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ANALYSIS OF BIOCHEMICAL OSCILLATOR

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PREFACE

This dissertation entitled "Analysis of Biochemical Oscillators" has been carried out in the School of Theoretical and Environmental Sciences, Jawaharlal Nehru University, New Delhi-110057. The work is original and has not been submitted in part or in full for any degree or diploma to any University or Institute.

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CONTENTS

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CHAPTER-I

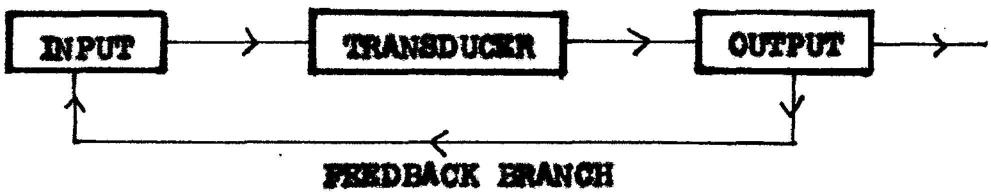
INTRODUCTION

Nature not only loves symmetry, it also loves periodicity. Though biological rythms are known to exist since antiquity, yet the existence of biological oscillators has been accepted only recently. A prominent view was that the rythms were due to external periodic effects like the daily changes of light and dark or similar changes in temperature, etc. This view was mainly expressed for oscillations having long period, because high frequency oscillations, like those involved in heartbeat, were obviously endogeneous in nature. Objections have been raised to the existence of endogeneous biological oscillators by connecting it to external factors like day and night and it has been criticized and discussed very much. But today the evidence in favour of "biological clocks" is beyond doubt and there are numerous examples of periodic or repetitive phenomena in living systems [11, Chap. 2, references quoted therein]. The opening and closing of flower petals at certain intervals and oscillations in the population of interacting biological species these two events indicate how biological rythm is present in all parts of the temporal organization in the systems.

In this article, emphasis will be on biochemical or metabolic oscillators. Here one finds oscillatory variation in the concentration of the biochemical species involved in the reaction. Recently there has been sudden rise in interest in biochemical oscillators among experimental and theoretical scientists due to the realization that enzyme systems, under certain circumstances, can generate fundamental rythms from which, it is speculated that, many properties of the cell and organism could be regulated and controlled. Chance and Pye have studied oscillations in the concentration of DPNH (Diphosphopyridine nucleotide) in yeast cells. Production of ATP and protein synthesis are also examples of biochemical oscillations. For experimental work reference [1] should be consulted.

The control studies in cells have led to a fundamental result that the concentrations of macromolecular species and their activities are regulated by specific control mechanisms involving feedback devices. Usually the small molecules of the metabolic system act as the feedback signals.

Feedback: In cybernetic terms, the interaction between the parts of the biological system is analysed as a flow of information and self-regulation is explained in terms of feedback. Feedback implies that a later state in a series of coupled processes may act backwards and modify an earlier link, thus changing the subsequent outcome. The process can be represented in a diagram Fig. 1.



Feedback Loop   Fig. 1

Feedback can be positive or negative. In the case of positive feedback the system tries to maintain or increase its own supply. In the case of negative feedback it tends to cut down its own supply and always tries to stop at a resting level. If disturbed it tries to return to that resting state. A common feature of the feedback control systems is the appearance of oscillations. This is also observed in some biochemical reactions. One of the most important biochemical reaction is the biosynthesis of protein. This reaction is controlled by feedback mechanism and clearly shows oscillatory behaviour in the concentration of the macromolecular species involved in the reaction.

The causal chain of the reaction is from DNA to RNA to protein to metabolite and it has been demonstrated that the metabolites act back upon gene activities in a precise manner and hence regulate further synthesis of protein. Now we discuss the protein synthesis reactions in details to have an insight in the feedback control mechanism involved in it.

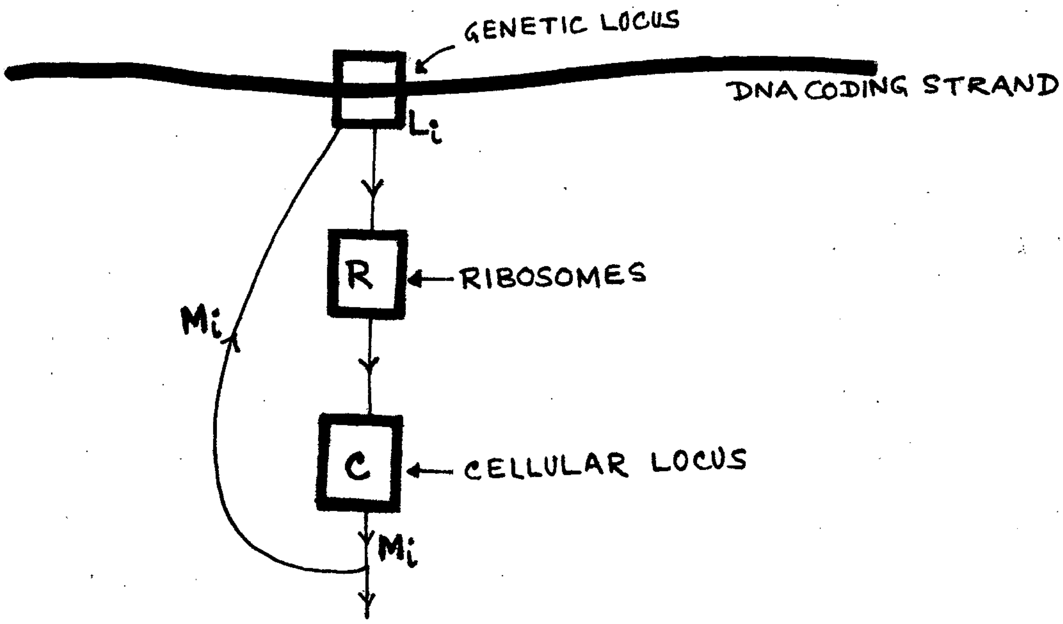
Protein Synthesis: The main task of DNA (Deoxyribonucleicacid) other than replication, is the synthesis of protein. DNA is not directly involved in the synthesis of protein, but instead the genetic information is transferred to another class of molecules, known as messenger RNA (mRNA) which then serve as the protein templates. RNA (ribonucleic acid) is similar to DNA except that RNA contains the sugar ribose instead of deoxyribose and the base Uracil (U) instead of Thymine (T). DNA serves as the template for RNA synthesis. It is a process during which the specific nucleotide sequence of the DNA dictates a complementary sequence in the RNA, U appearing on the RNA chain wherever A appears at the complementary site on the DNA.

In a nucleated cell, mRNA leaves the nucleus and gets attached to spherical, RNA containing particles, called Ribosomes, in the cytoplasm. All ribosomes contain both protein and ribosomal RNA (rRNA). The sequence of bases in the mRNA specifies the amino acid sequence of the protein to be synthesized. There is another type of RNA called transfer RNA (tRNA) which attaches to each amino acid (there are 20 kinds of them) and brings them to the appropriate sites on the mRNA chain (attached to the ribosome). This is done by means of a recognition process between a sequence of bases on the mRNA (the codon) and a sequence of bases on the tRNA (the anticodon). The function of the ribosomes is to orient properly the incoming complex of amino acid and tRNA and the template mRNA so that the genetic code can be read properly. When the amino acids arrive at the proper sites in the chain, they are linked together with the help of enzymes, by the formation of peptide bonds and synthesizes proteins.

The protein then serves its purpose (e.g. acts an enzyme) at some cellular locus where it generates some metabolic species. There is a regulatory or control mechanism to ensure that proteins are synthesized in the appropriate amounts for the proper functioning and multiplication of the cells. If the amount of metabolic species is greater than the required amount, the excess may be fedback to the genetic locus where it may act as a repressor or as a co-repressor (coupled with aporepressor). This in turn acts to bring down the synthesis of protein and so the synthesis of metabolic species. The whole process generates an oscillation in the concentration of the chemical species taking part in the reaction.

The three stages at which metabolites can affect the activities of macromolecules by specific interaction are the DNA stage, mRNA stage and the protein stage. This alters either DNA or RNA synthesis at the first stage, protein synthesis at the second stage and enzyme activity or it alters some other activity, such as contractibility at the last stage. In our case, we will consider the first stage of control, i.e. of gene activities, only.

The process of synthesis of protein is represented diagrammatically below:



Though this is an idealized model of a metabolic feedback control cycle, yet it includes the essential features of the real system. This model and the set of equations to be derived is due to Goodwin [2].

In the diagram, L_i represents one of the genetic locus which synthesizes mRNA in quantities represented by X_i. This mRNA then directs the synthesis of protein Y_i through the cellular structure R(ribosomes). The protein then travels to some cellular locus C, where it exerts an influence upon the metabolic state either by enzyme action or by some other means. The result of this activity is the generation of a metabolic species in quantity M_i. A part of M_i returns to the genetic locus and closes the control loop. Here (i.e. at the genetic locus) it is assumed to repress the activity of the gene, probably in association with a macromolecule, the aporepressor. Sometimes a separate operator locus exist for the control of genetic activity. In the present discussion that is also included as a part of L_i itself.

The rate equations for the concentration of the ith species of protein is of the form

$$\frac{dY_i}{dt} = f_i(X_i, Y_i, M_i) - g_i(Y_i, M_i) \qquad (1)$$

where f_i is the function describing the rate of synthesis of protein and g_i relates to the rate of its degradation. The simplest functions due to Goodwin are

$$\frac{dY_i}{dt} = \alpha_i X_i - \beta_i \qquad (2)$$

α and β are parameters containing rate constants. Here the of degradation has been taken as constant, i.e. no self damping present in the system. The first term in (2) represents mRNA controlled protein synthesis.

The concentration of metabolite M_i was assumed to be controlled by the concentration of protein Y_i, and so the equation is

$$\frac{dM_i}{dt} = \gamma_i Y_i - \delta_i \tag{3}$$

Again we have considered the rate of degradation of M_i to be constant.

The control equation for mRNA synthesis is more complicated due to the presence of the (repression by the) metabolite feedback. Under the assumption that the repressor complex acts on the DNA template by a surface absorption process similar to the action of inhibitors in enzymatic activity, Goodwin has written the rate equation for mRNA in the following nonlinear form

$$\frac{dX_i}{dt} = \frac{a_i}{A_i + k_i[M_i - S_i]} - b_i \tag{4}$$

The parameter k_i involves an equilibrium constant for the reaction between DNA and the repressor. For details of the derivation of (4), one may refer reference [2]. Here a_i, A_i, k_i and b_i are all constants. S_i in equation (4) is the storage capacity of the metabolic pool of ith species, and the excess $[M_i - S_i]$ is fed back to act as a repressor.

Goodwin has also proved, by taking into consideration of the idea of the relaxation times of the epigenetic and metabolic system in the equations (2), (3) and (4), that it can be simplified to two equations, (refer [2]) for a particular species,

$$\frac{dX}{dt} = \frac{a}{A + kY} - b \qquad (5)$$

$$\frac{dY}{dt} = \alpha X - \beta$$

It is possible to combine these two equations in (5) into a single equation and integrate to get the result

$$\alpha \frac{X^2}{2} - \beta X + bY - \frac{a}{k} \log(A + kY) = \text{constant.}$$

This equation defines a closed trajectory in the X-Y space and hence we can infer that the system described by the set of equations in (5) is oscillatory.

The system described above is generalised by introducing self damping terms in the rate equations for each variable. This means that mRNA and protein are turning over at rates proportional to their concentrations in the cell. This assumption is more realistic than the condition of constant degradation rates. The feedback signal can also be generalised by the quantity

$$[M_1 - S_1]^{\rho}$$

where ρ is a positive integer. (recently it has been conjectured that ρ can be a non integer also) and greater than one. The power ρ can be associated with what is called Hill number in enzyme kinetics which gives a quantity related to the number of binding sites for the feedback signal or the number of molecules of the metabolite taking part in the repression. So the general form of the three step control equations can be written as

$$\frac{dX_1}{dt} = \frac{a}{A + kX_3^{\rho}} - bX_1$$

$$\frac{dX_2}{dt} = \alpha X_1 - \beta X_2 \qquad (6)$$

$$\frac{dX_3}{dt} = \gamma X_2 - \delta X_3$$

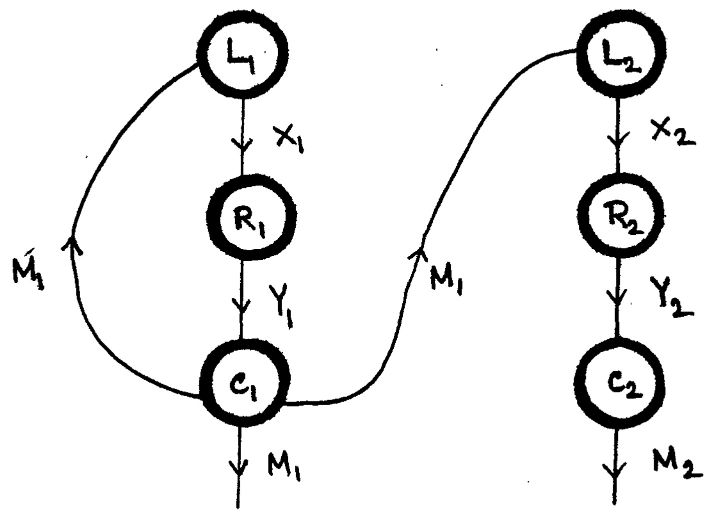
Equation (6) can be generalised to a n-step process

$$\frac{dX_1}{dt} = \frac{a}{A + kx_n^{\rho}} - b_1X_1$$

$$\frac{dX_j}{dt} = g_{j-1}\, x_{j-1} - b_jx_j \qquad (7)$$

where $j = 2,3,\ldots,n$ and $b_1 = g_j$, a, A, k, g_j and b_j are all positive reaction constants.

This was the simplest kind of model considered. There can be more complex control circuits. There are many structural proteins which are metabolically inactive (inert) and cannot generate a feedback repression signal, yet there is a control in its synthesis. The model for that can be as given below:



Here metabolite M_1, which is controlled by enzyme Y_1, acts to repress not only the activity of locus L_1, but also that of the locus L_2, the structural gene for the metabolically inactive protein Y_2. The set of equations describing the control scheme is (for $\rho = 1$)

$$\frac{dX_1}{dt} = \frac{a_1}{A_1 + k_1 Y_1} - b_1 X_1$$

$$\frac{dY_1}{dt} = \alpha_1 X_1 - \beta_1 Y_1$$

$$\frac{dX_2}{dt} = \frac{a_2}{A_2 + k_2 Y_1} - b_2 X_2$$

$$\frac{dY_2}{dt} = \alpha_2 X_2 - \beta_2 Y_2$$

The second system can be considered as a "driven" oscillator. The solutions become harder as one goes over to more complex models.

At this point let us consider certain points on the limitation of the theory.

All the variables has been treated as continuous variables without estimating the size of X_i and Y_i. It is possible that the amount of mRNA of a particular informational species may need stochastic representation. Nothing also has been said about systems with self-replicating mRNA species, the possible influence of metabolites at the ribosomal level, etc. However, the model has the essential features of the system and is also simple enough to study a class of biochemical reactions controlled by feedback mechanism.

Several attempts [3,4,5] have been made to see under what conditions the system (6) yields stable and periodic solutions. Most of the attempts involve computer simulation. The describing function technique has been used by P.E. Rapp [6,7] in first and second order only, where the author has found that for $\rho = 1$, no limit cycle exists for general n. For $\rho = 2$, he has found an inequality condition for the existence of stable limit cycle. Use of Lyapunov Analysis has also been done by Biswas, Pande and Rao [8] for arbitrary ρ and it has been found that for $n = 3$, ρ must be greater than 8 for a limit cycle to exist.

In another paper [9], the same authors have found a relation between n, i.e. the number of reaction steps, and ρ i.e. the number of molecules of the inhibitor. They have also found an additional constraint equation inter-relating the reaction constants.

Computer simulation has been extensively used in the study of biological and biochemical oscillators. Here one assumes some specific values for the parameters present in the differential equations representing the system and studies the behaviour of the variables. Goodwin has studied [3] the set of equations (6) for $\rho = 1$ in an analogue computer and found that there were periodic oscillations in the concentration variables. But it is shown by both Rapp and BPR that no oscillation is possible for $\rho = 1$ (for any n). Later Goodwin has also admitted that his results were artifactual. Thus computer simulation results are not very dependable unless they are supported theoretically.

In this paper, both describing function method and the method of Lyapunov analysis will be studied in relation to biochemical oscillators, comparing and contrasting the results obtained by the respective workers.

CHAPTER-II

DESCRIBING FUNCTION METHOD : ITS USE IN BIOCHEMICAL OSCILLATOR PROBLEM

This method has been extensively used for stability analysis and investigation of sustained non-linear oscillations in engineering problems of control system analysis. It is discussed elsewhere in detail [10,11]. P.E. Rapp [6] has used it to study the non-linear biochemical control equations introduced by Goodwin where one observes sustained oscillations.

This technique is applicable to systems where the non-linearity is isolated from the rest of the dynamics. The whole system is represented by a block diagram (Fig. 1), where G(p) is the linear component of the system (p is the differential operator $\frac{d}{dt}$) and f(z) is the nonlinear element, z being the independent variable. The linear part G(p) is known as the

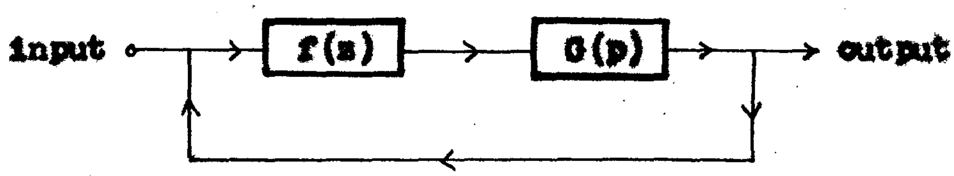


Fig. 1

transfer function. The basic interest is to determine whether the system can exhibit periodic oscillations. The problem may be reformulated so that it is required to determine the conditions that should be satisfied by $G(p)$ and $f(z)$ for the periodic oscillations to exist. To solve the problem by describing function approach, it is necessary to make the basic assumption that the variable $z = z(t)$ appearing in the nonlinear function $f(z)$ is sufficiently close to a sinusoidal oscillation; that is

$$z \simeq y + x \cos \omega t \tag{1.1}$$

where x is the amplitude of the oscillation and y is the mean value of the output, both being positive and real with the condition $y > |x| > 0$. ω is the frequency of the oscillation. Describing function analysis, therefore belong to those methods of solving nonlinear differential equations which are based upon an assumed solution. Then it is required to determine the conditions under which the desired oscillations occur.

Now if the variable z has the sinusoidal form, then the nonlinear function $f(z)$ is in general complex, but also a periodic function of time. Therefore $f(z)$ can be approximated by a fourier series of first order:

$$f(z) = \frac{a_0}{2} + a_1 \cos \omega t + a_2 \sin \omega t \tag{1.2}$$

where

$$a_0 = \frac{1}{\pi} \int_0^{2\pi} f(y + x \cos\theta) d\theta$$

$$a_1 = \frac{1}{\pi} \int_0^{2\pi} f(y + x \cos\theta)\cos\theta \, d\theta \qquad (1.3)$$

$$a_2 = \frac{1}{\pi} \int_0^{2\pi} f(y + x \cos\theta)\sin\theta \, d\theta$$

and $\theta = \omega t$.

To proceed further with this method we need the nonlinear differential equations describing the system. Here the reaction system to be studied is given by equations (7) with a little change in the parameters.

$$\frac{dS_1}{dt} = \frac{K}{1 + \alpha(S_n)^\rho} - b_1 S_1$$

$$\frac{dS_2}{dt} = g_1 S_1 - b_2 S_2$$

$$\vdots \qquad (1.4)$$

$$\frac{dS_n}{dt} = g_{n-1} S_{n-1} - b_n S_n$$

where $K = \frac{a}{A}$, $\alpha = \frac{k}{A}$ and S_j = concentration of the jth chemical species.

The above system of n equations has to be reduced to a single nth order equation in S_n, for the application of the describing function method.

The first equation of (1.4) can be written as

$$(p + b_1)S_1 = \frac{K}{1 + \alpha S_n^{\rho}} \quad \text{where} \quad p = \frac{d}{dt}$$

or

$$S_1 = \frac{K}{(1 + \alpha S_n^{\rho})(p + b_1)} \tag{1.5}$$

Using (1.5) in the second equation of (1.4), we get

$$(p + b_2)S_2 = g_1 \frac{K}{[1 + \alpha S_n^{\rho}]\,(p + b_1)}$$

or $$S_2 = \frac{g_1 K}{[1 + \alpha S_n^{\rho}]\,(p + b_1)(p + b_2)}$$

performing similar substitutions for n equations, the last equation of (1.4) becomes,

$$(p + b_1)(p + b_2)\ldots(p + b_n)S_n = \frac{(g_1\, g_2 \ldots g_{n-1})K}{[1 + \alpha S_n^{\rho}]} \tag{1.6}$$

The characteristic equation of (5) is

$$(p + b_1)(p + b_2)\ldots(p + b_n) = 0$$

whose eigenvalues are $-b_1, -b_2, \ldots, -b_n$, which are all negative since b_j's are all positive.

Equation (1.6) can be written in the form

$$\frac{1}{G(p)}\, z - f(z) = 0 \tag{1.7}$$

where

$$G(p) = \frac{1}{(p + b_1)(p + b_2)\ldots.(p + b_n)} = \prod_{i=1}^{n} \frac{1}{(p + b_i)} \tag{1.8}$$

$$f(z) = \frac{d_1}{1 + d_2\, z^{\rho}} \tag{1.9}$$

and $z = S_n$; $d_1 = (g_1\, g_2 \ldots.g_{n-1})K$, $d_2 = \alpha$. Thus equation (1.7) is the differential equation representing the system.

Now assuming periodic solution exists and using (1.2), equation (1.7) becomes

$$\frac{1}{G(p)}\,(y + x \cos \omega t) - \left(\frac{a_0}{2} + a_1 \cos \omega t + a_2 \sin \omega t\right) = 0$$

The balance equation corresponding to zeroeth harmonic is obtained by setting $\omega = 0$, i.e.,

$$\frac{1}{G(0)}\, y - \frac{a_0}{2} = 0$$

or

$$1 - G(0)\,\frac{a_0}{2y} = 0 \tag{1.10}$$

For first harmonic, the balance equation is (putting $p = i\omega$).

$$\frac{1}{G(i\omega)} x \cos \omega t - a_1 \cos \omega t - a_2 \sin \omega t = 0$$

Using the complex representation of cos ωt and sin ωt, one gets the following equation and its complex conjugate equation also:

$$\frac{1}{G(i\omega)} \frac{x}{2} e^{i\omega t} - \left(\frac{a_1 - ia_2}{2} \right) e^{i\omega t} = 0$$

or $$1 - (a_1 - ia_2) \frac{G(i\omega)}{x} = 0$$

or $$1 - G(i\omega) P_n(x, y(x)) = 0 \qquad (1.11)$$

where $P_n(x,y(x)) = \dfrac{a_1 - ia_2}{x}$

$P_n(x,y(x))$ is defined as the describing function of the non-linearity.

Equation (1.11) can be solved graphically by finding the intersection of the two curves defined by $G(i\omega)$ and $\dfrac{1}{P_n(x,y(x))}$ in the complex plane. If equation (1.11) has no solution, then no oscillations are possible, unless the system deviates significantly from the assumptions under which this method is applicable.

Now if $f(x)$ is a single valued function, then $a_2 = 0$, and $P_n(x,y(x))$ is real. The proof of this is the following:

Changing the variable to $\eta = y + x \cos\theta$, the integral defining a_2 becomes

$$a_2 = -\frac{1}{\pi x}\int_x^x f(\eta)d\eta$$

This is zero if $f(x)$ is a single valued function.

For symmetric $f(x)$, $a_o = 0$.

On the complex plane both $G(i\omega)$ and $\frac{1}{P_n(x,y(x))}$ are plotted as ω and x increases from zero to infinity. If the describing function contour ($\frac{1}{P_n(x,y(x))}$ contour) intersects the frequency response locus of the linear component ($G(i\omega)$ contour), then the balance equations (1.10) and (1.11) are satisfied and a limit cycle results, i.e. the system exhibits periodic oscillation. The stability of the limit cycle can also be predicted from the figure. Let us consider an example whose plot is given by Figure 2. A point is said to be inside the $G(i\omega)$ contour if

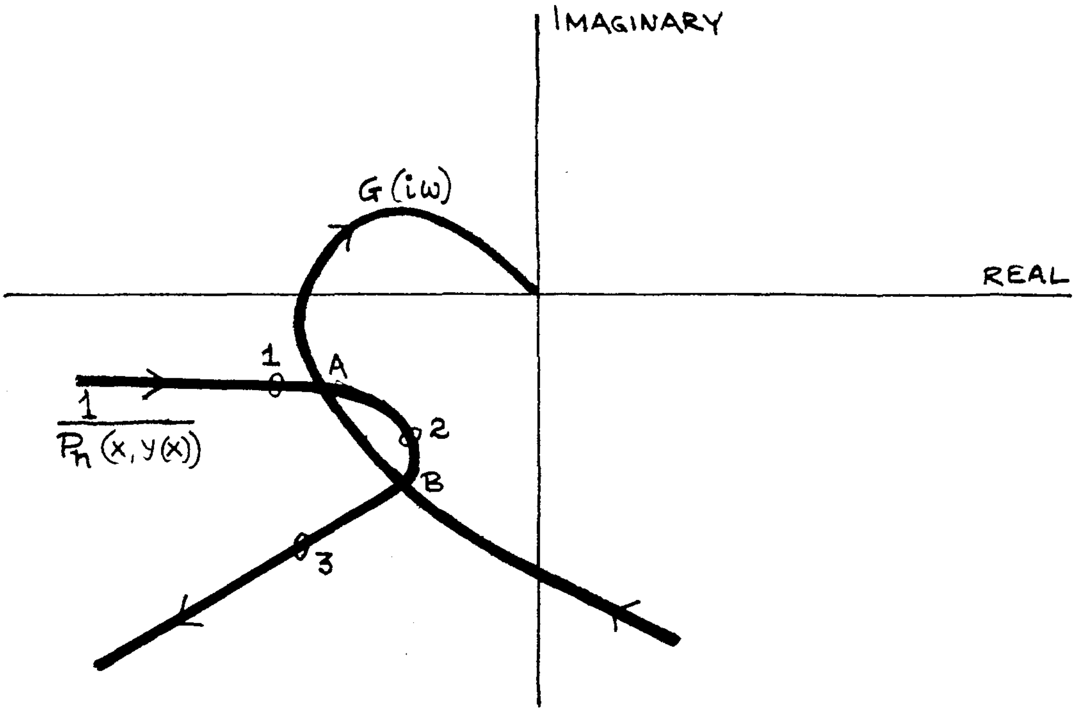


Fig. 2

it is to the right side of the curve. Each point of the $\frac{1}{F_n(x,y(x))}$ contour describes a state of the system. The system moves along the curve until it approaches a stable singular point or a stable limit cycle. If the point is inside the $G(i\omega)$ contour then the corresponding state is unstable and the amplitude x increases, i.e. the system moves along the $\frac{1}{F_n(x,y(x))}$ line in the direction of increasing x. A stable state arises when the point is not contained in the $G(i\omega)$ contour and the system moves along the $\frac{1}{F}$ curve in the direction of decreasing x. In the Figure A and B are intersection points giving rise to limit cycles. A system initially at points 1 and 3 will move to the left, the direction of decreasing x, since it is outside $G(i\omega)$ contour. A system at 2 will always move to the right, the direction of increasing x, since it is inside $G(i\omega)$ curve. Thus the intersection A corresponds to an unstable limit cycle, since if the system is perturbed to either left or right it will move away from A. Intersection B corresponds to a stable limit cycle because the perturbation will always tend the system to move towards B. The value of x at the intersection indicates the amplitude of the oscillation and it is such that y(x) is always greater than x, otherwise a limit cycle will not satisfy the condition of positivity of the concentrations.

This technique has got very good descriptive power to investigate the changes in behaviour of the system resulting from a variation in the value of a parameter (reaction constant, say). A change in a parameter causes both the curves to move relative to each other and to the co-ordinates, sometimes causing an intersection to appear or vanish. Sometimes only one of the curve move, making the interpretation of the effect of the change even easier. Without having point by point numerical solution of the differential equations, one can estimate the behaviour for parameter values more readily by plotting a few graphs. The internal structure of the equations under study can be interpreted readily by graphical means.

Accuracy of this method may be challanged because one is approximating the function by Fourier terms of zero and first order only. But the linear component acts in such a way that it suppresses the higher components and let the smaller frequency components to pass. In other words, it acts as a low pass filter. It has been observed that, given well behaved linear components the low pass filter effect becomes greater with increasing size of the system. Thus for the case of arbitrarily large linear component system, though the results cannot be stated with mathematical certainty, yet it can be argued that as an approximation it is probably accurate.

Now let us proceed with out problem. First we study $G(p)$ for general n:

The transfer function $G(p)$ is given by

$$G(p) = \frac{1}{(p + b_1)(p + b_2)\ldots.(p + b_n)}$$

So

$$G(i\omega) = \frac{1}{(b_1 + i\omega)(b_2 + i\omega)\ldots.(b_n + i\omega)} \tag{1.12}$$

At $\omega = 0$,

$$G(0) = \frac{1}{b_1 b_2 \ldots. b_n} = \frac{1}{c_o} \tag{1.13}$$

$G(0) > 0$ $\quad c_o > 0$ since b_i's are positive.

$G(i\omega)$ can be written in the form

$$G(i\omega) = M(\omega)e^{-i\phi(\omega)}$$

where $M(\omega)$ is the magnitude given by

$$M(\omega) = |G(i\omega)| = \sqrt{G(i\omega)G^*(i\omega)}$$

$$= \prod_{i=1}^{n} \frac{1}{(b_i^2 + \omega^2)^{\frac{1}{2}}} \tag{1.14}$$

and ϕ is the sum of the phase angles of the individual n species (superposition principle is valid since we are considering the linear component only).

$$\phi = \phi_1 + \phi_2 + \phi_3 + \ldots. + \phi_n = \tan^{-1}\left(\frac{\omega}{b_1}\right) + \tan^{-1}\left(\frac{\omega}{b_2}\right) + \ldots.$$

$$\ldots. + \tan^{-1}\left(\frac{\omega}{b_n}\right) \tag{1.15}$$

From equation (1.12) it is easy to see that $M(\omega)$ decreases monotonically to zero as ω increases from 0 to ∞.

Since b_j's and ω are positive, therefore $\left(\frac{\omega}{b_j}\right)$ is always a positive quantity. Hence ω always increases as ω increases from zero and the curve $G(i\omega)$ moves in a clockwise direction (since the phase factor is $e^{-i\phi}$). The first intersection with the real axis is at $\omega = 0$ where $G(0) = \frac{1}{c_0}$ is positive. Second intersection is at $\phi = \pi$ where

$$G(i\omega)\big|_{\phi = +\pi} = -M(\omega)\big|_{\phi=\pi} \quad (\quad e^{\pm i\pi} = -1)$$

Now both b_j and ω are positive, therefore $M(\omega)$ is always a non-negative quantity. Hence $-M(\omega)$ is negative and the second intersection of $G(i\omega)$ is with the negative real axis. As ω goes on increasing, magnitude of $G(i\omega)$, i.e. $M(\omega)$, decreases and continuous increasing of ϕ will give the crossing of $G(i\omega)$ with the real axis alternatively in positive ($\phi = 0, 2\pi, 4\pi$, etc.) and negative real axis. The contour for $G(i\omega)$ or the frequency response locus of the linear component is given in Fig. 3.

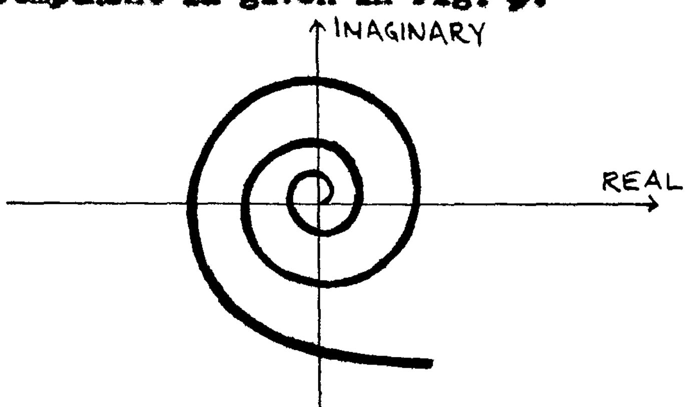


Fig. 3

To find the limit cycle for this problem, we need to calculate the describing function. Keeping all the parameters constant, one would get different contour for $\frac{1}{P_n(x,y(x))}$ for different ρ.

We first consider the case where $\rho = 1$.

Therefore $$f(z) = \frac{d_1}{1 + d_2 z} \qquad (1.16)$$

In this case $f(z)$ is a single valued function. Therefore $a_2 = 0$. The other fourier coefficients are,

$$a_0 = \frac{1}{\pi}\int_0^{2\pi} \frac{d_1}{1 + d_2(y + x\cos\theta)} d\theta$$

$$= \frac{d_1}{\pi}\int_0^{2\pi} \frac{d\theta}{(1+d_2 y) + (d_2 x)\cos\theta}$$

$$= \frac{d_1}{\pi}\int_0^{2\pi} \frac{d\theta}{a+b\cos\theta} \quad \text{where } a = 1 + d_2 y \text{ and } b = d_2 x$$

$$= \frac{d_1}{\pi}\frac{2\pi}{\sqrt{(a^2-b^2)}} = \frac{2d_1}{\{(1+d_2 y)^2-(d_2 x)^2\}^{\frac{1}{2}}} \qquad (1.17)$$

using the standard integral for $\int_0^{2\pi} \frac{d\theta}{a + b\cos\theta}$

$$a_1 = \frac{1}{\pi}\int_0^{2\pi} \frac{d_1 \cos\theta\, d\theta}{1 + d_2(y+x\cos\theta)} = \frac{d_1}{\pi}\int_0^{2\pi} \frac{\cos\theta}{a + b\cos\theta}$$

$$= \frac{d_1}{b\pi}\int_0^{2\pi} \frac{(a+b\cos\theta)d\theta}{a+b\cos\theta} - \frac{d_1}{b\pi}\int_0^{2\pi} \frac{a\, d\theta}{a+b\cos\theta}$$

$$= \frac{2d_1}{b} - \frac{2d_1 a}{b} \frac{1}{\sqrt{a^2 - b^2}}$$

$$= \frac{2d_1}{d_2 x} - \frac{2d_1}{d_2 x} \frac{(1 + d_2 y)}{[(1+d_2 y)^2 - (d_2 x)^2]^{\frac{1}{2}}} \tag{1.18}$$

Using (1.13) and (1.17), the balance of equation (1.10) gives y as a function of x,

$$1 - \frac{1}{c_o} \frac{2d_1}{[(1+d_2 y)^2 - (d_2 x)^2]^{\frac{1}{2}}} \frac{1}{2y} = 0$$

or,

$$\frac{d_1^2}{(c_o y)^2} \frac{1}{[(1+d_2 y)^2 - (d_2 x)^2]} = 1$$

or,

$$(c_o y)^2 (1+d_2 y)^2 - (c_o y)^2 (d_2 x)^2 - d_1^2 = 0 \tag{1.19}$$

The definition of $P_n(x, y(x))$ gives

$$P_n(x, y(x)) = \frac{a_1}{x}$$

Therefore

$$\frac{1}{P_n(x, y(x))} = \frac{x}{\frac{2d_1}{d_2 x} - \frac{2d_1}{d_2 x} \frac{(1 + d_2 y)}{[(1+d_2 y)^2 - (d_2 x)^2]^{\frac{1}{2}}}}$$

$$= \frac{d_2 x^2 [(1+d_2 y)^2 - (d_2 x)^2]^{\frac{1}{2}}}{2d_1 [(1+d_2 y)^2 - (d_2 x)^2]^{\frac{1}{2}} - 2d_1 (1+d_2 y)} \tag{1.20}$$

Our aim is to draw $\frac{1}{P_n(x,y(x))}$ curve and find the intersections with $G(i\omega)$ contour.

To draw $\frac{1}{P}$ curve, we do the following things:

a) Find out the limit of $\frac{1}{P}$ as $x \to 0$.

b) Show $\frac{1}{P}$ is a monotonically increasing function of positive x.

c) Evaluate the limit of $\frac{1}{P_n}$ as $x \to \infty$.

a) Let $y \to y_o$ as $x \to 0$, i.e. y_o is the value of y as x tends to zero.

Therefore $$\lim_{x \to 0} \frac{1}{P_n(x,y(x))} = \lim_{y \to y_o} \frac{1}{P(y)}$$

Now from (1.19) one gets, with $x = 0$,

$$(c_o y_o)(1 + d_2 y_o) - d_1 = 0 \tag{1.21a}$$

or $$c_o d_2 y_o^2 + c_o y_o - d_1 = 0$$

or $$y_o = \frac{-c_o + \sqrt{c_o^2 + 4c_o d_1 d_2}}{2c_o d_2} \tag{1.21b}$$

Equation (1.19) also gives

$$d_2 x^2 = \frac{(1+ d_2 y)^2}{d_2} - \frac{d_1^2}{d_2(c_o y)^2} \tag{1.22}$$

Therefore equation (1.20) becomes (using (1.19) and (1.22))

$$\frac{1}{P_n(x,y(x))} = \frac{\left[\frac{(1+d_2 y)^2}{d_2} - \frac{d_1^2}{d_2(c_o y)^2} \right] \frac{d_1}{c_o y}}{2d_1 \frac{d_1}{c_o y} - 2d_1(1 + d_2 y)}$$

or,
$$\frac{1}{P_n} = \frac{\dfrac{d_1}{c_o y}\left[\dfrac{(c_o y)^2(1+d_2 y)^2 - d_1^2}{(c_o y)^2}\right]}{d_1\left(\dfrac{2d_1}{c_o y} - 2(1+d_2 y)\right)}$$

$$= \frac{(c_o y)^2(1+d_2 y)^2 - d_1^2}{(c_o y)^2\, 2d_1 d_2 - 2(c_o y)^3\, d_2(1+d_2 y}$$

$$= \frac{((c_o y)(1+d_2 y) + d_1)((c_o y)(1+d_2 y) - d_1)}{-2d_2 (c_o y)^2 (c_o y(1+d_2 y) - d_1)}$$

$$= -\frac{((c_o y)(1+d_2 y) + d_1)}{(c_o y)^2\, 2d_2} \tag{1.23}$$

Taking the limit as $y \to y_o$ and using (1.21a) one gets

$$\lim_{y \to y_o} \frac{1}{P(y)} = -\frac{1}{2d_2}\left(\frac{d_1 + d_2}{\dfrac{d_1^2}{(1+d_2 y_o)^2}}\right) = -\frac{(1+d_2 y_o)^2}{d_1 d_2} \tag{1.24}$$

(b) If we can show that for $x_a < x_b$, $y(x_a)$ is less than $y(x_b)$, then $\frac{1}{P(x,y(x))}$ can be shown to be monotonically increasing function of x. One can do it by calculating $\frac{dy}{dx}$ and showing it to be positive, but here another method is used.

Solving equation (1.19) for x as a function of y,

$$x = \frac{1}{d_2}\left[(1+d_2y)^2 - \frac{d_1^2}{(c_oy)^2}\right]^{\frac{1}{2}} \tag{1.25}$$

This is an increasing function of y as d_2 is positive. From (1.23),

$$\frac{1}{P(y)} = -\frac{(c_oy)(1+d_2y)}{2d_2(c_oy)^2} - \frac{d_1}{2d_2(c_oy)^2}$$

$$= -\frac{1}{2c_o}\left(1+\frac{1}{d_2y}\right) - \frac{d_1}{2d_2(c_oy)^2} \tag{1.26}$$

If $y_a < y_b$, then

$$-\frac{1}{2c_o}\left(1+\frac{1}{d_2y_a}\right) < -\frac{1}{2c_o}\left(1+\frac{1}{d_2y_b}\right)$$

and (1.27)

$$-\frac{d_1}{2d_2(c_oy_a)^2} < -\frac{d_1}{2d_2(c_oy_b)^2}$$

therefore when $y_a < y_b$, then $\frac{1}{P(y_a)} < \frac{1}{P(y_b)}$

Therefore $\frac{1}{P_n(x,y(x))}$ or $\frac{1}{P(y)}$ is a monotonically increasing function of x.

(c) From (1.19) it is obvious that as $x \to \infty$, $y(x) \to \infty$. Thus

$$\lim_{x\to\infty} \frac{1}{P_n(x,y(x))} = \lim_{y\to\infty} \frac{1}{P(y)} = -\frac{1}{2c_o} \tag{1.28}$$

Thus we have found out the behaviour of the $\frac{1}{P_n(x,y(x))}$ curve as x increases from 0 to ∞. Now $\frac{1}{P_n(x,y(x))}$ starts of a negative number (equation (1.24)) and increases continuously along the negative real axis. The $G(i\omega)$ locus has been shown to spiral into the origin in a clockwise direction. Thus if any intersection between the two curves occurs it is of the form shown in Figure 4. But intersection of this type gives rise to unstable limit cycle.

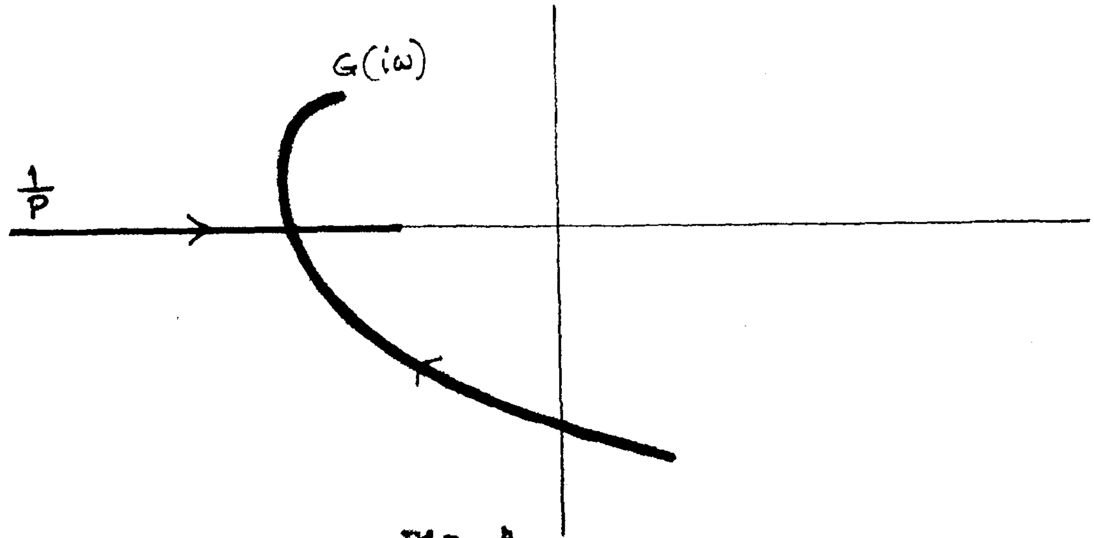


Fig. 4

Thus for $\rho = 1$ and general n, no stable limit cycle result for any values of the parameters b_j, g_1, α and k for the reaction system described by equation (1.4). Rapp in his paper has also chocked that for n = 3 and 4 even unstable limit cycles do not appear. Since protein synthesis is also a three step process (according to Goodwin), then there will be no limit cycle for $\rho = 1$.

For n = 3,

$$G(p) = \frac{1}{(p+b_1)(p+b_2)(p+b_3)} = \frac{1}{p^3 + c_2p^2 + c_1p + c_o}$$

where

$$c_o = b_1b_2b_3,\; c_1 = b_1b_2 + b_2b_3 + b_3b_4$$

and $\quad c_2 = b_1 + b_2 + b_3 \qquad (1.29)$

$$G(i\omega) = \frac{1}{(i\omega)^3 + c_2(i\omega)^2 + c_1(i\omega) + c_o} = \frac{1}{-i\omega^3 - c_2\omega^2 + c_1 i\omega + c_o}$$

$$= \frac{1}{(c_o - c_2\omega^2) - i(\omega^3 - c_1\omega)}$$

$$= \frac{(c_o - c_2\omega^2) + i(\omega^3 - c_1\omega)}{(c_o - c_2\omega^2)^2 + (\omega^3 - c_1\omega)^2} \qquad (1.30)$$

The points where $G(i\omega)$ curve intersects the real axis, there

$$I_m G(i\omega) = 0$$

or $\omega^2 - c_1 = 0$ or $\omega = 0$ or $\sqrt{c_1}$ (ω is positive)

For $\omega = 0$, $G(i\omega) = \frac{1}{c_o}$ and for $\omega = c_1$, $G(i\omega) = \frac{1}{c_o - c_1 c_2}$

From (1.29), $c_1 c_2 = (b_1 b_2 + b_1 b_3 + b_2 b_3)(b_1 + b_2 + b_3)$

$$= \Delta + 3c_o$$

where $\Delta = b_1(b_1 b_3 + b_1 b_2) + b_2(b_1 b_2 + b_2 b_3) + b_3(b_1 b_3 + b_2 b_3)$

and $\Delta > 0$, therefore b_j's are positive.

Therefore $-\Delta < 0$.

Now $$\frac{1}{-2c_o - \Delta} = -\frac{1}{2c_o + \Delta} > -\frac{1}{2c_o}$$

$$-\frac{1}{2c_o} < \frac{1}{c_o - c_1 c_2} \qquad (1.31)$$

Since both $\frac{1}{c_o}$ and $\frac{1}{c_o - c_1 c_2}$ are greater than the highest value of $\frac{1}{F_n(x, y(x))}$ (i.e. $-\frac{1}{2c_o}$), therefore there is no intersection between $G(i\omega)$ and $\frac{1}{P_n}$; hence there is no limit cycle of any kind stable or unstable.

For $n = 4$, the same thing happens. Therefore the results of the first paper of Rapp are as follows:

i) with $\rho = 1$, general n - no stable oscillation is possible.

and

ii) with $\rho = 2$, $n = 3,4$ - even unstable oscillation is impossible.

Hence, since biochemical oscillators execute stable oscillations only, therefore one must consider ρ greater than one cases.

Rapp in his second paper [7] has dealt with the case of $\rho = 2$. Let us discuss his results.

The frequency response locus will be the same as for $\rho = 1$, but the describing function contour will be different.

In this case,

$$f(z) = \frac{d_1}{1 + d_2 z^2} \tag{1.32}$$

and

$$G(p) = \frac{1}{(p+b_1)(p+b_2)\ldots.(p+b_n)}$$

The expressions for a_0 and a_1 are

$$a_0 = \frac{1}{\pi}\int_0^{2\pi} \frac{d_1}{1 + d_2(y + x\cos\theta)^2}\, d\theta$$

and

$$a_1 = \frac{1}{\pi}\int_0^{2\pi} \frac{d_1 \cos\theta\, d\theta}{1 + d_2(y + x\cos\theta)^2} \tag{1.33}$$

To derive the above integrals we make use of the following standard integrals:

$$\int_0^{2\pi} \frac{d\theta}{v + \cos\theta} = \frac{2\pi}{(v^2-1)^{\frac{1}{2}}} \qquad v \in R \text{ and } v > 1 \tag{1.34}$$

i.e. $\sqrt{v^2-1}$ is chosen from that branch of the two value function which is always positive when v is real and greater than one, and

$$\int_0^{2\pi} \frac{\cos\theta \, d\theta}{\alpha + \beta \cos\theta} = \frac{2\pi}{\beta} - \frac{2\pi\alpha}{\beta(\alpha^2-\beta^2)^{\frac{1}{2}}}, \quad \frac{\alpha}{\beta} \notin [-1,1] \tag{1.35}$$

Now, if $1 + (\alpha+\beta\cos\theta)^2 = 0$, then

$$\frac{1}{1 + (\alpha+\beta\cos\theta)^2} = \frac{1}{(\alpha+\beta\cos\theta + i)(\alpha+\beta\cos\theta - i)}$$

$$= \frac{1}{2i}\left[\frac{1}{\alpha + \beta\cos\theta - i} - \frac{1}{\alpha + \beta\cos\theta + i} \right]$$

$$= \frac{1}{(2+2i\alpha) + 2i\beta\cos\theta} - \frac{1}{(2i\alpha-2) + 2i\beta\cos\theta}$$

$$= \frac{1}{(2+2i\alpha) + 2i\beta\cos\theta} + \frac{1}{(2-2i\alpha) - 2i\beta\cos\theta} \tag{1.36}$$

The equation for a_o becomes, using $\alpha = \sqrt{d_2}y$ and $\beta = \sqrt{d_2}x$,

$$a_o = \frac{d_1}{\pi}\int_0^{2\pi} \frac{d\theta}{(1 + (\alpha+\beta\cos\theta)^2}$$

$$= \frac{d_1}{\pi}\left[\int_0^{2\pi} \frac{d\theta}{(2+2i\alpha)+2i\beta\cos\theta} + \int_0^{2\pi} \frac{d\theta}{(2-2i\alpha)-2i\beta\cos\theta}\right] \text{ using}(1.36)$$

$$= \frac{d_1}{\pi}\frac{1}{2i\beta}\left[\int_0^{2\pi} \frac{d\theta}{\frac{2+2i\alpha}{2i\beta}+\cos\theta} - \int_0^{2\pi} \frac{d\theta}{(\frac{-2+2i\alpha}{2i\beta})+\cos\theta}\right]$$

$$= \frac{d_1}{\pi}\frac{1}{2i\beta}\left[\frac{2\pi}{\sqrt{(\frac{1+i\alpha}{i\beta})^2-1}} - \frac{2\pi}{\sqrt{(\frac{-1+i\alpha}{i\beta})^2-1}}\right] \text{ using } (1.34)$$

$$= \frac{d_1}{i\beta}\left[\frac{1}{\sqrt{\frac{-(1+i\alpha)}{\beta^2}-1}} - \frac{1}{\sqrt{\frac{-(-1+i\alpha)^2}{\beta^2}-1}}\right]$$

$$\left\{\begin{array}{l} -1 = (-i)^2 \text{ for the first term and} \\ -1 = (i)^2 \text{ for the second term} \end{array}\right\}$$

$$= d_1\left[\frac{1}{\sqrt{(1+i\alpha)^2+\beta^2}} + \frac{1}{\sqrt{(-1+i\alpha)^2+\beta^2}}\right]$$

$$= d_1 \frac{(\sqrt{(1+i\alpha)^2+\beta^2} + \sqrt{(-1+i\alpha)^2+\beta^2})}{\sqrt{(\alpha^2-\beta^2+1)^2+4\beta^2}}$$

$$= \frac{d_1}{\sqrt{K}}\left[\sqrt{\frac{(1-\alpha^2+\beta^2)+2i\alpha}{K}} + \sqrt{\frac{(1-\alpha^2+\beta^2)-2i\alpha}{K}}\right]$$

where $K = \sqrt{(\alpha^2-\beta^2+1)^2+4\beta^2} = \sqrt{(d_2x^2-d_2y^2+1)^2+4d_2y^2}$

Therefore $$a_o = \frac{d_1}{K}\,[e^{i\gamma/2} + e^{-i\gamma/2}]$$

$$= \frac{2d_1}{K^{\frac{1}{2}}}\cos\gamma/2 \qquad (1.38)$$

where $$\cos\gamma = \frac{1-\alpha^2+\beta^2}{K} = \left(\frac{d_2x^2 - d_2y^2 + 1}{K}\right)$$

$$(1.39)$$

and $$\sin\gamma = \frac{2\alpha}{K} = \frac{2\sqrt{d_2}y}{K}$$

$$a_1 = \frac{d_1}{\pi}\int_0^{2\pi}\frac{\cos\theta\, d\theta}{1 + (\sqrt{d_2}y + \sqrt{d_2}x\cos\theta)^2}$$

$$= \frac{d_1}{\pi}\left[\int_0^{2\pi}\frac{}{(2+2i\alpha)+2i\beta\cos\theta} + \int_0^{2\pi}\frac{\cos\theta\, d\theta}{(2-2i\alpha)-2i\beta\cos\theta}\right] \text{ using } (1.39)$$

where $\alpha = \sqrt{d_2}y$ and $\beta = \sqrt{d_2}x$.

Substituting $a = 2 + 2i\alpha$, $a' = -2 + 2i\alpha$ and $b = 2i\beta$, one gets

$$a_1 = \frac{d_1}{\pi}\left[\int_0^{2\pi}\frac{\cos\theta\, d\theta}{a+b\cos\theta} - \int_0^{2\pi}\frac{\cos\theta\, d\theta}{a'+b\cos\theta}\right]$$

$$= \frac{d_1}{\pi}\left[\frac{2\pi}{b} - \frac{2\pi a}{b\sqrt{(a^2-b^2)}} - \frac{2\pi}{b} + \frac{2\pi a'}{b\sqrt{(a'^2-b^2)}}\right] \text{ using } (1.37)$$

$$= \frac{2d_1}{b}\left[\frac{a'}{\sqrt{(a'^2-b^2)}} - \frac{a}{\sqrt{(a^2-b^2)}}\right]$$

$$= \frac{2d_1}{2i\beta}\left[\frac{2i\alpha-2}{\sqrt{\{(-2+2i\alpha)^2-(2i\beta)^2\}}} - \frac{2+2i\alpha}{\sqrt{\{(2+2i\alpha)^2-(2i\beta)^2\}}}\right]$$

$$= \frac{2d_1}{\beta}\left[\frac{i\alpha-1}{\sqrt{\{(2i\beta)^2-(-2+2i\alpha)^2\}}} - \frac{i\alpha+1}{\sqrt{\{(2i\beta)^2-(2+2i\alpha)^2\}}}\right]$$

$(-i)^2$ taken out from the first term and $(i)^2$ from the second term.

$$= \frac{d_1}{\rho} \; \frac{(i\alpha-1)\sqrt{\{-\rho^2-(1+i\alpha)^2\}} + (i\alpha+1)\sqrt{\{-\rho^2-(-1+i\alpha)^2\}}}{K}$$

where K is given by (1.37)

$$= \frac{d_1}{\rho\sqrt{K}} \; [i\alpha[(i)\frac{\sqrt{\{(1+i\alpha)^2+\rho^2\}}}{\sqrt{K}} + \frac{(i)\sqrt{\{\rho^2+(-1+i\alpha)^2\}}}{\sqrt{K}}]$$

$$- (i)\frac{\sqrt{\{(i\alpha+1)^2+\rho^2\}}}{\sqrt{K}} - (i)\frac{\sqrt{\{\rho^2+(1+i\alpha)^2\}}}{\sqrt{K}}]]$$

$$= \frac{d_1}{\rho\sqrt{K}} \; [-\alpha\,[e^{i\gamma/2} + e^{-i\gamma/2}] \;\; -i[e^{i\gamma/2} - e^{i\gamma/2} \;\;]]$$

$$= \frac{d_1}{\rho\sqrt{K}} \; (2\sin\gamma/2 - 2\alpha\cos\gamma/2\,)$$ where $\sin\gamma/2$ and $\cos\gamma/2$ are given by (1.39)

Therefore $$a_1 = \frac{2d_1}{\sqrt{d_2}x\,K^2}\;\sin\;\gamma/2 - \sqrt{d_2}y\cos\gamma/2\,] \qquad (1.40)$$

Since the integrand of a_o is always positive then $\cos\gamma/2 > 0$. By equation (1.39), sign of $\sin\gamma$ is positive. Thus $\gamma/2$ is confined in the first quadrant $(0, \frac{\pi}{2})$ only.

The zeroeth balance equation (1.10) can be written for this case as

$$0 = 1 - \frac{d_1}{c_o y\,K^2}\,(\,\frac{1+\cos\gamma}{2}\,) \qquad (1.41)$$

where $\cos \gamma/2 = + \left(\frac{1 + \cos \gamma}{2} \right)$, and the form of $\frac{1}{p}$ is

$$\frac{1}{p} = \frac{\sqrt{d_2 x^2}\ K^{\frac{1}{2}}}{2d_1[\sin \gamma/2 - \sqrt{d_2}y \cos \gamma/2]} \tag{1.42}$$

Changing the variables to $w = d_2x^2$, $z = d_2y^2$,

$$A = d_1 \sqrt{d_2}/\sqrt{2}\ c_o \quad \text{and} \quad H = \frac{1}{\sqrt{2}\ d_1 \sqrt{d_2}}$$

equations (1.41) and (1.42) becomes

$$0 = 1 - \frac{A}{z^{\frac{1}{2}} K} (K + w - z + 1)^{\frac{1}{2}} = g(w,z) \tag{1.43}$$

$$\therefore \sqrt{\left(\frac{1+\cos\gamma}{2}\right)} = \sqrt{\left(\frac{K + w - z + 1}{2K}\right)} \tag{1.44}$$

and $$\frac{1}{p} = \frac{w\ K^{\frac{1}{2}}}{2d_1 \sqrt{d_2}\ [(\frac{K - w + z - 1}{2K})^{\frac{1}{2}} - \sqrt{z}(\frac{K + w - z + 1}{2K})^{\frac{1}{2}}]}$$

using the identities

$$\sin \gamma/2 = \sqrt{\left(\frac{1-\cos\gamma}{2}\right)} \text{ and } \cos \gamma/2 = \sqrt{\left(\frac{1+\cos\gamma}{2}\right)}$$

and (1.44)

Therefore $$\frac{1}{p} = \frac{HwK}{(K-w+z-1)^{\frac{1}{2}} - z^{\frac{1}{2}}(K + w - z + 1)^{\frac{1}{2}}} \tag{1.45}$$

where $$K = [(w-z+1)^2 + 4z]^{\frac{1}{2}} \tag{1.46}$$

The initial assumption of $y > x > 0$ now becomes $z > w > 0$. It is to be noted that equations (1.43) and (1.45) contain only two arbitrary positive constants A and H, whereas equations (1.41) and (1.42) contains three d_1, d_2 and c_o.

First one must determine if it is possible to use equation (1.43) to find $z(w)$. When this is impossible, a limit cycle behaviour is also impossible. For w positive and fixed, $g(w,z)$ in equation (1.43) can be treated as a function of z. To show that $g(w,z)$ is montonically increasing with increasing positive z, where $z > w$, one considers the following expression and proves it to be monotonically decreasing,

$$\frac{1}{z^{\frac{1}{2}}\ K^{\frac{1}{2}}}\quad \left(1 + \frac{w-z+1}{K}\right)^{\frac{1}{2}}$$

Since, $\quad \frac{\partial K}{\partial z} = (z-w+1) > 0 \quad \because z > w > 0$

then K is an increasing function of z. y and d_2 both are positive. Therefore $\frac{1}{z^{\frac{1}{2}}\ K^{\frac{1}{2}}}$ is monotonically decreasing function.

Let $\quad M = \left[1 + \frac{w-z+1}{K}\right]$

$$\frac{\partial M}{\partial z} = \frac{-K-(w-z+1)\left(\frac{z-w+1}{K}\right)}{K^2} \qquad \left(\because \frac{\partial K}{\partial z} = \frac{z-w+1}{K}\right)$$

$$= \frac{-K-[1-(w-z)^2]}{K^3} = \frac{-2-2w-2z}{K^3}$$

$$= -\left(\frac{2+2w+2z}{K^3}\right) < 0.$$

Therefore M is a monotonically decreasing function of z.

Therefore $g(w,z) = 1-\{$monotonically decreasing function of $z)$.

Hence $g(w,z)$ is a monotonically increasing function of z, when $z > w > 0$; and it increases to 1.

Let

$$J(w) = g(w,z, = w) = 1 - \frac{A}{w^{\frac{3}{2}}}\left[\frac{(1+4w)^{\frac{1}{2}}+1}{1+4w} \right]^{\frac{1}{2}} \tag{1.47}$$

At $w = 0$, $J(w) \rightarrow -\infty$.

For $w > 0$ and increasing, $J(w)$ increases monotonically to $+1$.

Thus $J(w)$ or $g(w,z = w)$ is negative for w at the interval $w = 0$ to $w = w_m$, where w_m is defined by $J(w_m) = 0$,

or

$$0 = 1 - \frac{A}{w_m^{\frac{3}{2}}}\left[\frac{(1+4w_m)^{\frac{1}{2}}+1}{1+4w_m} \right]^{\frac{1}{2}} \tag{1.48}$$

Since, the function $J(w)$ increases monotonically therefore the root w_m is unique. $w_m = d_2 x_m^2$, hence w_m is a quantity of physical interest as it is related to the maximum possible amplitude of the oscillation.

At $w = 0$, the value of z is z_o and $K = z+1$.

Therefore

$$g(0,z_o) = 1 - \frac{\sqrt{2}\,A}{z_o^{\frac{3}{2}}\,(z_o + 1)} = 0 \tag{1.49}$$

or, $$z_o^3 + 2z_o^2 + z_o - 2A^2 = 0 \tag{1.50}$$

This is a cubic equation and the solution is

$$z_o = \frac{1}{3}\{(1 + 27A^2) + [(1 + 27A^2)^2 - 1]^{1/2}\}^{1/3}$$
$$+ \frac{1}{3}\{(1 + 27A^2) - [(1 + 27A^2)^2 - 1]^{1/2}\}^{1/3} - \frac{2}{3} \tag{1.51}$$

Using equation (1.41), the balance equation gives for $x = 0$ as follows:

$$y_o^3 + \frac{1}{d_2} y_o - \frac{d_1}{d_2 c_o} = 0 \tag{1.52}$$

The solution is

$$y_o = \left[\frac{d_1}{2d_2 c_o} + \left(\frac{d_1^2}{4d_2^2\ c_o^2} + \frac{1}{27d_2^3}\right)^{1/2}\right]^{1/3}$$

$$+ \left[\frac{d_1}{2d_2\ c_o} - \left(\frac{d_1^2}{4d_2^2\ c_o^2} + \frac{1}{27d_2^3}\right)^{1/2}\right]^{1/3} \tag{1.53}$$

The physical significance of y_o can be obtained from the relation

$$z = S_n = y + x \cos wt$$

y_o is the value of the bias term when $x = 0$, i.e., there is no oscillation. Thus y_o is same as the equilibrium value of S_n.

Now we need to study the limit of $\frac{1}{F}$ and its monotonicity. For this we must find the derivative $\frac{dz}{dw}$. From equation (1.37), $L(w,z)$ can be defined as

$$L(w,z) = \frac{1}{zK^2}\ (K+w - z+1) = \frac{1}{A^2} = \text{constant} \tag{1.54}$$

$$\therefore \quad 0 = \frac{\partial L}{\partial z}\, dz + \frac{\partial L}{\partial w}\, dw$$

$$\therefore \quad \frac{dz}{dw} = -\left(\frac{\partial L}{\partial w}\right) / \left(\frac{\partial L}{\partial z}\right) \tag{1.55}$$

Now,
$$\frac{\partial L}{\partial w} = \frac{\partial}{\partial w}\left[\frac{[(w-z+1)^2 + 4z]^{\frac{1}{2}} + (w-z+1)}{z\{(w-z+1)^2 + 4z\}}\right]$$

Differentiating by quotient rule and rearranging one gets,

$$\frac{\partial L}{\partial w} = \frac{K^2 - (w-z+1)[(K + 2(w-z+1))]}{zK^4}$$

$$\frac{\partial L}{\partial z} = \frac{-K^3 + Kz(w-z-1) - K^2(w+1) - 4z(w-z+1) + 2z(w-z+1)^2}{z^2\, K^4}$$

Therefore equation (1.55) gives

$$\frac{\partial z}{\partial w} = -\left[\frac{z\{K^2 - (w-z+1)[K + 2(w-z+1)]\}}{-K^3 - K^2(w+1) + Kz(w-z-1) - 4z(w-z+1) + 2z(w-z+1)^2}\right]$$

$$= \frac{z\{K^2 - K(w-z+1) - 2(w-z+1)^2\}}{K^3 + K^2(w+1) + Kz(z-w+1) + 2z(w-z+1)[2-w+z-1]}$$

$$= \frac{z(K^2 + K(w-z+1-2w+2z-2) - 2(w-z+1)^2)}{K^3 + K^2(w+1) + Kz(z-w+1) + 2z(w-z+1)(z-w+1)}$$

$$= \frac{z\{K(K+w-z+1) - 2(w-z+1)(K+w-z+1)\}}{K^3 + K^2(w+1) \mp Kz(z-w+1) + 2z(w-z+1)(z-w+1)}$$

$$= \frac{z(K+w-z+1)[K-2(w-z+1)]}{K^3 + K^2(w+1) + Kz(z-w+1) + 2z(w-z+1)(z-w+1)} \qquad (1.56)$$

To derive an expression for $\frac{1}{P_0}$, the limit of the describing function as $w \to 0$, consider the equation (1.45). Both the numerator and denominator are zero, when $w = 0$, so we apply L'Hospital's rule.

Using equation (1.46) and (1.56), one gets,

$$\frac{dK}{dw} = \frac{w-z+1}{((w-z+1)^2 + 4z)^{\frac{1}{2}}}$$

$$\frac{dK}{dw}\Big|_{w=0} = \frac{1-z_o}{1+z_o} \quad , \quad z_o \text{ is the value of } z \text{ at } w = 0$$

and $$\frac{dz}{dw}\Big|_{w=0} = \frac{z_o(1+z_o-z_o+1)[1+z_o+2z_o-2]}{(1+z_o)^3 + (1+z_o)^2 + z_o(1+z_o)(1+z_o) + 2z_o(z_o+1)(1-z_o)}$$

$$= \frac{2z_o(3z_o-1)}{(z_o+1)(2+6z_o)} = \frac{z_o(3z_o-1)}{(z_o+1)(1+3z_o)}$$

These expressions are used to find the value of the derivatives of the numerator and the denominator of equation (1.45) at $w = 0$.

$$\text{Numerator} = N = HwK$$

$$\frac{dN}{dw}\Big|_{w=0} = H[K + w\frac{\partial K}{\partial w}]\Big|_{w=0} = H[z_o+1]$$

$$\text{Denominator} = D = (K-w+z-1)^{\frac{1}{2}} - z^{\frac{1}{2}}(K+w-z+1)^{\frac{1}{2}}$$

$$\frac{dD}{dw} = \frac{1}{2}\,\frac{(\partial K/\partial w - 1)}{(K-w+z-1)^{\frac{1}{2}}} - \frac{1}{2}\,\frac{z^{\frac{1}{2}}(\partial K/\partial w + 1)}{(K+w-z+1)^{\frac{1}{2}}}$$

$$\frac{dD}{dw}\Big|_o = \frac{1}{2}\left[\frac{\frac{1-z_o}{1+z_o} - 1}{(1+z_o+z_o-1)^{\frac{1}{2}}} - \frac{\sqrt{z_o}\left(\frac{1-z_o}{1+z_o}+1\right)}{(1+z_o-z_o+1)^{\frac{1}{2}}}\right]$$

$$= \frac{1}{2}\left(\frac{1}{(1+z_o)}\right)\left[\frac{1-z_o-1-z_o}{\sqrt{2z_o}} - \frac{\sqrt{z_o}(1-z_o+1+z_o)}{\sqrt{2}}\right]$$

$$= \frac{1}{2(1+z_o)} \left[\frac{-2z_o}{\sqrt{2z_o}} - \frac{2\sqrt{z}_o}{\sqrt{2}} \right]$$

$$= \frac{1}{2(1+z_o)} \quad \frac{-2z_o - 2z_o}{\sqrt{2z_o}} = \frac{-4z_o}{2\sqrt{2z_o}(1+z_o)}$$

$$\therefore \left.\frac{dD}{dw}\right|_o = \frac{-\sqrt{2z_o}}{1+z_o}$$

Therefore from equation (1.45), using the above relations, one gets,

$$\lim_{w \to 0} \frac{1}{P} = \frac{1}{P_o} = \frac{-H(z_o+1)^2}{\sqrt{2}\, z_o^{\frac{3}{2}}} \qquad (1.57)$$

Using equation (1.49), (1.57) can be written as,

$$\frac{1}{P_o} = \frac{-H\, 2A^2}{\sqrt{2}\, z_o^{\frac{1}{2}}\, z_o} = \frac{-\sqrt{2}\, HA^2}{z_o^{3/2}} \qquad (1.58)$$

It will now be shown that $\frac{1}{P}$ decreases monotonically with increasing positive w. In equation (1.45), since $z > w > 0$, therefore the numerator is always positive. The sign of $\frac{1}{P}$ is determined by the denominator. If we can show that

$$K - (w-z+1) < 3K-z(w-z+1) \qquad (1.59)$$

then $\frac{1}{P}$ is always negative.

The result is first obtained for the case $z < 1$, i.e $0 < w < z < 1$.

Now $\quad 4z(1-z)^2 < 4z(1-z+w)^2 \qquad \because \; w > 0$

or $\quad 4z(1-z)^2 < (1+z)^2(w-z+1)^2-(1-z)^2(w-z+1)^2$

Putting $\quad 4z = (1+z)^2 - (1-z)^2$

or $\qquad (1-z)^2[(w-z+1)^2 + 4z] < (1+z)^2(w-z+1)^2$

or $\qquad (1-z)K < (1+z)(w-z+1) \quad$ using (1.46)

or $\qquad K - (w-z+1) < Kz + z(w-z+1).$

Hence (1.59) is satisfied. This result also holds for the case $z = 1$. The third case is the situation when $1 < z < w+1$. It follows that

$$1 - z < 0 < w+1 - z$$

Since K and $(w+1-z)$ are positive,

$\therefore \qquad K(1-z) < 0 < (z+1)(w+1-z)$

$\therefore \qquad K-Kz < (w+1-z)z - (w+1-z)$ and (1.59) follows.

The proof for the case $z = w+1$ follows trivially, since most of the terms in (1.59) drop out. The last case is where $w+1 < z$.

Now, $\qquad 4z(w-z+1)^2 < 4z(z-1)^2$

or, $\qquad [(z+1)^2 - (z-1)^2](w-z+1)^2 < 4z(z-1)^2$

or, $\qquad 0 < (1+z)^2(w-z+1)^2 < K^2(z-1)^2 \qquad \left\{ \because K = \sqrt{(w-z+1)^2 + 4z} \text{ and } w > 0 \right\}$

or, $\qquad 0 < (1+z)(w-z+1) < K(z-1).$

Thus (1.59) follows.

The fine cases cited above covers all possible situations. Hence it is proved that the denominator of $\frac{1}{p}$ is always negative and so $\frac{1}{p}$ is always positive, if $z > w > 0$. To find the monotonicity of

$\frac{1}{P}$ we consider the function $|P|^2$.

$$|P|^2 = \frac{(K-w+z-1) + z(K+w-z+1) - 2\sqrt{z}\sqrt{K^2-(w-z+1)^2}}{H^2 w^2 K^2}$$

$$= \frac{K(z+1) - 1 - z^2 + 2z - 4z + w(z-1)}{H^2 w^2 K^2}$$

$$= \frac{K(z+1) - (z+1)^2 + w(z-1)}{H^2 w^2 K^2}$$

We can prove that $|P|^2$ is a monotonically decreasing function, then one can say that $\frac{1}{P}$ is a monotonically increasing function. But since $\frac{1}{P}$ is always negative, therefore it turns out to be a monotonically decreasing function. We need to show that $\frac{d|P|^2}{dw}$ is always negative.

$$\frac{d|P|^2}{dw} = -\frac{w}{D^2}\left((T_1 + T_2 K) + (T_3 + T_4 K)\frac{dz}{dw}\right)$$

where $D = H^2 w^2 K^2$

$$T_1 = -3w^2 - 8w^2 - 7w - 2z^4 - 8z^3 - 12z^2 - 8z - 2$$
$$-7wz + 7wz^2 + 7wz^3 - 8w^2z^2 + 3w^3z$$

$$T_2 = 3w^2 + 5w + 2z^3 + 6z^2 + 6z + 2 - 5wz^2 + 3w^2z$$

$$T_3 = w^2(-w^2 + 2w - z^2 + 2z + 3 + 2wz)$$

$$T_4 = w^2(-w + z - 3).$$

Now first we show that the denominator of $\frac{dz}{dw}$ is positive.

Let D = the denominator of $\frac{dz}{dw}$

$$= K^3 + K^2 \ (w+1) + Kz(z-w+1) + 2z(z-w+1)(w-z+1)$$

when $z > w > 0$, the expression

$$K^3 + K^2(w+1) + Kz(z-w+1) \tag{1.60}$$

is positive. If $z < w+1$, the remaining term of D is also positive. If $z = w+1$, then this term vanishes, leaving only the positive expression (1.60). It will now be shown that D is positive if $w+1 < z$. Equation (1.43) defines the function $z(w)$. Using that equation we get

$$\frac{zK^2}{A^2} - K = w-z+1 \ .$$

Let M be defined by

$$M = K^3 + K^2(w+1) + Kz(z-w+1) + 2z(z-w+1)(-K)$$

or $$M = K(K + Kw + w^2 + 2w + z-wz + 1).$$

For the case $w+1 < z$, the quantity $2z(z-w+1)$ is positive and $\frac{zK^2}{A^2}$ is positive, one concludes that M is strictly less than D.

If $w^2 + Kw + w-wz > 0$, (1.61)

then the proof is complete.

Since 4z is positive therefore it follows from the definition of K that,

$$|z-w-1| < K \tag{1.62}$$

The absolute value brackets can be dropped, since we are considering the case $z > w+1$. Therefore it follows that,

$$w^2 + 2w + w - wz > 0 \qquad \therefore \quad w > 0$$

Thus the inequality (1.61) is satisfied and the denominator of $\frac{dz}{dw}$ is positive.

Consider a quantity E given by

$$E = (T_1 + T_2K)D + (T_3 + T_4K)N$$

where N is the numerator of $\frac{dz}{dw}$,

or $$E = L_1 + L_2K \tag{1.63}$$

where,

$$\begin{aligned} L_1 = {} & 8z^7 + 40z^6 + 80z^5 + 80z^4 + 40z^3 + 8z^2 \\ & + 8wz - 24wz^3 - 120wz^4 - 136wz^5 - 48wz^6 \\ & - 24w^2z^3 + 144w^2z^4 + 120w^2z^5 + 8w^3z - 8w^3z^2 \\ & - 16w^3z^3 - 160w^3z^4 + 24w^4z - 56w^4z^2 + 120w^4z^3 \\ & + 24w^5z - 48w^5z^2 + 8w^6z \end{aligned}$$

and $$\begin{aligned} L_2 = {} & -8z^6 - 32z^5 - 48z^4 - 32z^3 - 8z^2 + 40wz^3 + 8wz^4 \\ & + 40wz^5 - 48w^2z^3 - 80w^2z^4 + 8w^3z - 16w^3z^2 \\ & + 80w^3z^3 + 16w^4z - 40w^4z^2 + 8w^5z \end{aligned}$$

The minimum of the function E on the interval $w = 0$ to $w = z$, where it is non-negative, is investigated via a numerical technique and found to be the value $E = 0$ at $w = 0$. At $z = w$,

$$L_1 = 64z^4 + 48z^3 + 8z^2$$

$$L_2 = -32z^3 - 8z^2$$

$$K = (1+4z)^{\frac{1}{2}} < (1+4z + 4z^2)^{\frac{1}{2}} = 1 + 2z$$

using the inequality bounding K, one sees that E must be strictly positive.

The numerical method employed by Rapp in his paper to find the minimum of E, has been developed by M.J.D. Powell.

Thus from all the results calculated above, one concludes that $\frac{1}{P}$ is monotonically decreasing with increasing positive w.

In this case ($\rho = 2$) the frequency response locus remains the same to the case with $\rho = 1$. The modulus and argument of $G(i\omega)$ decreases monotonically as positive w increases and the contour spirals into the origin in a clockwise direction. The smallest real value of $G(i\omega)$ is achieved at its first crossing with the negative real axis at frequency w_o.

The condition necessary for a limit cycle to exist is $G(iw_o) < \frac{1}{P_o}$

or
$$|P_o|\,|G(iw_o)| > 1$$

Using (1.58) for $\frac{1}{P_o}$, the limit cycle condition becomes

$$\frac{z_o^{3/2}}{\sqrt{2}\ HA^2}\,|G(iw_o)| > 1$$

We know that $G(0) = \frac{1}{c_o}$, the requirement for limit cycle condition becomes

$$R = \left(\frac{z_o^{3/2}}{\sqrt{2}\, HA^2 c_o} \right) \left| \frac{G(i\omega_c)}{G(0)} \right| > 1 \tag{1.64}$$

Now we will have to find the maximum value of $\left| \frac{G(i\omega_c)}{G(0)} \right|$

$$\frac{G(i\omega)}{G(0)} = \prod_{j=1}^{n} \frac{b_j}{(b_j + i\omega)} = \prod_{j=1}^{n} \frac{1}{(1 + i \tan \phi_j)}$$

where $\phi_j = \tan^{-1}(+ \frac{\omega}{b_j})$ as in equation (1.15)

$$\left| \frac{G(i\omega)}{G(0)} \right| = \prod_{j=1}^{n} \frac{1}{(1 + \tan^2\phi_j)^{\frac{1}{2}}} = \prod_{j=1}^{n} |\cos \phi_j| = \prod_{j=1}^{n} \cos \phi_j .$$

The last step follows from the fact that by definition of ϕ_j, $\cos \phi_j$ is always positive.

The object is to maximize the expression subject to the constraint that $\omega = \omega_c$, i.e. the argument of G is π, $\sum_j \phi_j = \pi$. For simplicity's sake, take the logarithm,

$$\log \left| \frac{G(i\omega)}{G(0)} \right| = \sum_{j=1}^{n} \log \cos \phi_j = \sum_{j=1}^{n} h(\phi_j)$$

where $h(\phi_j) = \log(\cos \phi_j)$, $\frac{d^2h(\phi)}{d\phi^2} = -\sec^2\phi$, a negative quantity which implies that h is concave. Thus for $0 < \theta < 1$,

$$\theta h(y) + (1-\theta)h(x) \leq h(\theta y + (1-\theta)x)$$

with equality holding only when $y = x$. Applying this relation n-1 times and the constraint on the argument gives

$$\sum_{j=1}^{n} h(\phi_j) \leq nh\left(\frac{\phi_1 + \ldots + \phi_n}{n}\right) = n \log \cos \frac{\pi}{n}$$

Taking antilog gives the bound on $\left|\frac{G(i\omega_c)}{G(0)}\right|$

$$\left|\frac{G(i\omega_c)}{G(0)}\right| \leq \left(\cos \frac{\pi}{n}\right)^n \tag{1.65}$$

when $\phi_j = \phi_\ell$ for all j and ℓ (equivalently $b_j = b_\ell$), the maximum value of the object function is obtained $[\cos \frac{\pi}{n}]^n$. The following table gives the maximum values

Table 1

n	$(\cos \frac{\pi}{n})^n$
3	.1250
4	.2500
5	.3466
6	.4219
7	.4819
8	.5380
9	.5713
10	.6054

Rapp has started from $n = 3$ because Griffith [13] had already shown that $n = 1$ and 2, there are no periodic solutions to the equations for positive reaction constants.

At the extremum $c_o = b^n$, which is unspecified. Let

$$U(A) = \frac{z_o^{3/2}}{\sqrt{2}\ HA^2c_o} = \frac{\sqrt{2}\ z_o^{3/2}}{A} \tag{1.66}$$

using the values of HAc_o which gives $\frac{1}{2}$

$$(HAc_o = \frac{d_1\sqrt{d_2c_o}}{\sqrt{2}\ d_1\sqrt{d_2}\sqrt{2}\ c_o} = \frac{1}{2}\ )$$

using $z_o = d_2y_o^2$, equations (1.52) and (1.53), we get

$$U(A) = \frac{\sqrt{2}\ d_2^{3/2}\ y_o^3}{A} = 2\{\frac{A}{A^2} + (\frac{4}{A^4} + \frac{8}{27A^6})^{1/2}\}^{1/3}$$

$$= \{\frac{2}{A^2} - (\frac{4}{A^4} + \frac{8}{27A^6})^{1/2}\}^{1/3} \tag{1.67}$$

As A tends to infinity, the limit of U(A) is two. From the fact that 2-U'(A) is positive, it is clear that 2 is the maximum value of U(A). Consulting Table 1, it is seen that for $n \leq 7$, the maximum value of R is less than one. This gives the result that given that all the reaction constants (Rapp forms a set S of all the reaction constants, i.e. $S = \{k, a, g_1, \ldots, g_{n-1}, b_1, \ldots, b_n\}$) are positive (i.e. the set S is permissible) and $z > w > 0$ (which gives condition for positive concentrations), there are no limit cycles.

Using the table, one can see that stable limit cycles (since $\frac{1}{P}$ is a decreasing function of positive x, the limit cycle will be stable) exist for n = 8,9 and 10, because there $R > 1$. If it can be shown that $(\cos\frac{\pi}{n})^n$ is monotonically increasing with increasing positive n, then the result can be generalised to all large n. Again we consider the function $n\log(\cos\frac{\pi}{n})$ and show that it is monotonically increasing with increasing n. It is also the same-thing to show that $\frac{1}{x}\log\cos x$ (where $x = \frac{\pi}{n}$) is monotonically decreasing with increasing x, where

$$0 < x < \frac{\pi}{2} \text{ , since } n > 2$$

This function can be re-stated as

$$\frac{1}{x}\log\cos x = \frac{1}{x}\int_0^x -\tan\eta \, d\eta \tag{1.68}$$

the right hand side is the mean value of $-\tan\eta$ taken over the interval 0 to x. The function is always negative and monotonically decreasing with increasing x, given that $0 < x < \frac{\pi}{2}$. In other words, the function $n \log\cos\frac{\pi}{n}$ is monotonically increasing with increasing x. Thus stable limit cycles result for $\rho = 2$ when $n \geq 8$. (1.69)

The next result, which Rapp has derived is a general procedure for constructing a large class of permissible S giving limit cycle behaviour from one such set.

Set A_{min} such that $R(A_{min}) = 1$. Now if $\left|\frac{G(i\omega)}{G(0)}\right|$ is held fixed and U(A) is shown to be a monotonically increasing function with increasing A, then $R > 1$ for all $A > A_{min}$ U(A) is monotonically increasing if 2-U(A) is monotonically decreasing

$$2-U(A) = \left[\frac{2}{A^2} + \left(\frac{4}{A^4} + \frac{8}{27A^6}\right)^{1/2}\right]^{1/3} + \left[\frac{2}{A^2} - \left(\frac{4}{A^4} + \frac{8}{27A^6}\right)^{1/2}\right]^{1/3} \tag{1.70}$$

The function K(A) defined by

$$K(A) + \frac{2}{A^2} = \left(\frac{4}{A^4} + \frac{8}{27A^6}\right)^{\frac{1}{2}}$$

is a positive monotonically decreasing function.

From (1.70),

$$\therefore \qquad 2-U(A) = [\frac{4}{A^4} + K(A)]^{1/3} - [K(A)]^{1/3} \tag{1.71}$$

The function $\delta(A)$, defined by

$$[K(A) + \frac{4}{A^4}]^{1/3} = [K(A)]^{1/3} + \delta(A)$$

is a decreasing function of A. Hence, since $\delta(A) = 2-U(A)$, therefore U(A) is a monotonically increasing function giving the desired result.

The condition for limit cycle can be expressed in terms of a physically realizable quantity y_o in the expression (1.67) which is nothing but the equilibrium value of S_n.

$$\therefore \qquad U(A) = \frac{\sqrt{2}\, d_2^{3/2}\, (S_n)_e^3 \sqrt{2}\, c_o}{d_1 \sqrt{d_2}} = 2\,\frac{d_2}{d_1}\,(c_o)(S_n)_e^3$$

Therefore the condition for existence of stable limit cycle is

$$\frac{2d_2c_o}{d_1}\;(S_n)_e^3 \left| \frac{G(i\omega)}{G(0)} \right| > 1 \tag{1.72}$$

When $b_j = b$ for all j, (1.72) becomes,

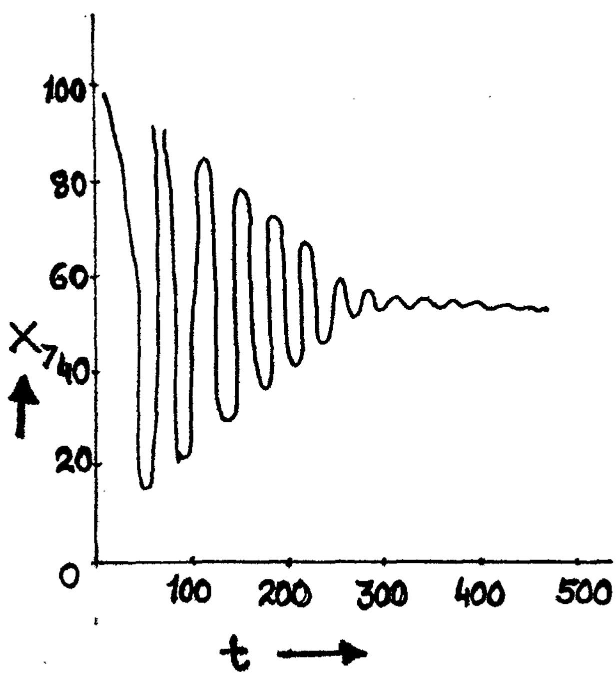
$$\frac{2d_2c_o}{d_1}\;(S_n)_e^3\,[\cos\frac{\pi}{n}]^n > 1 \tag{1.73}$$

After deriving the constraint equation, Rapp has discussed some numerical tests of the results obtained by him.

The following values of the constants were taken:

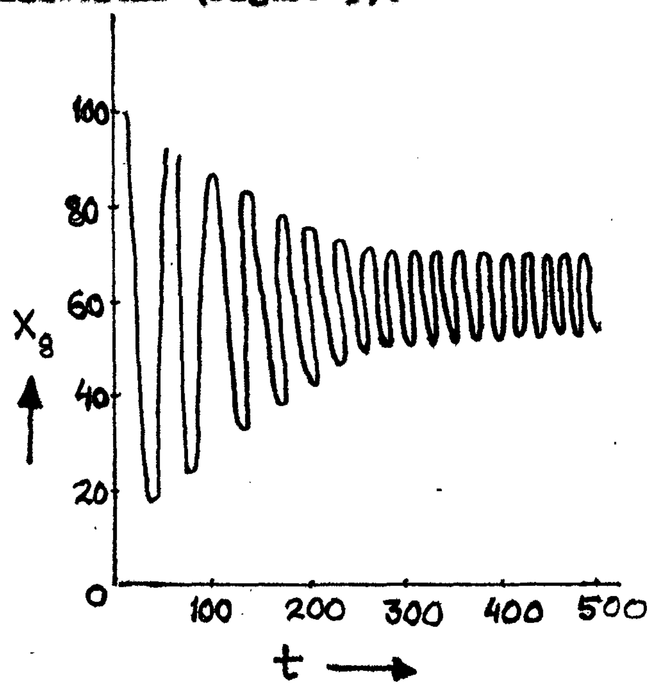
$K = 30{,}000$, $\alpha = 1$, $g_j = 1$, $x_j(0) = 1$ and $b_j = 1$ for all j.

This means, $d_1 = 30{,}000$, $d_2 = 1$, $c_o = 1$.
The example shown was at the boundary between limit cycle and non limit cycle situations ($n = 7$ and $n = 8$ systems). For the above values $A = 21213.205$ and $U(A)$ is strictly greater than 1.9887. Using table 1, one finds that for $n = 7$, the value of R is 0.9584, thus there should be no stable limit cycle for $n = 7$. For $n = 8$, $R = 1.0556$, indicating that there should be a stable limit cycle. The value of $x_n(t)$ as a function of time for each system was given. For $n = 7$ there was no stable oscillation (figure 8) and for $n = 8$ there was asymptotically stable oscillations (figure 9).



Seven dimensional system

Fig 8

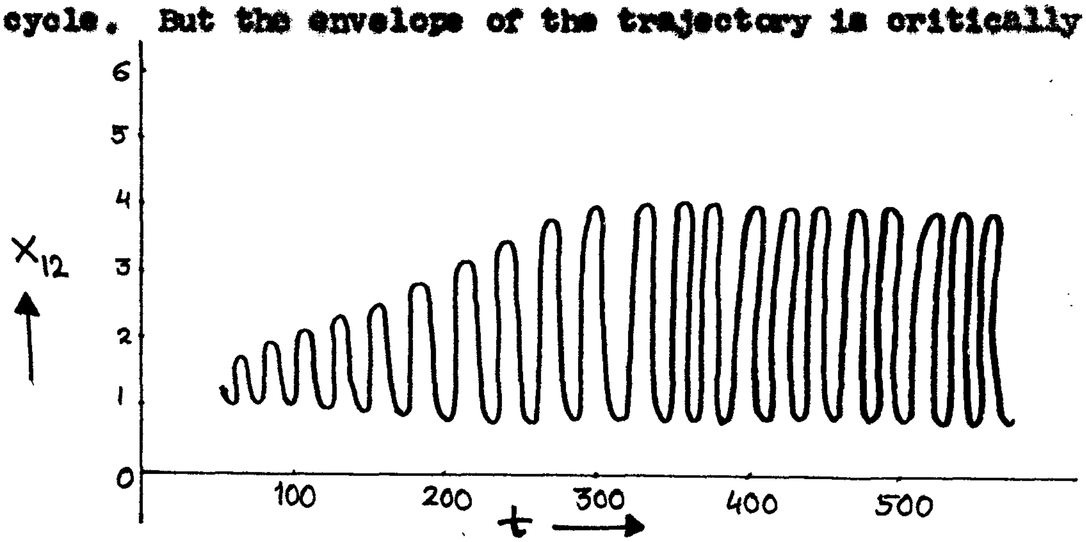


Eight dimensional system

Fig 9

The only difference is in the presence of one more reaction step, the reaction constants are the same. Thus clearly, stable limit cycles exist for reaction systems where the inhibition of the first step requires two molecules of inhibitor ($\rho = 2$). Comparing this result with the previous case of $\rho = 1$, where there existed no periodic solution for all n, Rapp concluded that "the distinction between a system that can oscillate and one that cannot is "hardwired" into the genetic structure". It doesn't depend on the numerical values of the reaction constants which are extremely sensitive to changes in the chemical environment.

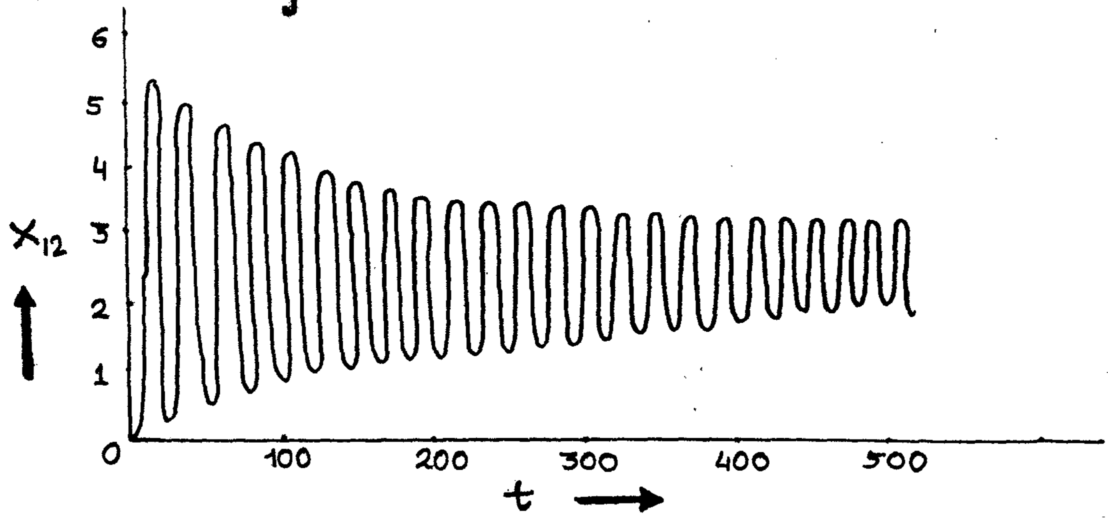
For larger system, the degree of accuracy increases since the linear component acts more effectively as a low pass filter. Rapp has predicted the value of the frequency for a system of $n = 12$, $K = 9$, $\alpha = 5$, $b_j = 1$, $g_j = 1$ and $x_j(0) = 1$ for all j, as 0.268 whereas the actual value is 0.263. Error is less than 2%. In this case, (Fig. 10), the trajectory builds up slowly to the stable limit cycle. But the envelope of the trajectory is critically



Twelve dimensional system $x_j(0) = 1$

<u>Fig. 10</u>

dependent on the initial conditions. Keeping all the values same, except changing $x_j(0) = 0.1$, fig. 11 results



Twelve dimensional system $x_j(0) = 0.1$

Fig. 11

The trajectory in this case climbs up to 5.5 concentration units and then decrease to the systems stable limit cycle. Using the above result Rapp has discussed how the biochemical oscillators can be used as developmental triggers. Suppose the twelve dimensional chemical oscillator is coupled to another system and served as an amplitude dependent trigger with a triggering level of 3.5. In the first system ($x_j(0) = 1$) it will reach the level only at $t = 300$, but in the second system ($x_j(0) = 1$) it occurs at $t < 20$.

So on the basis of the above results one can conclude that the describing function technique is correctly applied in this problem

The results of the second paper by Rapp may be summarized as (for $\rho = 2$).

a) For $n \leq 7$, the system of differential equations do not possess a limit cycle.

b) For $n \geq 8$, stable limit cycles exist.

c) The condition for a limit cycle to exist is

$$1 < \left| \frac{G(i\omega_o)}{G(0)} \right| \{2 - [\frac{2}{A^2} + (\frac{4}{A^4} + \frac{8}{27A^6})^{1/2}]^{1/3}$$

$$- [\frac{2}{A^2} - (\frac{4}{A^4} + \frac{8}{27A^6})^{1/2}]^{1/3}$$

where the expression in curly bracket is monotonically increasing with increasing positive A. This inequality is satisfied by all A greater than a minimum for which equality occurs.

d) The condition for limit cycle is obtained in terms of physically realizable $(S_n)_e$, the equilibrium value of the nth component.

$$1 < \frac{2d_2c_o}{d_1} (S_n)_e^3 \left| \frac{G(i\omega_o)}{G(0)} \right|$$

For a special case of $b_j = b$ for all j, this result simplifies to

$$1 < \frac{2d_2c_o}{d_1} (S_n)_e^3 [\cos \frac{\pi}{n}]^n$$

e) The expression for the equilibrium value of the nth species is also obtained in terms of reaction constants

$$y_o = (S_n)_e = [\frac{d_1}{2d_2c_o} + (\frac{d_1^2}{4d_2^2 c_o} + \frac{1}{27d_2^3})^{1/2}]^{1/3}$$

$$+ [\frac{d_1}{2d_2 c_o} - (\frac{d_1^2}{4d_2^2c_o^2} + \frac{1}{27d_2^3})^{1/2}]^{1/3}$$

f) It has been found out with representative examples, that the qualitative behaviour of the envelope of a solution can be extremely sensitive to the initial values even if the final periodic solution specified by the set S of reaction parameters remain the same.

Now, after reviewing Rapp's papers on the use of describing function method to the biochemical oscillator problem, we will go over to the Lyapunov technique of solving the equations and discuss the papers by Biswas, Pande and Rao in this connection.

CHAPTER III

LYAPUNOV ANALYSIS: ITS USE IN BIOCHEMICAL OSCILLATOR PROBLEM

Consider a dynamical system whose motion is governed by a system of differential equations

$$\dot{x}_i = X_i(x_1, x_2, \ldots, x_n); \quad i = 1, 2, \ldots, n \qquad (2.1)$$

where X_i are continuous functions of the variables x_i which may be regarded as the generalized co-ordinates. Suppose that a solution of this system is known to be

$$x_{io} = x_{io}(t)$$

which may be considered as a <u>nonperturbed solution</u>. Let $x_i(t)$ be the solution corresponding to an initial value $x_i(t_o) \neq 0$; it will be called a <u>perturbed solution</u>. Between the new and the old solutions, there exists a relation

$$x_i(t) = x_{io}(t) + \xi_i(t) \qquad (2.2)$$

where $\xi_i(t)$ are called the perturbations.

One assumes that $|\xi_i|$ are sufficiently small to be able to neglect their higher powers. If one inserts (2.2) in (2.1) and develops the functions X_i around the nonperturbed values $x_{io}(t)$ to the first order in ξ_i, one obtains a system of the variational equations

$$\dot{\xi}_i = \sum_{j=1}^{n} \left(\frac{\partial X_i}{\partial x_j} \right)_o \xi_i$$

in which the co-efficients of ξ_i are partial derivatives of the functions X_i with respect to the variables x_j into which the nonperturbed values x_{io} have been replaced after the differentiation. Since x_{io} is the known solution and x_i is the perturbed solution, an important case arises when all perturbation functions $\xi_i(t) \to 0$ for $t \to \infty$, in which case $x_i(t) \to x_{io}(t)$ as follows from (2.2). In this case the stability is called <u>asymptotic stability</u>. More detailed treatment can be found elsewhere [12].

Biswas, Pande and Rao (BPR) have made use of the above mentioned Lyapunov analysis to find the limit cycle behaviour of the set of differential equations given by equation (7).

In their first paper [8], BPR have analysed the $n = 3$ case (which can represent the protein synthesis reaction) with arbitrary power of the variable S_3 occuring in the nonlinear term. They have proved that ρ should be greater than 8 for the existence of the asymptotic periodic solutions. In addition to this they have also derived a constraint equation relating to the various rate constants. Explicit analytical solutions are also obtained.

In their second paper [8], BPR have generalized the matter in the first paper for the n-step feed-back control system. A general condition has been derived between ρ and n for asymptotically periodic solutions to exist. The constraint equation is also deduced for the n-step process.

Let us discuss the first paper now. The set of equations is

$$\left.\begin{aligned} \frac{dx_1}{dt} &= \frac{a}{A+kx_3^{\rho}} - bx_1 \\ \frac{dx_2}{dt} &= \alpha\, x_1 - \beta\, x_2 \\ \frac{dx_3}{dt} &= \gamma\, x_2 - \delta\, x_3 \end{aligned}\right\} \qquad (2.3)$$

For linearization by Lyapunov's technique, first the critical points of the equations are obtained by setting

$\frac{d\bar{x}_1}{dt} = 0$. This gives

$$\bar{x}_2 = \frac{\delta}{\gamma}\, \bar{x}_3 \qquad (2.4a)$$

$$\bar{x}_1 = \frac{\beta}{\alpha}\, \bar{x}_2 = \frac{\beta}{\alpha}\, \frac{\delta}{\gamma}\, \bar{x}_3 \qquad (2.4b)$$

and $$\frac{a}{A+k\bar{x}_3^{\rho}} = b\left(\frac{\beta\delta}{\alpha\gamma}\right)\bar{x}_3$$

or $$A\left(\frac{b\beta\delta}{\alpha\gamma}\right)\bar{x}_3 + k\left(\frac{b\beta\delta}{\alpha\gamma}\right)\bar{x}_3^{\rho+1} = a$$

or $$\left(\bar{x}_3^{\rho+1} + \frac{A}{k}\,\bar{x}_3\right) = \frac{a}{b}\,\frac{\gamma}{\delta}\,\frac{\alpha}{\beta}\,\frac{1}{k} \qquad (2.4c)$$

$\bar{x}_i$ is the critical or equilibrium concentration. Linearization is done by setting

$$x_i = \bar{x}_i + \varepsilon_i, \quad i = 1,2,3 \qquad (2.5)$$

where $$\varepsilon_3 \ll \bar{x}_3 \qquad (2.6)$$

The first two equations in equation (2.4) refer to epigenetic processes whereas the third equation represents a metabolic process. It is therefore possible that the relaxation time for x_3 is much less than that for x_1 and x_2. Consequently x_1 and x_2 need not assume values close to the equilibrium value $\bar{x}_i$ (i = 1,2), but x_3 may be close to $\bar{\bar{x}}_3$ and so equation (2.6) is satisfied.

Putting (2.5) in (2.3) and using (2.4), one gets,

$$\dot{\varepsilon}_1 = \frac{a}{A+k(\bar{\bar{x}}_3+\varepsilon_3)^{\rho}} - b(\bar{\bar{x}}_1+\varepsilon_1) \qquad (2.7)$$

$$= \frac{a}{A+k\,\bar{\bar{x}}_3^{\rho}(1+\frac{\varepsilon_3}{\bar{\bar{x}}_3})^{\rho}} - b\,\bar{\bar{x}}_1 - b\,\varepsilon_1$$

For $\varepsilon_3 << \bar{\bar{x}}_3$, binomial expansion leads to,

$$\dot{\varepsilon}_1 = \frac{a}{(A+k\,\bar{\bar{x}}_3^{\rho})\ (1+\frac{\rho\varepsilon_3\,\bar{\bar{x}}_3^{\rho-1}.k}{A+k\,\bar{\bar{x}}_3^{\rho}})} - b\,\bar{\bar{x}}_1 - b\,\varepsilon_1$$

Since $\rho\bar{\bar{x}}_3^{\rho-1}.\varepsilon_3.k << (A+k\,\bar{\bar{x}}_3^{\rho})$, then another binomial expansion gives (2.8)

$$\dot{\varepsilon}_1 = -b\varepsilon_1 - Q\varepsilon_3 \qquad (2.9)$$

where $Q = \frac{\rho k a\bar{\bar{x}}_3^{\rho-1}}{(A+k\bar{\bar{x}}_3^{\rho})^2}$ (2.10)

$$\dot{\varepsilon}_2 = \alpha\varepsilon_1 - \beta\varepsilon_1 \qquad (2.11)$$

$$\dot{\varepsilon}_3 = \gamma\varepsilon_2 - \delta\varepsilon_3 \qquad (2.12)$$

Thus the set of equations (2.3) has been linearized into the set

$$\dot{\varepsilon}_i = M_{ij}\varepsilon_j \; ; \quad i,j = 1,2,3 \qquad (2.13)$$

where M is a matrix given by,

$$M = \begin{pmatrix} -b & 0 & -Q \\ \alpha & \beta & 0 \\ 0 & \gamma & -\delta \end{pmatrix} \qquad (2.14)$$

The matrix equation $\dot{\varepsilon} = M\varepsilon$ can be solved by diagonalising M. Let T = diagonalising matrix for M. Then

$$T\dot{\varepsilon} = (TMT^{-1})T\varepsilon \qquad (2.15)$$

$(T\varepsilon)_i$ will be linear combination of ε_i. If ε_i have oscillatory behaviour, it is necessary for $(T\varepsilon)_i = \chi_i$ to have oscillatory behaviour. Hence the authors have demanded that M have two purely imaginary eigen-values (conjugate) and the third one is real. The three eigen-values of M are

$\mu_1 = +iI$, $\mu_2 = -iI$ and $\mu_3 = R$,

where I and R are both real.

The characteristic equation is

$|\mu E - M| = 0$ where E is a 3×3 unit matrix.

The eigen-values satisfy this equation which when expanded gives,

$$\mu^3 + \mu^2(b+\beta+\delta) + \mu(b\beta+\beta\delta+b\delta) + (b\beta\delta+Q\alpha\gamma) = 0 \qquad (2.16)$$

Let D be the diagonal matrix obtained from diagonalising M by T, i.e.,

$$T\,M\,T^{-1} = D \tag{2.17}$$

the form of D is

$$D = \begin{pmatrix} iI & 0 & 0 \\ 0 & -iI & 0 \\ 0 & 0 & R \end{pmatrix} \tag{2.18}$$

Since trace of the matrix is an invariant quantity, therefore

$$\mathrm{Tr}\,D = \mathrm{Tr}\,M$$

$$R = -(b+\beta+\delta) \tag{2.19}$$

This is a negative quantity.

Now, substituting one of the imaginary roots in equation (2.16) and collecting the real and imaginary parts, one gets

$$I^2 = (b\beta + b\delta + \rho\delta)$$

$$\text{and} \quad I^2 = \frac{b\beta\delta + Q\alpha\gamma}{b+\beta+\delta} \tag{2.20}$$

assuming $I \neq 0$.

From (2.20), one gets

$$Q = \frac{(b+\beta)(b+\delta)(\rho+\delta)}{\alpha\gamma} = N \tag{2.21}$$

Equating (2.12) and (2.21), one gets

$$N = \frac{\rho k a\, \bar{x}^{\rho-1}}{(A+k\bar{x}_3^{\rho})^2}$$

or $$Nk^2 \bar{\bar{x}}_3^{2\rho} + 2NAk\, \bar{\bar{x}}_3^{\rho} - \rho k a\, \bar{\bar{x}}_3^{\rho-1} + NA^2 = 0 \qquad (2.22)$$

Equation (2.4c) can be expanded as

$$k\bar{\bar{x}}_3^{\rho+1} + A\bar{\bar{x}}_3 = \frac{a}{b}\,\frac{\alpha}{\beta}\,\frac{\gamma}{\delta} \qquad (2.23)$$

Squarring,

$$A^2\bar{\bar{x}}_3^2 + k^2\bar{\bar{x}}_3^{2\rho+2} + 2Ak\bar{\bar{x}}_3^{\rho+2} = \left(\frac{a\alpha\gamma}{b\beta\delta}\right)^2$$

or $$A^2 + k^2\bar{\bar{x}}_3^{2\rho} + 2Ak\bar{\bar{x}}_3^{\rho} = \left(\frac{a\alpha\gamma}{b\beta\delta}\right)^2 \cdot \frac{1}{\bar{\bar{x}}_3^2}$$

Multiplying by N,

$$NA^2 + Nk^2\bar{\bar{x}}_3^{2\rho} + 2NAk\bar{\bar{x}}_3^{\rho} = N\left(\frac{a\gamma\alpha}{b\beta\delta}\right)^2 \frac{1}{\bar{\bar{x}}_3^2} \qquad (2.24)$$

(2.23) can also be divided throughout by $\bar{\bar{x}}_3^2$ and written as,

$$\bar{\bar{x}}_3^{\rho-1} + \frac{A}{k}\,\frac{1}{\bar{\bar{x}}_3} = \left(\frac{a\gamma\alpha}{b\beta\delta}\right)\frac{1}{k}\,\frac{1}{\bar{\bar{x}}_3^2} \qquad (2.25)$$

Using (2.24) and (2.25) in (2.22), one gets,

$$N\left(\frac{a\alpha\gamma}{b\beta\delta}\right)^2 \frac{1}{\bar{\bar{x}}_3^2} - \rho k a\left(\frac{a\alpha\gamma}{b\beta\delta}\right)\frac{1}{k}\,\frac{1}{\bar{\bar{x}}_3^2} + \rho k a\,\frac{A}{k}\,\frac{1}{\bar{\bar{x}}_3} = 0$$

or $$\bar{\bar{x}}_3 = \frac{\rho a\left(\frac{a\alpha\gamma}{b\beta\delta}\right) - N\left(\frac{a\alpha\gamma}{b\beta\delta}\right)^2}{\rho a A}$$

$$= \frac{a\gamma\alpha}{b\beta\delta A\rho}\left[\rho - \frac{N}{a}\,\frac{a\alpha\gamma}{b\beta\delta}\right]$$

or

$$\bar{x}_3 = \frac{a\gamma\alpha}{b\beta\delta A\rho} \left[\rho - \frac{(b+\beta)\ (b+\delta)\ (\beta+\delta)}{b\beta\delta} \right] \tag{2.26}$$

Since all the parameters are positive, therefore the positivity of the equilibrium concentration $\bar{x}_3$ gives the constraint:

$$\rho > F\ (b,\beta,\delta) \tag{2.27}$$

where $$F\ (b,\beta,\delta) = \frac{(b+\beta)(b+\delta)(\beta+\delta)}{b\beta\delta} \tag{2.28}$$

The minimum value of ρ is obtained by minimising $F(b,\beta,\delta)$. $\frac{\partial F}{\partial b} = 0 = \frac{\partial F}{\partial \beta} = 0 = \frac{\partial F}{\partial \delta}$ leads to the condition $b = \beta = \delta$. [Since the reaction parameters are all positive, the second derivatives are also positive, hence it corresponds to minima].

$$\therefore \quad F(b,\beta,\delta)_{min} = 8, \quad \text{from} \tag{2.28}$$

Hence $\rho > 8$ (2.29)

So far a three step feed back control process the power of the concentration of the inhibitor molecule must be greater than 8. In other words, more than 8 molecules of the inhibitor is required to suppress the $x_1 \to x_2$ reaction. The authors have also found a relation for k with all the other reaction parameters. (2.4c) can be written as,

$$\bar{x}_3^{\rho} + \frac{A}{k} = \frac{a\alpha\gamma}{kb\beta\delta} \cdot \frac{1}{\bar{x}_3} \tag{2.30}$$

Using (2.26), one gets

$$\bar{x}_3^{\rho} = \left(\frac{a\alpha\gamma}{Ab\beta\delta} \right)^{\rho} \left[1 - \frac{(b+\beta)(\beta+\delta)(b+\delta)}{\rho b\beta\delta} \right]^{\rho} \tag{2.31}$$

Using (2.31) and (2.26) in (2.30), one gets

$$\left(\frac{a\alpha\gamma}{Ab\rho\delta}\right)^{\rho}\left[1-\frac{(b+\rho)(b+\delta)(\rho+\delta)}{\rho b\rho\delta}\right]^{\rho}$$

$$=\frac{A}{k}\left\{\left[1-\frac{(b+\rho)(\rho+\delta)(b+\delta)}{\rho b\rho\delta}\right]^{-1}-1\right\}$$

$$=\frac{A}{k}\left[\frac{1-1+\dfrac{(b+\rho)(b+\delta)(\rho+\delta)}{\rho b\rho\delta}}{1-\dfrac{(b+\rho)(b+\delta)(\rho+\delta)}{\rho\rho b\delta}}\right] \tag{2.32}$$

$$=\frac{A}{k}\,\frac{(b+\rho)(b+\delta)(\rho+\delta)}{\rho b\rho\delta}\left[1-\frac{(b+\rho)(\rho+\delta)(b+\delta)}{\rho b\rho\delta}\right]^{-1}$$

$\therefore$ Equation (2.32) becomes,

$$k=\frac{A}{\rho b\rho\delta}\left(\frac{a\alpha\gamma}{Ab\rho\delta}\right)^{-\rho}\left[1-\frac{(b+\rho)(b+\delta)(\rho+\delta)}{\rho b\rho\delta}\right]^{-\rho-1}(b+\rho)(b+\delta)(\rho+\delta) \tag{2.33}$$

Returning to (2.17), the matrix equation becomes,

$$\dot{\chi}=D\chi \tag{2.34}$$

where D is given by (2.18).

This gives,

$$\begin{aligned}\chi_1 &= C_1e^{iIt}\\ \chi_2 &= C_2e^{-iIt}\\ \chi_3 &= C_3e^{Rt}\end{aligned} \tag{2.35}$$

where C_1, C_3 & C_3 are arbitrary constants. With the help of diagonalising matrix

$$T = \begin{pmatrix} K_1 & \frac{(b+iI)}{\alpha} K_1 & \frac{(b+iI)(\beta+iI)}{\alpha\gamma} K_1 \\ K_2 & \frac{(b-iI)}{\alpha} K_2 & \frac{(b-iI)(\beta-iI)}{\alpha\gamma} K_2 \\ K_3 & \frac{(R+b)}{\alpha} K_3 & -\frac{Q}{\delta+R} K_3 \end{pmatrix} \tag{2.36}$$

where K_1, K_2 and K_3 are again arbitrary. Using thefact that $(Ts)_i = \xi_i$ along with (2.35), one gets the equations for e_i:

$$\left.\begin{aligned}
e_1 &= \frac{\eta A_1}{I(I^2+R^2)} \operatorname{Cos}\,(It+\phi-\phi_1) + Ge^{Rt}\,\frac{(\beta\delta-bR)}{(I^2+R^2)} \\
\text{where}\quad A_1 &= [I^2(b^2-R^2)^2 + (bI^2+\beta\delta R)^2]^{\frac{1}{2}}, \\
\tan^{-1}\phi_1 &= \frac{(bI^2+\beta\delta R)}{I(b^2-R^2)}\,; \\
e_2 &= \frac{\eta A_2}{I(I^2+R^2)} \operatorname{Cos}\,(It+\phi-\phi_2) + Ge^{Rt}\,\frac{\alpha(\delta+R)}{(I^2+R^2)} \\
\text{where}\quad A_2 &= \alpha\,[\,I^2(\delta+R)^2 + (R\delta-I^2)^2\,]^{\frac{1}{2}}, \\
\tan^{-1}\phi_2 &= \frac{(R\delta-I^2)}{I(\delta+R)}\,; \\
e_3 &= \frac{\eta A_3}{I(I^2+R^2)} \operatorname{Cos}\,(It+\phi-\phi_3) + Ge^{Rt}\,\frac{\gamma\alpha}{(I^2+R^2)} \\
\text{where}\quad A_3 &= \alpha\gamma\,[\,I^2+R^2\,]^{\frac{1}{2}}, \\
\tan^{-1}\phi_3 &= R/I.
\end{aligned}\right\} \tag{2.37}$$

From equations (2.37) one can see that the solutions are not purely periodic, it is a superposition of two terms, one purely oscillatory and the other an exponentially damped quantity (R is a negative quantity). Asymptotically, the exponential term disappears and pure oscillation remain.

To check the validity of the assumptions involved ((2.6) & (2.8)) in the analysis, BPR has chosen some numbers (led by Goodwin's article in "Advances in Enzyme Regulations" Ed. C.Webster, Vol. 3) for the various parameters and shown that for $b = \beta = \delta = \alpha = \gamma = 1$, $\rho = 9$ (minimum allowed value), $a = 500$ and $A = 10$, the maximum of e_3 asymptotically is

$$(e_3)_{max} = \frac{A_3}{I(I^2+R^2)} = \frac{1}{6}$$

and $$\bar{x}_3 = \frac{a}{9A} = \frac{50}{9}$$

$$\therefore \quad e_3 \ll \bar{x}_3$$

Equation (2.33) becomes

$$k = \frac{8}{9} a \left(\frac{a}{9A} \right)^{-10} = 1.584 \times 10^{-5}$$

this gives $$\frac{\rho e_3 k \bar{x}_3^{\rho-1}}{A + k\bar{x}_3^{\rho}} = .24$$

$\therefore$ Both the assumptions (2.6) and (2.8) were satisfied. They have also checked the validity for cases when the exponential term in (2.37) is non-negligible and found out that it is still satisfied. The small value of k obtained by them has also been discussed. They have remarked that since $\tilde{x}_3 \simeq 5$, therefore $\tilde{x}_3^{\rho}$ is a huge quantity. Hence, for the nonlinear term to be significant, k must be small.

In their second paper B P & R have considered the n-step process in which the n^{th} constituent acts in such way that the $x_1 \to x_2$ reaction is suppressed. They have generalised all the results obtained in the first paper. We discuss their second paper now.

The n-step reaction network are described by the system of equations

$$\left.\begin{aligned} \dot{S}_1 &= \frac{a}{A+kS_n^{\rho}} - b_1 S_1 \\ \dot{S}_2 &= g_1 S_1 - b_2 S_2 \\ &\vdots \\ \dot{S}_n &= g_{n-1} S_{n-1} - b_n S_n \end{aligned}\right\} \tag{2.38}$$

Linearization is done by setting,

$$S_i = \overline{S_i} + e_i ; \qquad i = 1,2,\ldots,n \tag{2.39}$$

The "critical points" $\overline{S_i}$ are obtained by setting $\frac{d\overline{S_i}}{dt} = 0$ in equation (2.38), which gives

$$\bar{S}_n^{\rho+1} + \frac{A}{k}\bar{S}_n = \frac{a}{k}\,\frac{\prod_{i=1}^{n-1} g_i}{\prod_{i=1}^{n} b_i} \tag{2.40}$$

Substituting (2.39) in (2.38), one gets

$$\begin{aligned}
\dot{e}_1 &= \frac{a}{A+k(\bar{S}_n+e_n)^{\rho}} - b_1\,(\bar{S}_1 + e_1) \\
\dot{e}_2 &= g_1 e_1 - b_2 e_2 \\
&\vdots \\
\dot{e}_n &= g_{n-1} e_{n-1} - b_n e_n \quad .
\end{aligned} \tag{2.41}$$

Equation for $\dot{e}_1$ is then linearised by doing appropriate Taylor series expansion around $\bar{S}_n$ and keeping terms only upto first order. This gives (as before)

$$\dot{e}_1 = -b_1 e_1 - Q e_n \tag{2.42}$$

where $$Q = \frac{\rho k a\,\bar{S}_n^{\rho-1}}{(A+k\bar{S}_n^{\rho})^2} \tag{2.43}$$

Deriving the above relations the following assumptions have been taken as valid

$$e_n \ll \bar{S}_n \tag{2.44}$$

and $\rho\, e_n k \bar{S}_n^{\rho-1} \ll (A+k\bar{S}_n^{\rho})$.

In matrix form, equation (2.41) and (2.42) can be written as

$$\dot{\varepsilon}_i = M_{ij}\varepsilon_j \quad ; \quad i,j = 1,2,\ldots\ldots,n \tag{2.45}$$

where M is now a nxn dimensional matrix:

$$M = \begin{pmatrix} -b_1 & 0 & \cdots & \cdots & -Q \\ g_1 & -b_2 & 0 & & 0 \\ 0 & g_2 & -b_3 & \cdots & 0 \\ \vdots & \vdots & \vdots & \vdots & \vdots \\ 0 & \cdots & \cdots & g_{n-1} & -b_n \end{pmatrix} \tag{2.46}$$

The characterisitic equation for diagonalisation of M is

$$\prod_{i=1}^{n} (b_i+\lambda) + Q \prod_{i=1}^{n-1} g_i = 0 \tag{2.47}$$

where λ are the roots of the equation.

Let T = diagonalising matrix for M;

$$\therefore \quad TMT^{-1} = D, \tag{2.48}$$

where D is the diagonal matrix.

Then equation (2.45) can be written as

$$\dot{\chi} = D\chi \tag{2.49a}$$

$$\text{where } \dot{\chi} = T\dot{\varepsilon} \text{ and } \chi = T\varepsilon. \tag{2.49b}$$

In order that ε_i have periodic solutions (or consequently χ_i be oscillatory, the matrix M must have pure imaginary eigen-values. Since it is sufficient to have asymptotically periodic solutions, then at least one pair of eigenvalues of M should be pure imaginary and the remaining ones can be either negative real or complex with negative real parts.

Let the pure imaginary roots be

$$\lambda = \pm iI \tag{2.50}$$

where I is real.

Therefore equation (2.47) can be written as

$$\exp\left[i \sum_{i=1}^{n} \theta_i \right] \prod_{i=1}^{n} \eta_i + Q \prod_{i=1}^{n-1} g_i = 0 \tag{2.51}$$

where $(b_i + iI) = \eta_i e^{i\theta_i}$ (2.52)

with $\eta_i = (b_i^2 + I^2)^{\frac{1}{2}}$ and $\theta_i = \tan^{-1}\left(\frac{I}{b_i}\right)$ (2.53)

and $0 < \theta_i < \pi/2$ (2.54)

From (2.51), separating the real and imaginary parts, one gets

$$\prod_{i=1}^{n} \eta_i \cos \sum_{i=1}^{n} \theta_i = -Q \prod_{i=1}^{n-1} g_i$$
$$\prod_{i=1}^{n} \eta_i \sin \sum_{i=1}^{n} \theta_i = 0 \tag{2.55}$$

These two equations give (assuming $\eta_i \neq 0$)

$$\sum_{i=1}^{n} \theta_i = (2i+1)\Pi, \quad i = 0,1,2,\ldots \tag{2.56}$$

and

$$Q = \frac{\prod_{i=1}^{n} \eta_i}{\prod_{i=1}^{n-1} g_i} \tag{2.57}$$

Equation (2.43) and (2.57) give

$$\frac{\prod_{i=1}^{n-1} g_i}{\prod_{i=1}^{n} \eta_i} \quad \rho \, ka \, g_n^{\rho-1} = (A + k \, g_n^{\rho})^n \tag{2.58}$$

Equations (2.40) and (2.58) give

$$g_n = \frac{a}{A\rho} \frac{\prod^{n-1} g_i}{\prod^{n} b_i} \left[\rho - \frac{\prod^{n} \eta_i}{\prod^{n} b_i} \right] \tag{2.59}$$

Condition for g_n being positive is

$$\rho > \frac{\prod^{n} \eta_i}{\prod^{n} b_i} \tag{2.60}$$

since all other quantities are positive. To find the minimum of ρ, the function $\frac{\prod \eta_i}{\prod b_i}$ is minimised.

Putting $\frac{\partial}{\partial b_m} \left(\frac{\prod \eta_i}{\prod b_i} \right) = 0, \quad m = 1, 2, \ldots n$ (2.61)

one gets, (Appendix (A))

$$\frac{1}{b_m} = \frac{b_m}{\eta_m^2} + I \frac{\partial I}{\partial b_m} \sum^{n} \frac{1}{\eta_i^2} \tag{2.62}$$

Differentiating (2.56) with respect to b_m and using (2.53), one gets

$$\frac{\partial I}{\partial b_m} \sum_{i=1}^{n} \frac{b_i}{\eta_i^2} = \frac{I}{\eta_m^2}$$

$$\therefore \quad \eta_m^2 \frac{\partial I}{\partial b_m} = K_1, \quad \text{where } K_1 = I \left(\sum_{i} \frac{b_i}{\eta_i^2} \right)^{-1} = \text{Constant.}$$

Substitution of this equation in equation (2.62), gives

$$\eta_m^2 = b_m^2 + K_2\, b_m \tag{2.63}$$

where $K_2 = I\, K_1 \sum_{i=1}^{n} \frac{1}{\eta_i^2}$ = Constant.

From (2.63) and (2.53),

$$b_m = I^2/K_2 \tag{2.64}$$

This is true for all m, hence the minimum corresponding to the function $(\Pi\, \eta_i / \Pi\, b_i)$ is obtained when,

$$b_1 = b_2 = \ldots\ldots = b_n ; \; \eta_1 = \eta_2 = \ldots\ldots = \eta_n \tag{2.65}$$

Consequently, equation (2.53) gives,

$$\theta_1 = \theta_2 = \ldots\ldots = \theta_n \tag{2.66}$$

Equation (2.53) also gives that

$$\frac{\eta_i}{b_i} = \sec\, \theta_i . \tag{2.67}$$

Hence

$$\left(\frac{\prod_{i=1}^{n} \eta_i}{\prod_{i=1}^{n} b_i} \right)_{min} = [\prod_{i=1}^{n} \text{Sec}\ \theta_i]_{min}$$

$$= \text{Sec}^n \left(\frac{\Pi}{n} \right) \qquad (2.68)$$

Since $0 < \theta_i < \pi/2$ and $\sum_{i=1}^{n} \theta_i = (2\ell+1)\Pi$ (2.69)

∴ Equation (2.60) finally gives

$$\rho > \sec^n \left(\frac{\Pi}{n} \right) \qquad (2.70)$$

As in the case of n = 3, a constraint on K is also obtained here.

Equations (2.40) and (2.58) can be exploited to give the following result:

$$k = A \left[-1+\rho \left(\rho - \frac{\prod^{m} \eta_i}{\prod^{n} b_i}\right)^{-1} \right] \left[\frac{a}{A\rho} \frac{\prod^{n-1} g_i}{\prod^{n} b_i} \left(\rho - \frac{\prod^{n} \eta_i}{\prod^{n} b_i}\right) \right]^{-\rho} \qquad (2.71)$$

Since ρ is constrained by (2.70), therefore the expression for k in equation (2.71) leads to a new constraint on the rate coefficient which controls the feed-back. The relevance of this result is the same as for the case of n = 3.

CHAPTER IV

DISCUSSIONS

New, since we have discussed the works of Rapp and BPR in details, we can compare the merits and demerits of the methods used and results obtained.

It is, of course, undoubtedly true that Lyapunov analysis has given the results in a more elegant and straight forward way than the describing function method.

Rapp, in his two papers, has derived the solutions of the set of differential equations representing feed back control systems for $\rho = 1$ and $\rho = 2$ only. He has also indicated that the cases of $\rho = 3$ and 4 are in progress. For each value of ρ, one has to go through tedious mathematical calculations to derive the analytic forms for the describing function. The integrals and solutions of equations become more difficult as one goes to higher powers of ρ. Apparently it seems that there can be no straight forward generalisation for the case of any ρ.

On the other hand, BPR have derived the general condition for oscillatory solutions in a simple but fairly rigorous way by using Lyapunov analysis. Their results agree totally with Rapp's.

For n = 7, the inequality (2.70) gives

$$\rho > \operatorname{Sec}^7 \left(\frac{\pi}{7} \right) = \operatorname{Sec}^7 (25^\circ 43') = 2.072$$

and for $n = 8$, it gives

$$\rho > \text{Sec}^8 \left(\frac{\pi}{8} \right) = \text{Sec}^8 (22^\circ 30') = 1.850$$

These two results show that for $\rho = 2$, n must be equal to or greater than 8 for a stable limit cycle to appear. Thus the result agrees with Rapp's equation (1.69).

From BPR's analysis, it is also obvious that ρ can never be less than one; (sec 0 = 1 at $n = \infty$ only and $0 < \theta_1 < \frac{\pi}{2}$ and ρ is positive), hence there can be no asymptotically stable periodic solution for $\rho = 1$. This result also agrees with the results of the first paper of Rapp, where he says that no stable limit cycle can result for $\rho = 1$, general n.

Both Rapp and BPR have derived a relation for the equilibrium value of the n^{th} chemical species which acts as the inhibitor for the feed back control process.

Rapp has shown in equation (1.53) that the equilibrium value of S_n (i.e. y_o or (S   ) for $\rho = 2$ is dependent on the reaction parameters only. BPR, in equations (2.26) and (2.59) have given explicit solutions for the equilibrium value of S_3 and S_n along with the constraints (2.29) and (2.70) on ρ. That means, when ρ is given, one can find the values of (S_3) or (S_n) explicitly. In Rapp's paper y_o or (S_n) (for $\rho = 2$) has been derived from the zeroeth balance equation (1.41) which involves the integral a_o which in turn is a function of

ρ(=2 here). But in DPR's work one can see easily how the equilibrium value $(S_n)_e$ depends on ρ.

One of the principal results obtained by Rapp is that when $\left|\frac{G(i\omega)}{G(0)}\right|$ is kept fixed in the inequality

$$U(A)\left|\frac{G(i\omega)}{G(0)}\right| > 1$$

stable limit cycle condition is also satisfied for all A ($A = d_1\sqrt{d_2}/\sqrt{2}\, c_o$) greater than a $A_{minimum}$, where U(A) is given equation (1.67). That means the condition for stable limit cycle is dependent on the value of A_{min}. Now, A is a function of the reaction parameters d_1, d_2 and c_o which in turn depend on α, k, g_j's and b_j's. Thus the values of the parameters also determine the condition for stable oscillations when the reaction steps (n) are kept fixed.

The expression for k in terms of other reaction parameters have been obtained by DPR in both of their papers. This is an additional constraint which controls the feedback. Equations (2.33) and (2.71) shows clearly how k is dependent on the other reaction parameters and also on ρ.

Thus the value of k changes with ρ even when the other parameters are kept constant. To understand it we must discuss the physical significance of k.

While deriving the nonlinear term in $\dot{X}_1$ equation of (G) i.e. $\frac{a}{A + kx_3}$ (for $\rho = 1$), Goodwin [2] has assumed that the metabolite remains in a steady state relative to the epigenetic species, because the relaxation time of the metabolic system is much larger than that of the epigenetic system. The parameter $k = \frac{m\gamma}{\delta}$ refer [2]. γ and δ are obtained from the control equations for X_3, i.e. $\frac{dX_3}{dt} = \gamma X_2 - \delta X_3$, where X_2 is the protein or enzyme.

γ involves the rate of enzyme action, the substrate concentration and other constants. δ is the rate constant for the degradation of the metabolite. The constant m is a complicated function of many elementary constants which relate to the equilibrium constants of the processes like:

i) reaction between the repressors and the DNA templates

ii) reaction of precursors for RNA synthesis, namely nucleotides, with the free DNA templates,

iii) if the repressor do not act directly to repress mRNA synthesis but do so by combining with an aporepressor, then m also contains the equilibrium constant for that complicated reaction.

We have already seen that k is a function of the other parameters, but its dependence on m might give a clue of its dependence on ρ. Generally the physical interpretation of ρ is the following: it represents the number of molecules of the inhibitor required to block the X_1 to X_2 reaction, or it is related to the number of binding sites for X_n on X_1. From Goodwin's discussion on m, it is fairly correct to infer that m is, in a way, related to the binding affinity of X_n to X_1, or in other words, it is related to the various ways the inhibitor reacts with X_1 to repress $X_1 \rightarrow X_2$ reaction.

So it seems to be natural for m to depend on the number of binding sites or on the number of molecules of the inhibitor, ρ. Consequently, it is rather convincing that k should also depend on ρ. Thus the relation obtained by BPR for k is not only useful to make the nonlinear term significant, ~~but also has the nonlinear term significant~~, but also has physical meaning. When all the other reaction parameters are constant, even then k will change with ρ. This is an important result obtained by BPR.

Rapp in his second paper, has given another important and biologically significant result. It is the dependence of the envelope of the solutions on initial values. He has shown by taking representative examples that, keeping all the parameters the same, the qualitative behaviour of the envelope of the solutions change drastically with the change in initial concentrations (x_j values). Asymptotically both have the same sort of curve but in one case it slowly builds up and in the other it shoots up for a very short time and then smoothens to the stable form. This may be very important in the case of developmental triggers. Chemical oscillators coupled to different systems may trigger the developmental mechanisms to start at different level of concentrations. Thus the finding by Rapp is quite significant.

BPR in their first paper (n = 3 case) have derived the oscillatory solutions explicitly (equation (2.37)). The solutions contain the eigenvalues I and R. Knowing them, one can have the exact numerical solutions of the equations. From equation (2.68), knowing n and b_1 one can find η_1 and consequently I from equation (2.53). For n = 3, the values chosen by the authors were

$$b = \rho = \alpha = \delta = \gamma = 1$$

Therefore from equation (2.20) $I^2 = 3$, or $I = \pm\sqrt{3}$.
From (2.19), R = -3, using the values of I and R, the solutions s_1, s_2 and s_3 can be written. For a n-step process, the frequency can be found, when all the ρ's are equal, by using equation (2.68). For n = 12 and all $\rho_i = 1$, equation (2.68) gives $(1 + I^2)^6 = \sec^{12}(\frac{\pi}{12})$ or I = 0.268. This value is the same as obtained by Rapp in his second paper. However, it is interesting to note that when the ρ's are all equal, the frequency of oscillation asymptotically is independent of ρ and the other parameters like a, A, k, α_1, etc. and depends only on ρ and n.

Since the frequency we have calculated depends on the assumption that there is only one pair of purely imaginary roots then this is similar to the approximation in the describing function method of keeping just the first harmonic of the Fourier expansion of f(z). The existence of more than one pair of imaginary roots would imply the existence of more than one frequency of oscillation, and consequently one could expect the appearance of harmonics.

In describing function analysis it is not possible to get the analytical oscillatory solutions of the concentrations of the biochemical species involved, but it gives the criterial for asymptotically stable oscillations.

On the other hand, Lyapunov analysis not only gives explicit analytic solutions where the appearance of the term e^{Rt} (R is negative) shows the existence of damping (which asymptotically becomes insignificant) giving rise to stable pure sinusoidal oscillations, but it also gives a general condition on ρ and n for the existence of stable oscillations. Thus, after reviewing and discussing both the methods, we comment that Lyapunov's method is algebraically more tractable and gives general results concerning all ρ and n.

APPENDIX-A

MINIMIZATION OF THE FUNCTION $\dfrac{\prod_i \eta_i}{\prod_i b_i}$

$$\eta_i = (b_i^2 + I^2)^{\frac{1}{2}}$$

$$\frac{\partial}{\partial b_m}\left(\frac{\prod_i (b_i^2 + I^2)^{\frac{1}{2}}}{\prod b_i} \right) = \frac{1}{\prod b_i}\left[\frac{\partial}{\partial b_m} \prod (b_i^2 + I^2)^{\frac{1}{2}} \right]$$

$$+ \prod \eta_i \frac{\partial}{\partial b_m} \frac{1}{\prod b_i}$$

$$= -\frac{\prod \eta_i}{\prod b_i} \frac{1}{b_m} + \frac{1}{\prod b_i}\left[\prod_i{}' \eta_i \frac{\partial}{\partial b_m} \eta_m + \sum_i{}'' \prod_i{}' \eta_i \frac{\partial}{\partial b_m} \eta_i \right]$$

$$= -\frac{\prod \eta_i}{\prod b_i} \frac{1}{b_m} + \frac{1}{\prod b_i} \prod \eta_i \frac{1}{2}\frac{1}{\eta_m^2}\left(2 b_m + 2 I \frac{\partial I}{\partial b_m}\right)$$

$$+ \frac{1}{\prod b_i} \prod \eta_i \sum_i \frac{1}{\eta_i^2} \frac{1}{2} 2 I \frac{\partial I}{\partial b_m}$$

$$= \frac{\prod \eta_i}{\prod b_i}\left[-\frac{1}{b_m} + \frac{b_m}{\eta_m^2} + I \frac{\partial I}{\partial b_m} \sum \frac{1}{\eta_i^2} \right] \tag{a}$$

For extremum,

$$\frac{\partial}{\partial b_m}\left(\frac{\prod \eta_i}{\prod b_i} \right) = 0 \tag{b}$$

or

$$\frac{1}{b_m} = \frac{b_m}{\eta_m^2} + I \frac{\partial I}{\partial b_m} \sum_i \frac{1}{\eta_i^2} \tag{2.62}$$

SECOND DERIVATIVE

$$\frac{\partial}{\partial b_p}\frac{\partial}{\partial b_m}\left(\frac{\Pi\eta_i}{\Pi b_i}\right) = \frac{\partial}{\partial b_p}\left[\frac{\Pi\eta_i}{\Pi b_i}\left(\frac{b_m}{\eta_m^2} + I\frac{\partial I}{\partial b_m}\sum_i \frac{1}{\eta_i^2} - \frac{1}{b_m}\right)\right]$$

$$\text{First term} = \frac{\Pi\eta_i}{\Pi b_i}\frac{\partial}{\partial b_p}\left(\frac{b_m}{\eta_m^2} + I\frac{\partial I}{\partial b_m}\sum \frac{1}{\eta_i^2} - \frac{1}{b_m}\right)$$

$$\text{Second term} = \left(\frac{b_m}{\eta_m^2} + I\frac{\partial I}{\partial b_m}\sum \frac{1}{\eta_i^2} - \frac{1}{b_m}\right)\frac{\partial}{\partial b_p}\frac{\Pi\eta_i}{\Pi b_i} = 0$$ from the condition of extremum.

$$\therefore \text{First term} = \frac{\Pi\eta_i}{\Pi b_i}\left[\frac{\delta_{mp}}{\eta_m^2} - \frac{2b_m}{\eta_m^3}\frac{\partial\eta_m}{\partial b_p} + \frac{\partial I}{\partial b_p}\frac{\partial I}{\partial b_m}\sum_i\frac{1}{\eta_i^2} + I\frac{\partial^2 I}{\partial b_p \partial b_m}\sum_i\frac{1}{\eta_i^2}\right.$$

$$\left. + I\frac{\partial I}{\partial b_m}\frac{\partial}{\partial b_p}\left(\sum_i \frac{1}{\eta_i^2}\right) + \frac{1}{b_m^2}\delta_{mp}\right] \qquad \text{(c)}$$

Since $\eta_m = (b_m^2 + I^2)^{\frac{1}{2}}$

$$\therefore \frac{\partial\eta_m}{\partial b_p} = \frac{1}{2\eta_m}\left(2b_m\delta_{mp} + 2I\frac{\partial I}{\partial b_p}\right) \qquad \text{(d)}$$

calculation of $\frac{\partial I}{\partial b_p}$:

From equations (2.53) and (2.56)

$$\sum_i \frac{\partial\theta_i}{\partial b_m} = \sum_i \frac{\partial}{\partial b_m}\tan^{-1}\left(\frac{I}{b_i}\right) = 0$$

or $$\frac{1}{1+\frac{(b_m)^2}{I^2}}\frac{\partial}{\partial b_m}\left(\frac{b_m}{I}\right)+\sum_i{}'\frac{1}{1+\frac{b_i^2}{I^2}}\frac{\partial}{\partial b_m}\left(\frac{b_i}{I}\right)$$ (' indicates all term excluding $i = m$)

or $$\frac{I^2}{\eta_m^2}\left(I - b_m\frac{\partial I}{\partial b_m}\right)+\sum_i{}'\frac{I^2}{\eta_i^2}b_i - \frac{1}{I^2}\frac{\partial I}{\partial b_m} = 0$$

or $$\frac{I}{\eta_m^2} - \frac{b_m}{\eta_m^2}\frac{\partial I}{\partial b_m} - \sum_i{}'\frac{b_i}{\eta_i^2}\frac{\partial I}{\partial b_m} = 0$$

or $$\frac{\partial I}{\partial b_m}\sum_i\frac{b_i}{\eta_i^2} = \frac{I}{\eta_m^2}$$

or $$\frac{\partial I}{\partial b_m} = \frac{I}{\eta_m^2}\left[\sum_i\frac{b_i}{\eta_i^2}\right]^{-1} \qquad (e)$$

At minimum, i.e. when $b_1 = b_2 = \ldots = b_n = b$, all η_i's are equal and can be written as η.

$$\therefore \left(\frac{\partial I}{\partial b_m}\right)_{min} = \frac{I}{\eta^2}\left(\frac{nb}{\eta^2}\right)^{-1} = \frac{I}{nb} \qquad (f)$$

Using (f) in (d), at minimum,

$$\left(\frac{\partial \eta_m}{\partial b_p}\right)_{min} = \frac{1}{2}\left(2b\,\delta_{mp} + \frac{2I^2}{nb}\right) = \frac{1}{\eta}\left(b\,\delta_{mp} + \frac{I^2}{nb}\right) \qquad (g)$$

$$\frac{\partial}{\partial b_p}\left(\sum_{i=1}^{n}\frac{1}{(b_i^2 + I^2)}\right) = \sum_{i=1}^{n}\left[-\frac{1}{(b_i^2 + I^2)^2}\left(2b_i\,\delta_{ip} + 2I\frac{\partial I}{\partial b_p}\right)\right]$$

$$= -\frac{2b_p}{(b_p + I^2)^2} - \sum_{i=1}^{n} 2I\left(\frac{\partial I}{\partial b_p}\right)\frac{1}{\eta_i^4}$$

At minimum, when $b_1 = b_2 = \ldots\ldots = b_n = b$

$$= -\frac{2b}{\eta^4} - \frac{2I^2}{nb}\ \frac{n}{\eta^4} = -\frac{2b}{\eta^4} - \frac{2I^2}{b\eta^4} \qquad \text{(h)}$$

From equation (e), one gets by differentiating w.r.t. b,

$$\frac{\partial^2 I}{\partial b_p \partial b_m}\left(\sum_1 \frac{b_i}{\eta_i^2}\right) + \frac{\partial I}{\partial b_m}\left[\frac{\partial}{\partial b_p}\left(\sum_1 \frac{b_i}{\eta_i^2}\right)\right] = \frac{\partial I}{\partial b_p}\ \frac{1}{\eta_m^2} + I\frac{\partial}{\partial b_p}\left(\frac{1}{\eta_m^2}\right)$$

or

$$\frac{\partial^2 I}{\partial b_p \partial b_m} = \left(\frac{\partial I}{\partial b_m}\ \frac{1}{\eta_m^2} - \frac{\partial I}{\partial b_m}\left[\frac{1}{\eta_p^2} - 2\sum_{i=1}^{n} \frac{b_i}{\eta_i^3}\ \frac{\partial}{\partial b_p}(\eta_i)\right]\right.$$

$$\left. - I2\frac{1}{\eta_m^3}\frac{\partial}{\partial b_p}(\eta_m)\right]\left(\sum_{i=1}^{n} \frac{b_i}{\eta_i^2}\right)^{-1}$$

Using (f) and (g), one gets the minimum of the double derivative of I, as

$$\left(\frac{\partial^2 I}{\partial b_p \partial b_m}\right)_{min} = \left[\frac{I}{\eta^2 nb} - \frac{I}{nb}\left[\frac{1}{\eta^2} - \left(2\sum_1 \frac{b_i}{\eta_i^3}\ \frac{1}{2\eta_i}\left(2b_i\,\delta_{ip} + 2I\frac{\partial I}{\partial b_p}\right)\right)_{min}\right.\right.$$

$$\left. - \frac{I}{\eta^4}\left(2b\,\delta_{mp} + \frac{2I^2}{nb}\right)\right)\left(\frac{n^2}{nb}\right)\Big]$$

$$= \left[\frac{I}{nb\eta^2} - \frac{I}{nb\eta^2} + \frac{I}{nb}\left(\frac{2b^2}{\eta^4} + \frac{2I^2}{nb}\ \frac{nb}{\eta^4}\right)\right.$$

$$\left. - \frac{2I}{\eta^4}\,b\delta_{mp} - \frac{2I^3}{nb\eta^4}\right]\ \frac{n^2}{nb}$$

$$= \left[\frac{2Ib}{n\eta^4} - \frac{2Ib}{\eta^4}\delta_{mp} \right] \frac{\eta^2}{nb} = \frac{2I}{\eta^2 n^2} - \frac{2I\delta_{mp}}{n\eta^2}$$

$$\therefore \left(\frac{\partial^2 I}{\partial b_p \partial b_m} \right)_{min} = \frac{2I}{\eta^2 n^2} - \frac{2I\delta_{mp}}{n\eta^2} \qquad (i)$$

Therefore using (c), (f) and (i) the second derivative becomes (at the minimum):

$$\frac{\partial}{\partial b_p}\frac{\partial}{\partial b_m}\left(\frac{\Pi\eta_i}{\Pi b_i} \right) = \frac{\eta^n}{b^n}\Big[\frac{\delta_{mp}}{\eta^2} - \frac{b}{\eta^4}\left(b\delta_{mp} + \frac{I^2}{nb}\right)$$

$$+ \frac{I^2}{n^2 b^2}\,\frac{n}{\eta^2} + \frac{In}{\eta^2}\left(\frac{2Ib}{n\eta^4} - \frac{2Ib}{\eta^4}\delta_{mp}\right)\frac{\eta^2}{nb}$$

$$- \frac{2I^2}{nb}\left(\frac{b}{\eta^4} + \frac{I^2}{b\eta^4} \right) + \frac{\delta_{mp}}{b^2} \Big]$$

$$= \frac{\eta^n}{b^n}\Big(\delta_{mp}\left[\frac{1}{\eta^2} - \frac{b^2}{\eta^4} - \frac{2I^2}{\eta^4} + \frac{1}{b^2} \right]$$

$$- \frac{I^2}{n\eta^4} + \frac{I^2}{nb^2\eta^2} + \frac{2I^2}{n\eta^4} - \frac{2I^2}{n\eta^4} + \frac{2I^4}{nb^2\eta^4} \Big)$$

$$= \left(\frac{\eta}{b} \right)^n \left[\delta_{mp}\,\frac{(b^4 + I^4 + I^2 b^2)}{\eta^4 b^2} + \frac{3I^4}{nb^2\eta^4} \right] \qquad (j)$$

The second derivative is a matrix D^n_{mp}. The diagonal elements of D_{mp} are obtained from (j) when $m = p$, and other terms give the non diagonal elements. It is obvious that all the diagonal elements are equal and also all the non-diagonal elements are equal to each other. The off-diagonal elements are:

$$\left(\frac{\eta^2}{b^2}\right)^{\eta/2} \frac{I^4}{nb^2\eta^4} = (\sec^n \frac{\pi}{n}) \frac{3b^4(\frac{I^4}{b^4})}{nb^2 \frac{\eta^4}{b^4} b^4}$$ using equations (2.68) and (2.53)

$$= \sec^n \frac{\pi}{n} \frac{3b^4 \tan^4(\frac{\pi}{n})}{nb^2 b^4 \sec \frac{\pi}{n}}$$

$$= \frac{\sec^n(\frac{\pi}{n})}{b^2} \frac{3\sin^4(\frac{\pi}{n})}{n} \qquad (k)$$

The diagonal element is:

$$\left(\frac{\eta^2}{b^2}\right)^{\eta/2} \left[\frac{(b^2+I^2)^2 - I^2 b^2}{b^2(b^2+I^2)^2} + \frac{3I^4}{nb^2\eta^4} \right]$$

$$= \sec^n(\frac{\pi}{n}) \left[\frac{b^4[\sec^n(\frac{\pi}{n}) - \sec^n(\frac{\pi}{n})]}{b^6 \sec^n(\frac{\pi}{n})} + \frac{3\sin^4(\frac{\pi}{4})}{nb^2} \right]$$

$$= \sec^n(\frac{\pi}{n}) \left(\frac{\sec^2 \frac{\pi}{n} - 1}{b^2 \sec^2(\frac{\pi}{n})} + \frac{3\sin^4 \frac{\pi}{n}}{nb^2} \right)$$

$$= \sec^n(\frac{\pi}{n}) \left[\frac{\sin^2 \frac{\pi}{n}}{b^2} + \frac{3\sin^4(\frac{\pi}{4})}{nb^2} \right]$$

$$= \frac{\sec^n(\pi/n)}{b^2} \sin^2(\frac{\pi}{n}) \left[1 + \frac{3\sin^2(\frac{\pi}{n})}{n}\right] \qquad (1)$$

The condition for minimum is that the determinant $|D^n_{mp}| > 0$ for all n where $b_1 = b_2 = \ldots = b_n$.

From equations (k) and (l), one can easily see that all elements are positive (n is always greater than 1 and 2) and the diagonal elements are larger than the non-diagonal ones. Let A be the diagonal element and B the off-diagonal ones. Then

$$|D^n| = \begin{vmatrix} A & B & B & \cdots\cdots & B \\ B & A & B & \cdots\cdots & B \\ B & B & A & B \cdots\cdots & B \\ \vdots & \vdots & \vdots & \vdots & \vdots \\ B & B & B & \cdots\cdots & A \end{vmatrix} \qquad \text{where } A > B > 0$$

To show that $|D^n|$ is positive we do the following:

Consider n = 2,

$$D^2 = \begin{vmatrix} A & B \\ B & A \end{vmatrix} = A^2 - B^2 > 0, \qquad \text{since } A > B.$$

n = 3,

$$D^3 = \begin{vmatrix} A & B & B \\ B & A & B \\ B & B & A \end{vmatrix} = AD^2 - 2BR_2$$

$$\text{where } R_2 = \begin{vmatrix} B & B \\ B & A \end{vmatrix} = AB - B^2$$

$$\therefore \quad D^3 = A(A^2 - B^2) - 2AB^2 + 2B^3$$

or $$D^3 = A^3 - AB^2 - 2AB^2 + 2B^3$$

$$= A^3 + AB^2 - 2A^2B + 2A^2B + 2B^3 - 4AB^2$$

$$= A(A^2 + B^2 - 2AB) + 2B(A^2 + B^2 - 2AB)$$

$$= (A + 2B)(A-B)^2 > 0$$

$$D^4 = \begin{vmatrix} A & B & B & B \\ B & A & B & B \\ B & B & A & B \\ B & B & B & A \end{vmatrix} = AD^3 - 3BR_3$$

where

$$R_3 = \begin{vmatrix} B & B & B \\ B & A & B \\ B & B & A \end{vmatrix}$$

$$\therefore \quad D^4 = A[(A + 2B)(A-B)^2] - 3B(B(A^2-B^2)-2B(AB-B^2))$$

$$= A(A+2B)(A-B)^2 - 3B^2(A-B)[A+B - 2B]$$

$$= A(A+2B)(A-B)^2 - 3B^2(A-B)^2$$

$$= (A-B)^2[A^2 + 2AB - 3B^2]$$

$$= (A-B)^2[A^2 + AB + 3AB - 3B^2]$$

$$= (A-B)^3 (A + 3B) > 0.$$

Let us assume that

$$D^n = (A-B)^{n-1} [A + (n-1)B]$$

Then $\quad D^{n+1} = AD^n - n\,BR_n$.

Now $\quad R_2 = B(A - B)$

$$R_3 = B(A - B)^2$$

$$\vdots$$

$$R_n = B(A - B)^{n-1}$$

$$\therefore \quad D^{n+1} = AD_n - B_n\, B(A - B)^{n-1}$$

$$= A(A-B)^{n-1}[A + (n-1)B] - B_n\, B(A-B)^{n-1}$$

$$= [A-B]^n[A + nB]$$

Thus we see that all

$$D^n > 0 \text{ when } b_1 = b_2 = \dots = b_n = b$$

Therefore the condition for minimum is satisfied.

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