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Dynamic Characteristics of Enzymatic Feedback Systems

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In order to find out the basic and general structure common to enzymatic feedback-systems, which are able to maintain the output of whole system at a desired level with rejecting an external perturbation, dynamic characteristics of various enzymatic feedback-systems were studied by means of computer simulation. Under a constant input, an oscillating feedback-system composed of a single large-sized feedback-loop, was found to be relatively unstable for the external perturbation. On the other hand, a non-oscillating feedback-system, which includes several short-sized feedback-loops, had a capability to retain the end product concentration at a constant level against external perturbation. From the results of the simulation, necessary conditions for the basic structure were discussed.

INTRODUCTION

The general response of a nonlinear enzymatic feedback-system to oscillatory input has been reported in the previous paper (Okamoto *et al.,* 1977); the frequency-response curve of a given enzymatic feedback-system showed a single peak or double peaks, and their positions in the frequency units shifted with the change in the feedback constant. This fact seemed to be one of the characteristics of nonlinear feedback-systems.

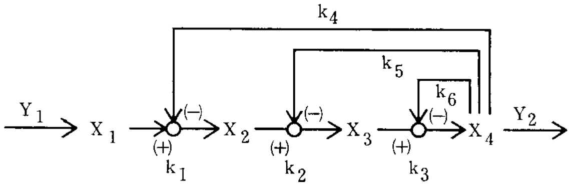
The nonlinear feedback-system dealt with in the previous study (Okamoto *et al.,* 1977) included only a single feedback-loop. In living organisms, however, there may be various structures of feedback-systems, and these appear to have commonly multiple feedback-loops. Since Stadtman (1970) has first classified the mode of feedback regulation in biosynthetic path ways, many problems on the feedback regulation have been discussed in detail. However, a large part of the dynamic characteristics of feedback-systems seem to be remained unexplained.

In the present study, the dynamic characteristics of various enzymatic systems including several feedback-loops were simulated, and the structure of the feedback control system most stable to external perturbation was examined. This will be of value for understanding the relationships between the structure and controlling capability of feedback-system against external perturbation.

COMPUTATION

The following four types of models were subjected to the computational simulation as typical biochemical feedback-system. In each scheme, X_i represents the reactant or intermediate, and Y_1 and Y_2 are the input and output of the system, respectively.

Type A



Scheme 1.

This type of scheme is called "simple end product inhibition" (Stadtman, 1970). k_1, k_2 and k_3 represent the reaction rate constants of corresponding steps, and k_4, k_5 and k_6 are considered to be feedback constant controlling the weight of contribution of the X_4 concentration. The detailed mode of operation at the summing points of the feedback-loops was described in a previous paper (Okamoto et al., 1976).

The rate equation of Scheme 1 in the form of simultaneous differential equation may be written as:

$$\begin{cases} \dfrac{dX_1}{dt} = Y_1 - (k_1/k_4X_4)X_1 \\ \dfrac{dX_2}{dt} = (k_1/k_4X_4)X_1 - (k_2/k_5X_4)X_2 \\ \dfrac{dX_3}{dt} = (k_2/k_5X_4)X_2 - (k_3/k_6X_4)X_3 \\ \dfrac{dX_4}{dt} = (k_3/k_6X_4)X_3 - Y_2 \end{cases} \tag{1}$$

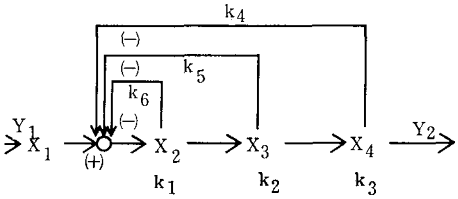
The value of Y_2 (output) was assumed to be constant ($Y_2 = A$) during the reaction, while Y_1 (input) was assumed to be given by the following equation:

$$Y_1 = A + B\sin(\omega t) \tag{2}$$

where B and ω denote the amplitude and the angular velocity (defined as frequency of sinusoidal curve), respectively.

Type B

This type of scheme (Scheme 2) is termed "multivalent inhibition of unifunctional enzyme" (Umbarger, 1969). The step of $X_1 \rightarrow X_2$ in the scheme is inhibited not only by the end product X_4 but also by other intermediates X_3



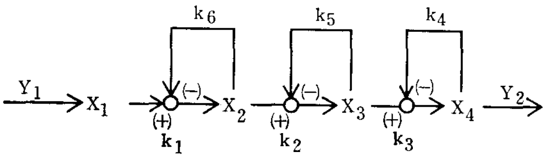
Scheme *2.*

and X_2. k_i **is** of the same notation as in Scheme 1.

The rate equation of Scheme 2 may be written as:

$$\begin{cases} \frac{dX_1}{dt}=Y_1-[k_1/(k_4X_4k_5X_3k_6X_2)]\,X_1 \\ \frac{dX_2}{dt}=[k_1/(k_4X_4k_5X_3k_6X_2)]\,X_1-k_2X_2 \\ \frac{dX_3}{dt}=k_2X_2-k_3X_3 \\ \frac{dX_4}{dt}=k_3X_3-Y_2 \end{cases} \tag{3}$$

Type C



Scheme 3.

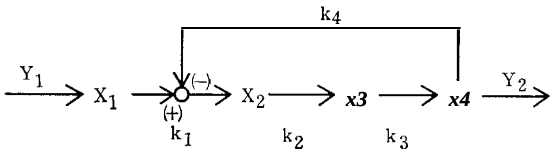
This type of scheme called "sequential inhibition" (Stadtman, 1970). In this scheme, $X_1 \rightarrow X_2$ step is inhibited not by the end product X_4 but by the intermediate X_2. However, $X_2 \rightarrow X_3$ step is inhibited by X_3 and the neighboring step $X_3 \rightarrow X_4$ is by the end product X_4. Eventually, $X_1 \rightarrow X_2$ step is inhibited by the end product indirectly.

The rate equation of Scheme 3 may be expressed as follows:

$$\begin{cases} \frac{dX_1}{dt}=Y_1-(k_1/k_6X_2)X_1 \\ \frac{dX_2}{dt}=(k_1/k_6X_2)X_1-(k_2/k_5X_3)X_2 \\ \frac{dX_3}{dt}=(k_2/k_5X_3)\,X_2-(k_3/k_4X_4)\,X_3 \\ \frac{dX_4}{dt}=(k_3/k_4X_4)X_3-Y_2 \end{cases} \tag{4}$$

Type D

The following feedback-system which had been subjected to the previous study (Okamoto et al., 1977) was also dealt with for comparison.



Seheme 4.

The numerical solution of the simultaneous differential equation was performed by means of either the MRKGM subprogram, in which the restriction of non-negative concentration of reactants was added to the Runge-Kutta-Gill method (Okamoto et *al.,* 1975), or the ADSL (Analog to Digital Simulation Language) application program.

All computations were carried out by a FACOM 230-75 digital computer in the Computer Center of Kyushu University.

METHODS

The following methods were adopted to characterize the dynamic behavior of each scheme.

1) Frequency-response test

It has been well known that the frequency-response test is one of the most valuable methods for the characterization of general linear feedback-systems. This method is concerning with the question how the amplitude ratio of the output to input changes with the increase in angular velocity or frequency of the sinusoidal input. The details of the application to the enzymatic system of this test were reported in the previous paper (Okamoto et al., 1977).

2) Changes in damping constant (Higgins, 1967)

There is evidence that a feedback control system may play a major role in the occurrence of oscillation of the concentration of an intermediate in enzymatic reaction-system *in vivo.* For the analysis of oscillatory behavior,

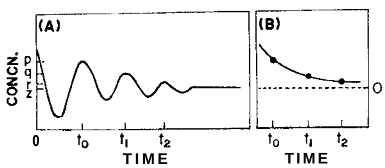


Fig. 1. Typical pattern of damped oscillation with Scheme 4. A constant input was applied to the scheme. (A) Time-course, (B) Damping curve. p, q, r are concentrations of peaks and z is stationary concentration.

a damping factor has been often used for descriptive purposes. This factor represents the damping rate of oscillation. Figure 1 (A) shows a typical damped oscillatory pattern of an intermediate *vs* time observable with Scheme 4. The co-ordinates $(t_0, p), (t_1, q)$ and (t_2, r) indicate the top points of this curve, and z is the stationary value of the damped oscillation. Figure 1 (B) shows plot of the top points *vs* time assuming that the value of z (stationary value) is zero. The curve thus obtained can be expressed by a single exponential function as follows:

$$D(t)=ae^{-\lambda t} \tag{5}$$

where a and A are arbitrary coefficients. As is mentioned above, damping factor d can be defined as:

$$\mathbf{d} = \frac{D\left(t+\frac{2\pi}{\omega}\right)}{D(t)} \tag{6}$$

where ω is the angular velocity of oscillation. By substituting Eq. (5) to (6) and taking natural logarithm,

$$d=e^{-\lambda\left(\frac{2\pi}{\omega}\right)} \tag{7}$$

$$-ln\, d=\frac{2\pi\lambda}{\omega} \tag{8}$$

are obtained. $ln\, d$ is called damping constant and represented by δ; i.e.,

$$\delta=-ln\, d \tag{9}$$

The δ-value is zero for sustained oscillation, negative for divergent oscillation and infinity for non-oscillation. If there is a strong damping and period of transient response is short, δ takes positive and large value. The value of λ is obtained by a regression analysis of the exponential curve, and $2\pi/\omega$ is measured as the time interval between neighboring two peaks.

3) Changes in stationary value of end product

This is concerning with the change in stationary value of the end product X_4 with the increase in angular velocity ω of the input Y_1 under fixing A- and B-values in Eq. (2). The stationary value of the end product X_4 is expressed by α, when the input Y_1 is held at a constant value, $(Y_1=A)$. When Y_1 has a sinusoidal mode with a period of 24.0 sec as represented by Eq. (10), the stationary value of X_4 (averaged on the values at top and bottom of waved curve) is expressed by β.

$$Y_1=A+B\sin\left(\frac{2\pi}{24.0}\right)t \tag{10}$$

Then, the relative deviation Q between a and β can be defined by:

$$Q=\frac{|\beta-\alpha|}{\alpha}\times 100 \tag{11}$$

In this paper, the change in the stationary value of the end product X_4 due to external perturbation was evaluated by the Q-value.

RESULTS AND DISCUSSION

Dynamic behavior of Type D

Figure 2 shows the Bode diagram for Scheme 4 with changing value of k_4 under conditions of $A=0.701$, $B=0.50$ in Eq. (2). The broken line indicates the frequency-response of the reaction system without any feedback-loop (corresponding to the case of $k_4=0$ in Scheme 4). The detailed characteristic features on the Bode diagram were summarized in the previous paper (Okamoto *et al.*, 1977).

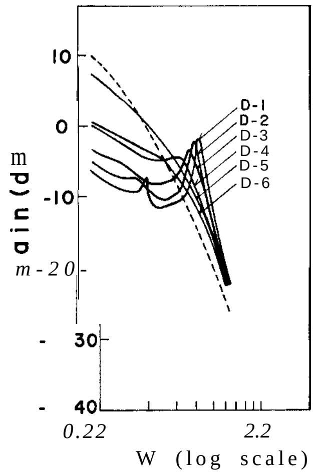


Fig. 2. The Bode diagrams for Scheme 4. Curve D-1: $k_4=7.50$, D-2: $k_4=6.33$, D-3: $k_4=5.00$, D-4: $k_4=3.30$, D-5: $k_4=0.063$, D-6: $k_4=3.00$. The broken line corresponds to $k_4=0$ (no feedback-loop).

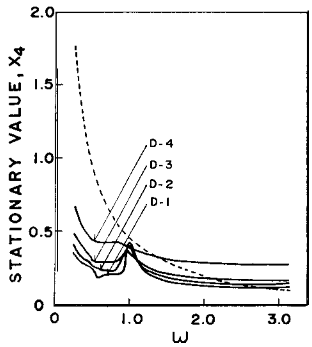


Fig. 3. Effect of ω- and k_4-values on stationary value of X_4 in Scheme 4. Curve D-1: $k_4=7.50$, D-2: $k_4=6.33$, D-3: $k_4=5.00$, D-4: $k_4=3.30$. The broken line corresponds to $k_4=0$.

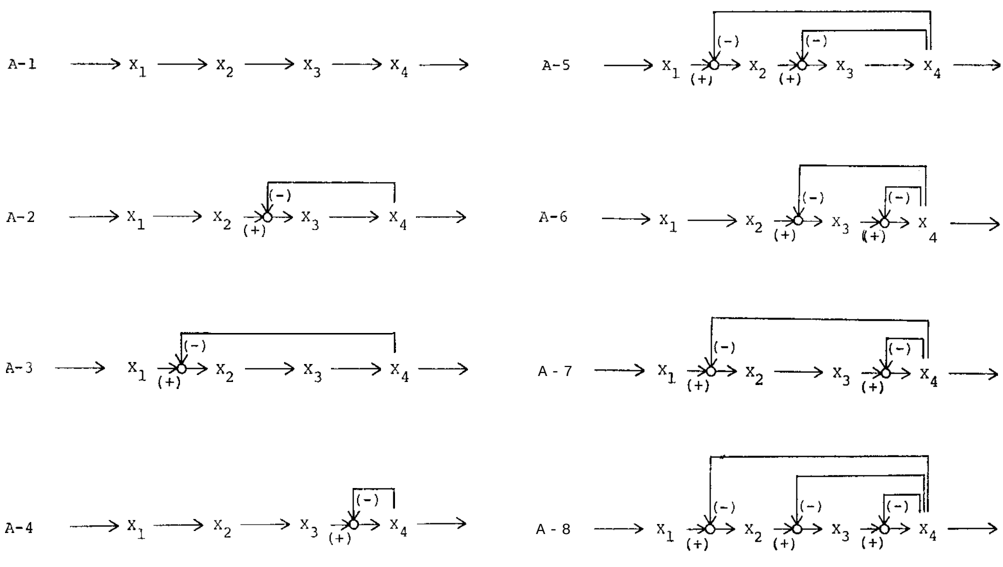
Figure 3 shows the effect of ω– and k,-values on the stationary value of X_4 in Scheme 4. The broken line indicates the value for the system without a feedback-loop. As is evident from Fig. 3, the feedback control system has a potency to hold a constant stationary value of X_4 against the various oscillatory inputs, except for inputs with certain o-values which brought about peaks on the frequency-response curve (see Fig. 2).

Table 1 indicates the relation between the damping constant δ and the relative deviation Q of the stationary value of X_4 for Scheme 4 under various oscillatory inputs. With Scheme 4, it was observed that a sustained oscillation can be realized with a certain k_4–value (Okamoto ***et al.,*** 1976). As is evident from Table 1, the damping constant δ decreased with increase of the k_4–value, and the sustained oscillation on X_4 was achieved by a large k_4–value. However, with the increase in k_4–value, the stationary value of X_4 became smaller than that in system without feedback-loop (see Fig. 3), whereas

Table 1. δ– and Q-values of Scheme 4.

k_4	Damping constant (δ)	Relative deviation (Q)
0. 0		3862.90
0. 063	5. 790	201.25
3: 00	1.365	202.34
3.50	1.256	203.81
4.50	0.824	206.97
5.00	0.637	213.46
6.33	0.330	216.83
7.50	0.116	263.81

Table 2. Assumed schemes related to Type A.



(Scheme 1)

the relative deviation Q of the stationary value of X_4 became larger (see Table 1). Thus, it can be concluded that with the constant input, a simple feedback system exemplified by Scheme 4 has relatively small-capability to retain the constant stationary value of end product against external perturbation.

Type A

Assumed schemes related to Type A are listed in Table 2. The Bode diagram of various schemes related to Type A is shown in Fig. 4. Each frequency-response curve has no peak except for curve A-3 (same as Scheme 4). Figure 5 shows the effect of the o-value in oscillating input Y_1 on the change

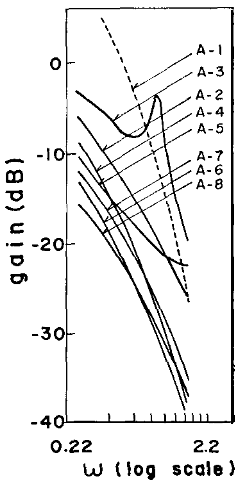


Fig. 4. Bode diagram for schemes related to Type A. Notations on curves are same as that in Table 2.

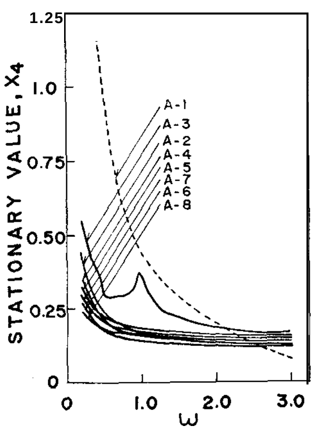


Fig. 5. Effect of o-value on stationary value of X_4 in schemes related to Type A. Notations are same as in Fig. 4.

Table 3. Q-values of schemes listed in Table 2.

Scheme	Relative deviation (Q)
A-1	3862.90
A-2	265.88
A-3	213.46
A-4	183.26
A-5	108.64
A-6	102.37
A-7	92.41
A-8	66.08

in the stationary value of X_4. The peak on the curve A-3 shows that this scheme (Scheme 4) exhibits the oscillation on X_4 under the constant input. From judgement of this fact, it is easily presumed that other schemes listed in Table 2 can not produce oscillation on any reactant under the constant input. Table 3 shows the relative deviations Q of the stationary value of X_4 in schemes related Type A against external perturbation. There is an obvious relation between the number of feedback-loops and the capability of homeostatic control of feedback-systems. Among the schemes including two feedback-loops, one having concurrently both the largest and the smallest loops (Scheme A-7) provided the most effective control in respect to the Q-value.

Type B

Analized model schemes related to Type B are listed in Table 4. The frequency-response curves of these schemes are shown in Fig. 6. Curves B-2 and B-3 exhibits a peak, respectively, while curves B-4 and B-5 are of monotonous profile. The effect of the o-value on the stationary value of X_4 is shown in Fig. 7. The curves have a similar disposition to those shown in Fig. 6.

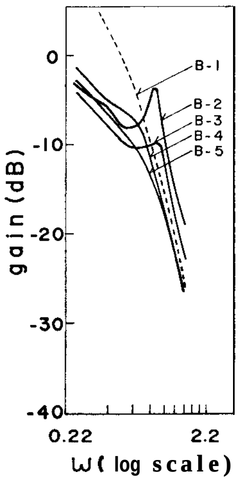
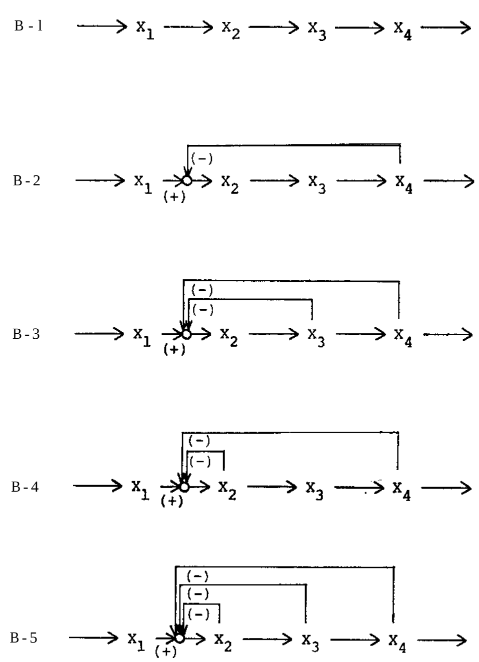


Fig. 6. Bode diagram for schemes related to Type B. Schemes are shown in Table 4.

Table 4. Assumed model schemes related to Type B.



(Scheme 2)

Curves B-2, –3 also show a respective peak around $\omega=1.0$.

Table 5 indicates the relative deviation of the schemes listed in Table 4. The non-oscillating feedback-systems (B-4 and B-5) were more stable to exter-

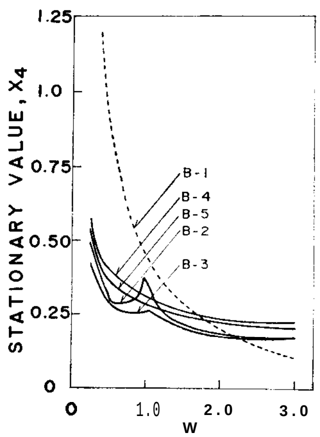


Fig. 7. Effect of o-value on stationary value of X_4 in schemes related to Type B. Schemes are shown in Table 4.

Table 5. δ-and Q-values of schemes of Type B.

Scheme	Relative deviation (Q)	Damping constant (δ)
B-1	3862.90	
B-2	213.46	0.637
B-3	197.65	1.059
B-4	192.34	2.143
B-5	186.82	2.818

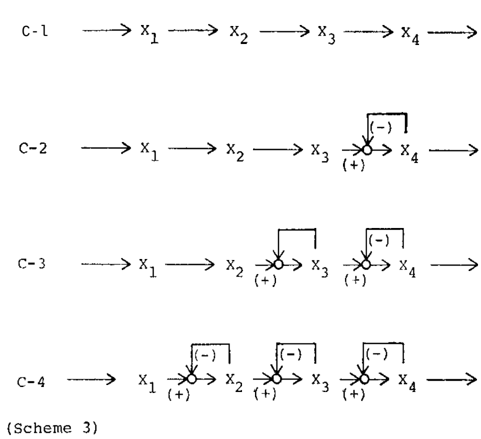
nal perturbation than the oscillating feedback-systems (B-2 and B-3). The schemes including multiple feedback-loops are of advantage for the homeostatic control. These features are also observable for those of Types A and D.

Type C

The same simulations as above were performed for schemes related to Type C (Table 6) and the results are summarized in Figs. 8 and 9, and Table 7. As shown in Table 7, the feedback-system having several short-sized loops is most suitable for the homeostatic control; for instance, in the case of Scheme C-4 (same as Scheme 3), the stationary value of X_4 was kept almost unchanged against a great change in o-value of the input (see Fig. 9).

Figure 10 shows the typical oscillatory pattern of X_4 in Scheme 3 under conditions of $Y_1 = 0.701 + 0.5 \sin \frac{2\pi}{12.0}$. Curve 2 is the time-course of the input Y_1, and the broken line (curve 1) shows the time-course of X_4 in a system without feedback-loop (Scheme C-l). Curve 3 is the time-course of X_4 in Scheme 4 and curve 4 is that in Scheme 3. Scheme 3 includes three short-sized feedback-loops, and does not oscillate when a constant input is applied. As is evident from Fig. 10, the amplitude of X_4 in Scheme 3 is much smaller than that of X_4 in Scheme 4. Therefore, it can be concluded that Scheme 3 is the best system in a sense that Scheme 3 has a capability of maintaining the concentration of X_4 at a constant level independent of change in w-value

Table 6. Assumed schemes related to Type C.



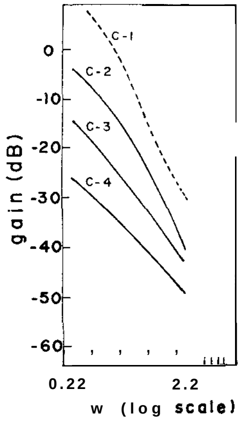


Fig. 8. Bode diagram for schemes of Type C. Schemes are listed in Table 6.

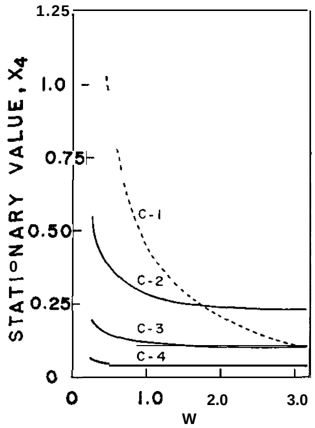


Fig. 9. Effect of o-value on stationary value of X_4 in the schemes of Type C shown in Table 6.

Table 7. Q-values of schemes of Type C.

Scheme	Relative deviation (Q)
C-1	3862.90
C-2	181.96
c-3	102.32
c-4	65.59

of the oscillatory input and has a good potency to rectify the oscillatory input.

The results of the simulation on the dynamic characteristics of various enzymatic feedback-systems are summarized as follows :

i) There are intimate relationships between the oscillatory behavior and the feedback-loop size. ii) Feedback-system which oscillates with constant input

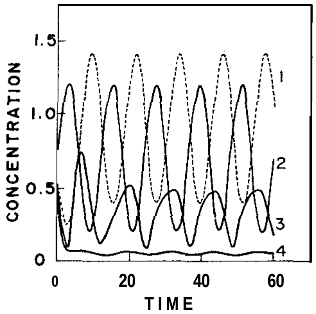


Fig. IO. Effect of number of feedback-loops on amplitude of X_4. Input Y_1 is assumed to be $0.701+0.5\,sin(2\pi/12.0)$. Curve 1 (broken line) shows the time-course of X_4 in case of no feedback-loop, Curve **2:** time-course of Y_1, Curve 3: time-course of X_4 in Scheme 4, Curve 4: time-course of X_4 in Scheme 3.

can not keep the stationary value of end product at a desired level against external perturbation. iii) In general, the more the number of feedback-loops increases, the feedback-system becomes the more effective for the homeostatic control. iv) The feedback-system including multiple short-loops is more of advantage for the homeostatic control than that including only a single long-loop. As far as tested, Scheme 3 (C-4) seems to be the best feedback-system suggesting that the feedback-system having many conpensating circuits (i.e. the feedback-loops do not overlap each other) may be most sophisticated system for the homeostatic control. v) An oscillation under constant input is produced by a feedback scheme including a long-loop. Contrarily smoothing of oscillatory input is realized by a feedback scheme having many short-loops.

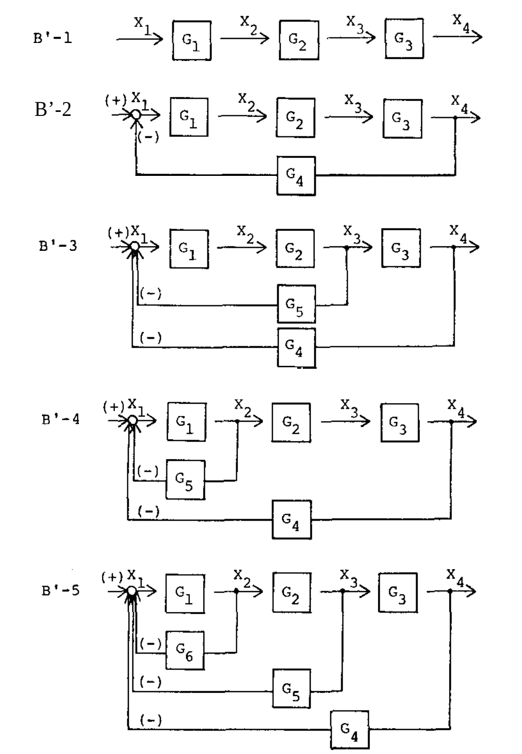
Nonlinearity

The qualitative difference in the frequency-response between nonlinear and linear feedback-systems was further examined, since an enzymatic feedback-system is essentially of nonlinear. The linear feedback-systems corresponding to the schemes of Type B (Table 4) were set up and shown in Table 8. The results are shown in Fig. 11 (counterpart: Fig. 6). On plotting of gain *vs* o-value, linear systems exhibit in general rather gentle shape than nonlinear system. It should be, however, noted that nonlinear systems with much complicated loops exhibited an unstable behavior in contrast to the case of linear systems.

Linear systems corresponding to the schemes of Type C (Table 6) are shown in Table 9. The Bode diagram of the linear systems are shown in Fig. 12. Contrary to above, the behavior of linear system is quite similar to that of counterpart nonlinear system.

The role of the enzymatic feedback-system in metabolic regulation is thought to maintain the concentrations of the product and intermediates at the desired level. Recently, the oscillation of the concentration of metabolite has been frequently observed by many investigators, and consequently it has gradually been believed that the oscillation of the concentration of metabolite

Table 8. Linear feedback-system corresponding to Type B. Counterpart schemes *are* listed in Table 4.



$G_1 = k_1/s$, $G_2 = k_2/s$, $G_3 = k_3/s$, $G_4 = k_4$, $G_5 = k_5$, $G_6 = k_6$

k_1: Rate constant

s: Complex variable

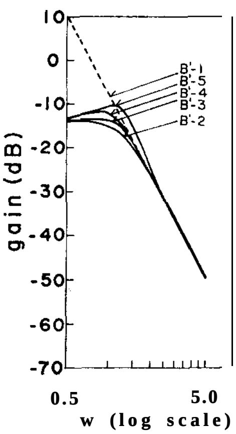
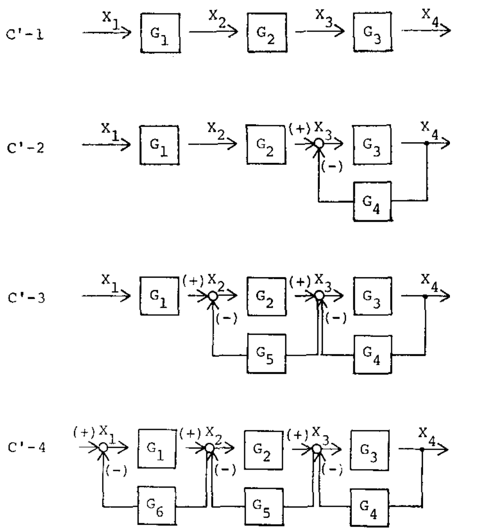


Fig. 11. Bode diagram for schemes listed in Table 8. Schemes are counterparts of schemes in Table 4.

Table 9. Linear feedback-system corresponding to Type C, Counterpart schemes are in Table 6.



$G_1 = k_1/s$, $G_2 = k_2/s$, $G_3 = k_3/s$, $G_4 = k_4$, $G_5 = k_5$, $G_6 = k_6$

k_1: Rate constant

s : Complex variable

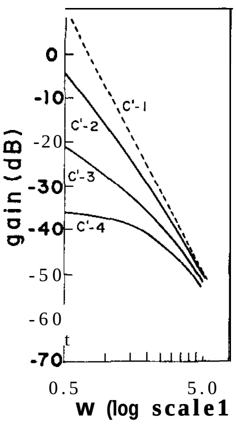


Fig. 12. Bode diagram for schemes listed in Table 9. Schemes are counterparts of schemes in Table 6.

is common and basic features in dynamic behavior of biochemical systems in vivo. Such the facts forced to investigate the oscillatory behavior of the feedback-system, because the input of the feedback-system is generally of oscillatory nature. Thus, in the present study, the response of feedback-system to the oscillatory input was examined. As a result, as described above, it was found that the gain and the deviation of stationary concentration of the systems for sinusoidal inputs were strongly dependent on the structure of the feedback-system.

The required response of feedback-system to oscillatory input would be different according to the role and situation of enzymatic system in metabolic pathway; someone is required to amplify the oscillation amplitude and other to rectify the oscillation. Now, there is no information on the behavior of real enzymatic feedback-system to oscillatory input, However, it is easily presumed that the structure of feedback-system decides strictly the oscillatory or dynamic behavior of the system.

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