p_2, i\sqrt{p_1}, -i\sqrt{p_1}) + \begin{bmatrix} \sum_{j=1}^{3}\sum_{h=1}^{3} a_{jh}^1 Z_j Z_h \\ \sum_{j=1}^{3}\sum_{h=1}^{3} a_{jh}^2 Z_j Z_h \\ \sum_{j=1}^{3}\sum_{h=1}^{3} a_{jh}^3 Z_j Z_h \end{bmatrix} + \begin{bmatrix} \sum_{j=1}^{3}\sum_{h=1}^{3}\sum_{k=1}^{3} b_{jhk}^1 Z_j Z_h Z_k \\ \sum_{j=1}^{3}\sum_{h=1}^{3}\sum_{k=1}^{3} b_{jhk}^2 Z_j Z_h Z_k \\ \sum_{j=1}^{3}\sum_{h=1}^{3}\sum_{k=1}^{3} b_{jhk}^3 Z_j Z_h Z_k \end{bmatrix} \tag{5.8}
$$

The coefficients a_{jh}, b_{jhk} are complex and symmetrized, that is

$$
a_{jh}^i = a_{hj}^i, \quad b_{\{jhk\}}^i = \text{identical} \quad (i, j, h, k = 1, 2, 3) \tag{5.9}
$$

where $\{jhk\}$ denotes any permutation of j, h and k. Here

$$
a_{11}^l = A_l - \frac{(-1)^l B_l}{\gamma\gamma_1\gamma_2 a_0^3}
$$

$$
a_{22}^l = A_l + \frac{(-1)^l B_l}{\gamma_1\gamma_2 a_0^3 x}
$$

$$
a_{33}^l = A_l + \frac{(-1)^l B_l}{\gamma_1\gamma_2 a_0^3 \bar{x}}
$$

$$
a_{21}^l + a_{12}^l = 2A_l + \frac{(-1)^l B_l}{\gamma_1\gamma_2 a_0^3}\left(\frac{2}{x} - \frac{1}{\gamma}\right)
$$

$$a^i_{32} + a^i_{23} = 2A_i + \frac{(-1)^i 2B_i}{\gamma_1\gamma_2 a_0^3}\left(\frac{1}{\bar{x}} + \frac{1}{x}\right)$$

$$a^i_{31} + a^i_{13} = 2A_i + \frac{(-1)^i B_i}{\gamma_1\gamma_2 a_0^3}\left(\frac{2}{\bar{x}} - \frac{1}{y}\right)$$

$$A_1 = \frac{1}{a_0(2y+x)(2y+\bar{x})}\left[3x\bar{x} - \frac{2\rho_c y_{10}}{\gamma_3 a_0^2}\right]$$

$$A_2 = \bar{A}_3 = \frac{x}{y a_0(x-\bar{x})(2y+x)}\left[3 + \frac{\rho_c y_{10}}{\gamma_3 a_0^2 \bar{x}}\right]$$

$$B_1 = \frac{2\rho_c}{\gamma_3 a_0^2(2y+x)(2y+\bar{x})}, \quad B_2 = -\bar{B}_3 = \frac{\rho_c x}{y\gamma_3 a_0^2(x-\bar{x})(2y+x)}$$

$$b^i_{111} = C_i + \frac{(-1)^i D_i}{y\gamma_1\gamma_2 a_0^3}$$

$$b^i_{222} = \overline{b^i_{333}} = C_i - \frac{(-1)^i 2D_i}{\gamma_1\gamma_2 a_0^3 x}$$

$$b^i_{112} + b^i_{121} + b^i_{211} = 3C_i + \frac{(-1)^i 2D_i}{\gamma_1\gamma_2 a_0^3}\left(\frac{1}{y} - \frac{1}{x}\right)$$

$$b^i_{113} + b^i_{131} + b^i_{311} = 3C_i + \frac{(-1)^i 2D_i}{\gamma_1\gamma_2 a_0^3}\left(\frac{1}{y} - \frac{1}{\bar{x}}\right)$$

$$b^i_{122} + b^i_{212} + b^i_{221} = 3C_i + \frac{(-1)^i D_i}{\gamma_1\gamma_2 a_0^3}\left(\frac{1}{y} - \frac{4}{x}\right)$$

$$b^i_{133} + b^i_{313} + b^i_{331} = 3C_i + \frac{(-1)^i D_i}{\gamma_1\gamma_2 a_0^3}\left(\frac{1}{y} - \frac{4}{\bar{x}}\right)$$

$$b^i_{223} + b^i_{232} + b^i_{322} = 3C_i - \frac{(-1)^i 2D_i}{\gamma_1\gamma_2 a_0^3}\left(\frac{2}{x} - \frac{1}{\bar{x}}\right)$$

$$b^i_{233} + b^i_{323} + b^i_{332} = 3C_i - \frac{(-1)^i 2D_i}{\gamma_1\gamma_2 a_0^3}\left(\frac{1}{x} + \frac{2}{\bar{x}}\right)$$

$$\Sigma b^i_{hjk}\,(h,j,k = 1,2,3;\ h \neq j \neq k) = 6C_i + \frac{(-1)^i 2D_i}{\gamma_1\gamma_2 a_0^3}\left(\frac{1}{y} - \frac{2}{x} - \frac{2}{\bar{x}}\right)$$

$$C_1 = \frac{2}{a_0^2(2y+4x)(2y+\bar{x})}\left[\frac{\rho_c y_{10}}{\gamma_3 a_0^2} - 2x\bar{x}\right]$$

$$C_2 = \bar{C}_3 = -\frac{x\bar{x}}{a_0^2(x-\bar{x})(2y+x)}\left[4 + \frac{\rho_c y_{10}}{y\gamma_3 a_0^2 \bar{x}}\right] \qquad (5.10)$$

$$D_1 = \frac{2\rho_c}{\gamma_3 a_0^3(2y+x)(2y+\bar{x})}$$

$$D_2 = -\bar{D}_3 = \frac{\rho_c x\bar{x}}{y\gamma_3 a_0^3(x-\bar{x})(2y+x)\bar{x}}$$

where $x = y + im\sqrt{p_1}$, $i = 1, 2, 3$.

Theorem 4. Fundamental Brujno theorem (Brujno 1971)

There exists a reversible complex change of variables (normalizing transform)

$$Z_i = W_i + \Sigma\alpha^i_{lm} W_l W_m + \Sigma\beta^i_{lmn} W_l W_m W_n + \ldots \quad (i = 1, 2, 3)$$

$$(\alpha^i_{ml} = \alpha^i_{lm}, \beta_{i(lmn)} = \text{identical}; i, l, m, n = 1, 2, 3) \tag{5.11}$$

which reduces the system (5.8) to the normal form

$$\frac{dW_i}{dt'} = \lambda_i W_i + W_i \sum_Q g_{iQ} W_1^{q_1} W_2^{q_2} W_3^{q_3} \quad (i = 1, 2, 3) \tag{5.12}$$

such that g_{iQ} are non-vanishing only for those Q that satisfy the resonant equation

$$(\Lambda, Q) = \lambda_1 q_1 + \lambda_2 q_2 + \lambda_3 q_3 = 0 \tag{5.13}$$

Here $\Lambda = (\lambda_1, \lambda_2, \lambda_3)'$ is a vector formed from the diagonal elements of the linear part of the system (5.8) and $Q = (q_1, q_2, q_3)'$ is a vector with integer components, and the set $\mathcal{M}_i$ of Q for the ith equation is

$$\begin{aligned} &q_1, \ldots, q_{i-1}, q_{i+1}, \ldots, q_n \geqslant 0 \\ &q_i \geqslant -1, \quad \sum_1^3 q_i \geqslant 1 \end{aligned} \tag{5.14}$$

System (5.12) with this property is called a normal form.

In the case under consideration

$$(\Lambda, Q) = \lambda_1 q_1 + \lambda_2 q_2 + \lambda_3 q_3 = -p_2 q_1 + i(q_2 - q_3) = 0 \tag{5.15}$$

This implies

$$q_1 = 0, \quad q_2 = q_3 \tag{5.16}$$

which means $q_1 + q_2 + q_3 = 2q_3$ and the terms with even powers vanish, while for the odd $(2r + 1)$st power terms we have $q_2 = q_3 = r$ $(r = 1, 2, \ldots)$.

The normal form (5.12) can now be written as

$$\left.\begin{aligned} \frac{dW_1}{dt'} &= -p_2 W_1 + W_1 \sum_{r=1}^{\alpha} g^1_r W_2^r W_3^r \\ \frac{dW_2}{dt'} &= i\sqrt{p_1} W_2 + W_2 \sum_{r=1}^{\alpha} g^2_r W_2^r W_3^r \\ \frac{dW_3}{dt'} &= -i\sqrt{p_1} W_3 + W_3 \sum_{r=1}^{\alpha} g^3_r W_2^r W_3^r \end{aligned}\right\} \tag{5.17}$$

which, within cubic terms, becomes

$$\left.\begin{aligned} \frac{dW_1}{dt'} &= -p_2 W_1 + g^1_1 W_1 W_2 W_3 \\ \frac{dW_2}{dt'} &= i\sqrt{p_1} W_2 + g^2_1 W_2^2 W_3 \\ \frac{dW_3}{dt'} &= -i\sqrt{p_1} W_3 + g^3_1 W_2 W_3^2 \end{aligned}\right\} \tag{5.18}$$

Since only the symmetrized coefficients of the normal form are distinct from zero, and in the present problem the normal forms have no second-power terms, we have (Starzhinskii 1980)

$$\xi^{i}_{lmp} = b^{i}_{lmp} + \frac{2}{3} \sum_{j=1}^{3} [a^{i}_{jl}\alpha^{j}_{mp} + a^{i}_{jm}\alpha^{j}_{pl} + a^{i}_{jp}\alpha^{j}_{lm}] \tag{5.19}$$

where ξ^{i}_{lmp} denote the symmetrized coefficients of the third-order terms of the normal form (5.17) and where $i, l, m, p = 1, 2, 3$.

Hence,

$$\left.\begin{aligned} g^{1}_{1} &= 6\xi^{1}_{123} = 6b^{1}_{123} + 4 \sum_{j=1,2,3} (a^{1}_{1j}\alpha^{j}_{23} + a^{1}_{2j}\alpha^{j}_{31} + a^{1}_{3j}\alpha^{j}_{12}) \\ g^{2}_{1} &= 3\xi^{2}_{223} = 3b^{2}_{223} + 2 \sum_{j=1,2,3} (2a^{2}_{2j}\alpha^{j}_{23} + a^{2}_{3j}\alpha^{j}_{22}) \\ \text{and} \quad g^{3}_{1} &= 3\xi^{3}_{323} = 3b^{3}_{323} + 2\left[\sum_{j=1,2,3} (2a^{3}_{3j}\alpha^{j}_{32} + a^{3}_{2j}\alpha^{j}_{33})\right] \end{aligned}\right\} \tag{5.20}$$

Now putting values of each term in the right-hand side of (5.20) from (5.9) and comparing each term of g^{2}_{1} and g^{3}_{1}, it can be easily shown that $g^{2}_{1} = \overline{g^{3}_{1}} = -g^{3}_{1}$. Multiplying the second equation of (5.18) by W_3 and the third by W_2 and then adding them, we obtain

$$\frac{dW_2 W_3}{dt} = 0$$

which implies

$$W_2 W_3 = C_1 \text{ (constant)} \tag{5.21}$$

where $C_1 = |W_2(0)|^2$.

Substituting (5.21) in the first equation of (5.18) gives

$$\frac{dW_1}{dt'} = (C_2 - p_2)W_1 \quad (C_2 = g^{1}_{1}C_1) \tag{5.22}$$

This implies that the system decomposes into cylinders $C_2 =$ const. in the neighbourhood of its equilibrium point. Each cylinder has an equator representing a limit cycle which is stable for $C_2 < p_2$ and unstable for $C_2 > p_2$. For some suitable choice of the parameter γ and initial value $W_2(0)$ it is possible to have $C_2 < p_2$, in which case the system (5.1), and hence the original system (2.1), possesses a stable limit cycle.

6. Discussion

In this paper, a model of the epigenetic mechanism has been constructed. The epigenetic mechanism involves transcription of different size classes of RNA, each controlled by end-product feedback inhibition, according to the Jacob–Monod operon concept (1961), followed successively by *m*RNA-ribosome complex (template) formation and protein synthesis during embryonic development. The model proposed is a six-dimensional multiple-loop control network based on recognized physiological principles. Using singular perturbation theory, this six-dimensional system has been reduced to a three-dimensional one. Stability and oscillatory properties of this system have been investigated using and extending the studies of Mees and Rapp (1978) in a generalized multiple-loop negative feedback biochemical system and of Tapaswi and Bhattacharya (1981) in an eight-dimensional multiple-loop negative feedback epigenetic control system where, unlike the present case under investigation, not all the feedbacks were exerted by the same end product. With the help of a Lyapunov function it has been shown, as a particular case of Mees and Rapp, that the system is globally stable.

As already discussed in the Introduction, RNA and protein synthesis during embryonic development is an oscillatory process. Hence, the mathematical study of oscillation in this system is a very relevant project. We have investigated this important property by applying the Nyquist criterion. Using the Hopf bifurcation theorem and Nyquist criterion we have shown that the system possesses a limit cycle oscillation for some critical values of the parameters. We have also established the stability of the limit cycle by power series expansion of each equation of (5.1) around the equilibrium up to third order, and then applying Brujno's theorem on normal transformation which reduces the non-linear system to a normal form of three equations, two of which constitute a complex conjugate pair. The solutions of the reduced normal form thus become surprisingly simple and give rise to cylinders, the equator of each of which represents a stable limit cycle for some certain values of the parameters and initial conditions.

This work deals with a real biological system which, like other realistic systems, is very difficult for mathematical analysis. In this paper we have attempted to tackle the problem efficiently with standard mathematical tools.

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