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Okamoto, Masahiro

Laboratory of Sericultural Chemistry, Faculty of Agriculture, Kyushu University

Aso, Yoichi

Laboratory of Sericultural Chemistry, Faculty of Agriculture, Kyushu University

Hayashi, Katsuya

Laboratory of Sericultural Chemistry, Faculty of Agriculture, Kyushu University

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Oscillatory Behavior of Enzymatic Feedback System

Masahiro Okamoto, Yoichi Aso and Katsuya Hayashi

Laboratory of Sericultural Chemistry, Faculty of Agriculture,
Kyushu University, Fukuoka

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It has been well recognized that many enzymatic chain reactions and metabolisms in living organisms exhibit an oscillatory character with respect to the concentration of an intermediate. Some kinds of oscillation observable on the metabolic intermediate were presumed to be due to the feedback control system in the enzymatic chain reaction, which can produce the time-retardation function.

Analog computer simulation in the present study revealed an appropriateness of the above assumption. Since the feedback system has been recognized to be the universal mode for the regulation of enzymatic chain reactions *in vivo*, it was concluded that the oscillatory behaviors in enzymatic chain reactions may not be particular but general one.

INTRODUCTION

Many investigators have experimentally proved that oscillatory chemical reactions exist in the homeostatic regulation systems (Goodwin, 1965; Higgins *et al.*, 1973; Sompayrac and Maaløe, 1973). The various oscillatory behaviors observable in living organisms such as secretions of hormones, beat of heart, circadian rhythms or interacting population (Pavlidis, 1973) may probably be fundamentally caused by chemical events. Therefore, the quantitative explanation of the mechanism of chemical oscillation *in vivo* may provide some information for understanding the mechanisms by which the highly organized oscillations are generated.

An oscillatory phenomenon in physical or technological system, as seen in the behavior of a pendulum, is essentially due to the inertia term. A chemical system is characterized to have no inertia term. Chemical reactions can occur or stop at the moment, not producing time-retardation by the inertia term. Therefore, in a system having no inertia term, the periodic response would be caused by another factors which are able to act on a system in the same way as the physical inertia term.

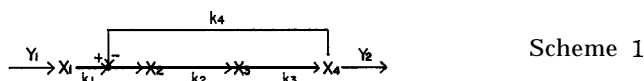
On the other hand, it is well known that a typical oscillation with sine or cosine curve is easily generated by a looped (feedback) electronic circuit. A looped pathway is considered to be a retardation-realizing device, corresponding to the effect of the physical inertia term. From this fact, it is expected that the chemical oscillation would be derived from a chemical system involving a looped step or feedback system. Actually, Morales and McKay (1967) have first clarified the relationship between the feedback control system and the biochemical oscillation.

Many reactions in living organisms are characterized to exhibit a nonlinear behavior. Feedback control systems *in vivo* could not simply be analyzed by the transfer function. The most suitable method for analyzing such the systems is thought to be one involving the numerical computation or the computer simulation of the mathematical model.

The present report deals mainly with the oscillatory characteristics of biochemical feedback system; the parameter-set in the feedback system which can realize the oscillation, changes in the stationary concentrations of the intermediates caused by an external perturbation and the conditions under which the concentration of the final intermediate in the feedback loop was maintained in constant value without suffering the effect of the perturbation, were specially investigated by the computer simulation.

COMPUTATION

The following reaction scheme was assumed to be a typical biochemical feedback system, where X_i represents the reactant or intermediate, Y_1 and Y_2 are input and output of the system and both are assumed to be constant values during the reaction. k_1, k_2 and k_3 represent the reaction rate constants of



corresponding step. k_4 is considered to be a feedback constant controlling the weight of contribution of the concentration of X_4 to feedback summing point. At the summing point between X_1 and X_2 , following operation is assumed:

$$Z = \frac{k_1}{k_4 X_4} \quad (1)$$

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

$$\left\{ \begin{array}{l} \frac{dX_1}{dt} = Y_1 - (k_1/k_4 X_4) X_1 \\ \frac{dX_2}{dt} = (k_1/k_4 X_4) X_1 - k_2 X_2 \\ \frac{dX_3}{dt} = k_2 X_2 - k_3 X_3 \\ \frac{dX_4}{dt} = k_3 X_3 - Y_2 \end{array} \right. \quad (2)$$

The set-up diagram of the equation (2) for analog computer is shown in Fig. 1. Bold lines indicate the feedback path. The simulations were conducted with changing the loop size, values of the rate constants and the initial concentration of X_4 . All computations were performed by a Hitachi analog computer Model ALS-220 in the Biotron Institute of Kyushu University.

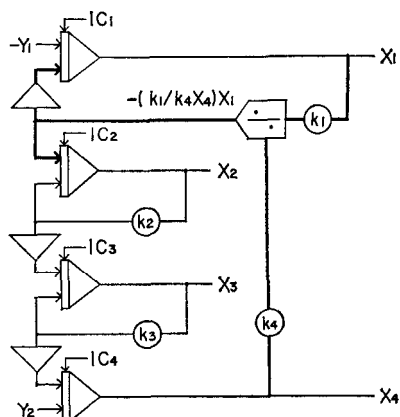


Fig. 1. Set-up diagram of equation (2) for analog computer.

RESULTS AND DISCUSSION

Pattern of oscillation

The typical patterns of concentration of X_i vs time and phase diagram, which are sampled from various patterns obtained by the present simulation, are shown in Figs. 2-6 to classify roughly the oscillatory patterns.

Figure 2 shows a non-oscillatory pattern: (A) is the computed time-course of the reaction product in the feedback system, X_4 , and (B) the phase diagram (trajectory) between two intermediates, for instance X_3 and X_4 . The ordinate of (B) indicates the concentration of an intermediate and the abscissa the concentration of another intermediate. With the analysis of phase diagram, the transition behavior of the system can be predicted according to the shape of the trajectory. In Fig. Z(B), the trajectory converged to a certain point, the lower end of the curve.

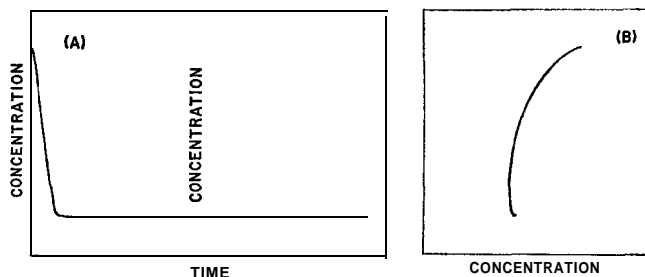


Fig. 2. Non-oscillatory pattern. (A) Time-course, (B) phase diagram.

Figure 3 shows a typical pattern of damped oscillation. The trajectory converges to a center point with drawing a swirl. The sustained oscillation is shown in Fig. 4. In this case, the average value of X_4 at the stationary state is lower than the value of initial X_4 . The trajectory of this type draws a limit

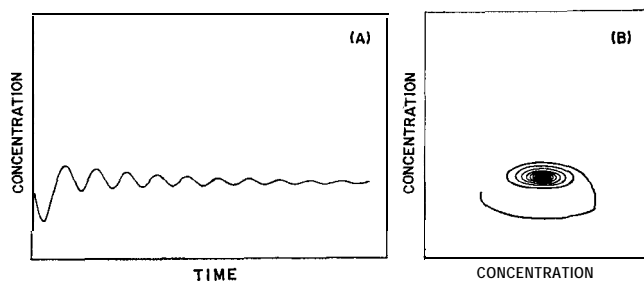


Fig. 3. Damped oscillation. (A) Time-course, (B) phase diagram. In (B), the trajectory converges to a center point.

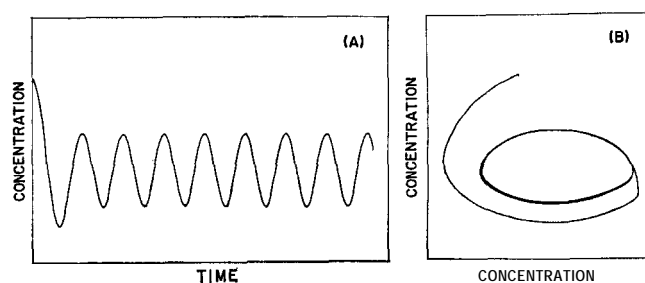


Fig. 4. Sustained oscillation. In phase diagram, starting point is at the upper center. This type of oscillation is called sustained inside.

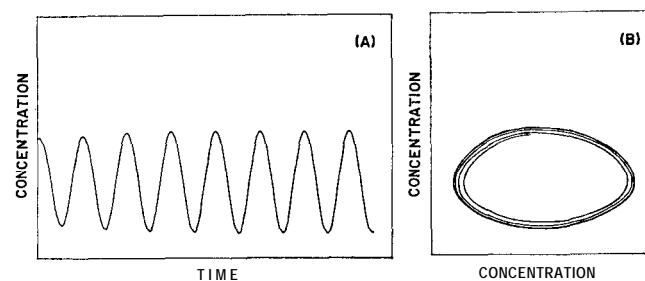


Fig. 5. Sustained oscillation. In phase diagram, starting point is in limited cycle. This type of oscillation is called sustained outside.

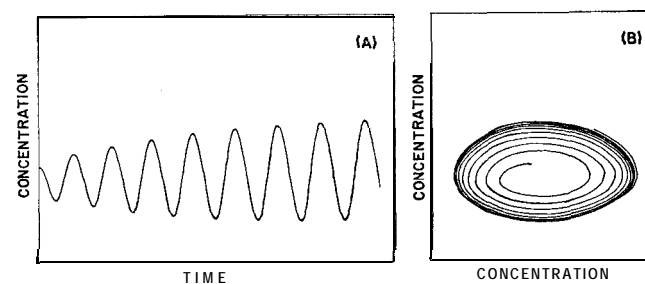
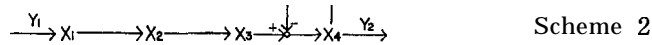


Fig. 6. Divergent oscillation. In phase diagram, starting point is inside of swirl.

cycle. Figure 5 also shows a pattern of sustained oscillation. However, this holds a higher average value of X_4 at the stationary state than the initial value of X_4 . The limit cycle in this case is at the outside of the starting point. Figure 6 shows the typical pattern of divergent oscillation. The trajectory diverges with drawing a swirl outward from the starting point. The oscillating patterns in Figs. 3-6 are respectively represented as Damped, Sustained inside, Sustained outside and Divergent.

Position of summing point and oscillation

It is assumed in general that an end-product of enzymatic chain reaction inhibits some enzyme in the same chain by negative feedback, keeping itself the stationary concentration at the desired constant level. In this simulation, therefore, the feedback system is assumed to be framed in the way that the end-product X_4 is fed back to a preceding reaction step. In the case of that



the summing point of feedback was between X_3 and X_4 (Scheme 2), the time-course of X_4 was entirely similar to that shown in Fig. 2(A), holding a non-oscillatory constant value at the stationary state. The typical pattern in the case of that X_4 inhibits the enzyme which catalyzes the reaction between X_2 and X_3 is shown in Fig. 7. The X_4 showed the damped oscillation.

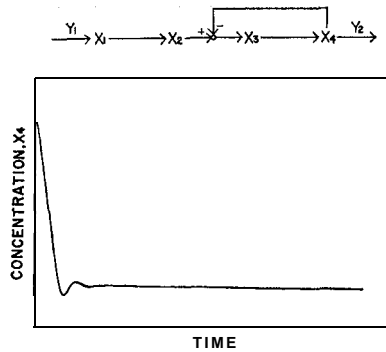


Fig. 7. Damped oscillation. The scheme drawn on the figure was analyzed.

In order to see the effect of another operation (equation (3)] at the summing point, a scheme shown on Fig. 8 was also assumed.

$$Z = k_1 - k_3 X_3 \tag{3}$$

This system realizes only negative feedback because the non-negativity of Z value, while in the equation (1) the region of Z value is $Z \leq k_1$, indicating the possibility of realizing a negative or positive feedback. The computed results are shown in Fig. 8. The end-product of the system, X_3 , shows also a kind of damped oscillation in the transition state. Thus, the difference in the operation mode at the summing point did not change the basic oscillatory behavior of the system, if only the negative feedback was considered.

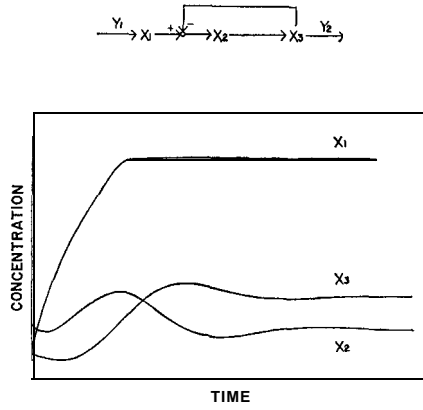


Fig. 8. Damped oscillation. The scheme drawn on the figure was analyzed with equation (3) assuming the operation at the summing point is represented by

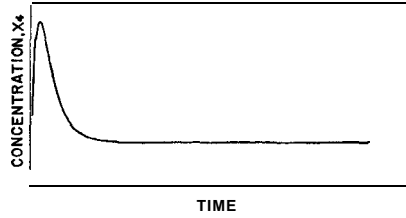


Fig. 9. Typical non-oscillatory pattern. Scheme 1 was analyzed.

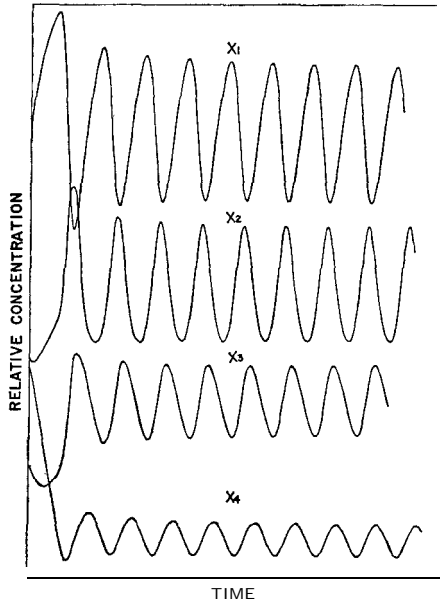


Fig. 10. Typical oscillatory pattern of Scheme 1.

The time-courses of X_i in the system in which the summing point is between X_1 and X_2 showed various profiles according to the parameter-set used for the computation. Under a certain parameter-set, X_i kept a constant value after overshoot the initial value, as shown in Fig. 9. With another parameter-set, the sustained oscillations of all X_i were observed as shown in Fig. 10. Thus, it was found that for the appearance of an obvious oscillation there was a limitation of the loop size, when the positive feedback was not taken into the consideration. In the following computer simulations, only Scheme 1 was analyzed.

k_1/k_4 Ratio and oscillation

The simulations of Scheme 1 were performed with various combinations of k_1 and k_4 under fixing the values of other parameters to clarify the effects of k_1 and k_4 on the oscillatory behavior of the system. The sustained oscillations were observed with the combinations listed in Table 1. The detailed oscillatory profiles are shown in Fig. 11. From these results, it is obvious that for the appearance of the sustained oscillation, k_1/k_4 ratio should be limited to a narrow range, while the individual k_1 and k_4 are allowed to have a considerably extensive

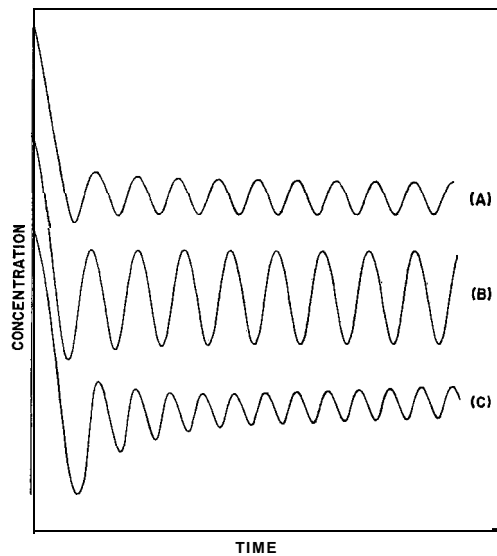


Fig. 11. Sustained oscillation. Parameters listed in Table 1 were used.

Table 1. Relation between k_1/k_4 value and sustained oscillation.

k_1	k_4	k_1/k_4 Value	Initial X_4	Fig. 11
0.300	8.20	0.08	0.543	(A)
0.453	6.33	0.07	0.543	(B)
0.050	0.72	0.07	0.543	
0.025	0.36	0.07	0.543	(C)

value. In order to determine the allowable range for k_1/k_4 ratio, the computations were performed with changing the k_4 value under the fixed value of k_1 . The results are listed in Table 2. It is clear that the only very narrow range of the ratio, **0.07-0.09**, is essential for the generation of the sustained oscillation.

Table 2. Effect of k_1/k_4 value on oscillation behavior.

k_1	k_4	k_1/k_4 Value	Initial X_4	
0.453	1.16	0.39	0.543	Damped
0.453	4.21	0.11	0.543	Damped
0.453	5.45	0.09	0.543	Sustained
0.453	7.00	0.07	0.543	Sustained
0.453	8.00	0.06	0.543	Divergent

Effect of initial concentration of X_4 on oscillation

The combinations of k_1 and k_4 are divided into two classes under fixing the k_1/k_4 ratio at 0.07.

- (A) k_4 value is below 1.0 (example: $k_1/k_4=0.025/0.36$).
- (B) k_4 value is above 1.0 (example ; $k_1/k_4=0.435/6.33$).

In each case, the oscillatory behavior of the system was computed with changing the initial concentration of X_4 . This situation is corresponding to that the external perturbation was introduced to the system so as to increase or decrease the value (concentration) of X_4 . In the case of (A), the oscillatory behavior with changing the initial concentration of X_4 from 0.0 to 1.0 is shown in Table 3. The initial value of X_4 above 0.5 has broken the sustained oscillation of X_4 . The average value of X_4 at the stationary state was changing downward with increase in the initial value of X_4 . In the case of (B), the results are listed in Table 4. The average value of X_4 at the stationary state was changing upward in proportion to the increase in the initial value of X_4 .

For both cases, (A) and (B), the ratio of k_1/k_4 was the same, although the real value of k_1 and k_4 were different from each other. The unified rate constant at the summing point, $Z = k_1/(k_4X_4)$ was the same for both the cases, when it was compared under the same initial value of X_4 . Furthermore, as can be

Table 3. Influence of the initial concentration of X_4 on the oscillation behavior (A) ($k_1 = 0.025, k_4 = 0.36$).

Initial X_4	Average X_4 at stationary state	Pattern of X_4	Pattern corresponds to
0.0	0.075	Sustained	Fig. 4
↓	↓	↓	
0.5	0.050	Sustained	Fig. 4
↓	↓	↓	
0.6	0.020 ↓	Damped ↓	Fig. 2
↓	↓	↓	
0.7	0.010	Damped	Fig. 2
↓	↓	↓	

Table 4. Influence of the initial concentration of X_4 on oscillation behavior (B) ($k_1 = 0.453$, $k_4 = 6.33$).

Initial X_4	Average X_4 at stationary state	Pattern of X_4	Pattern corresponds to
0.0	0.040	Damped	Fig. 2
↓	↓	↓	
0.4	0.075	Sustained	Fig. 4
↓	↓	↓	
0.5	0.090 ↓	Sustained	Fig. 4
↓	↓	↓	
0.8	0.125	Damped	Fig. 2
↓	↓	↓	
0.9	0.135	Damped	Fig. 2
↓	↓	↓	

seen in the last equation in the simultaneous differential equation (2), X_4 was consumed by Y_2 but not by the feedback loop. These considerations suggest us that the response of the system against the initial value (external perturbation) would be the same for both cases, in spite of the differences in the k_1 and k_4 values. The real computations indicated quite different patterns on the stationary values of X_4 as shown already, contrary to the expectation. In the case of (A), X_4 value at the stationary state decreased with the increase in the initial value, whereas in the case of (B), just the reverse was observed. The reason, why such the different responses were derived under the same value of Z , can not be clarified at the present time.

Effect of k_2 value on oscillation

The effect of k_2 value in the step of $X_2 \rightarrow X_3$ on the oscillatory behavior of the system was estimated by the simulation under the conditions that $k_1/k_4 (=0.07)$ and the initial value of $X_4 (=0.543)$ were fixed. As listed in Table 5, the increase in the k_2 value accompanied the change in the X_4 pattern in order of divergent, damped, sustained, damped. The sustained oscillation on X_4 was observable in a narrow range of k_2 value, 0.916-2.0. In order to estimate the effect of the combination of k_2 value with the initial value of X_4 , the computations were performed with changing the initial value of X_4 under selected k_2

Table 5. Effect of k_2 value on oscillation behavior.

k_2	Pattern of X_4
↓	↓
0.90	Divergent
↓	↓
1.50	Damped
↓	↓
2.00	Sustained
↓	↓
3.00	Sustained
↓	↓
3.47	Damped
↓	↓
	Damped

values, **0.916**, **2.00** and 3.00. The results are listed in Table 6. With $k_2 = 0.916$, the pattern of X_4 changed continuously from divergent to damped oscillation and the stationary value of X_4 changed downward with increase in the initial value of X_4 .

Table 6. Relation between k_2 value and the initial concentration of X_4 .

k_2	Initial X_4				Change in average X_4 at stationary state	
	0.0	→ 0.5	→ 1.0			
0.916	Div.	→ Sus.	→ Sus.	→ Dam.	→ Dam.	Downward
2.00	Sus.	→ sus.	→ sus.	→ sus.	→ sus.	Scarcely
3.00	Dam.	→ Dam.	+ Dam.	→ Dam.	→ Dam.	Scarcely

Div., Divergent ; Dam., Damped oscillation ; Sus., Sustained oscillation.

It is noteworthy that the oscillation profiles were quite stable for $k_2 = 2.00$ and 3.00. The profiles were not altered by the increase in the initial value of X_4 . Furthermore, the stationary values of X_4 in the case of $k_2 = 2.00$ and 3.00 were shifted very scarcely with increase in the initial value of X_4 . Thus, the feedback control system is able to maintain the concentration of a certain intermediate at constant value against the external perturbation.

Effect of k_3 value on oscillation

The effect of k_3 value which is the rate constant for the step of $X_3 \rightarrow X_4$ on the oscillatory behavior of the system was estimated under fixing the value of $k_1/k_4 = 0.07$ and the initial value of $X_4 = 0.543$. The computed results are summarized in Table 7. Similar to the effect of k_2 , the increase in the k_3 value accompanied the alteration of pattern of X_4 . The effect of the initial concentration of X_4 on the pattern was examined with fixing the k_3 value at 0.700, 0.746, 2.83 and 3.50, respectively. In the cases of $k_3 = 2.83$ and 3.50, the patterns did not change with increase in the initial value of X_4 and the average X_4 value at the stationary state was also remained unchanged as shown in Table 8.

There seems to be no clear evidences conflicting to the argument that the feedback system is a prime mode to control the metabolism or the enzymatic chain reactions *in vivo*. However, it does not necessarily mean that any type

Table 7. Effect of k_3 value on oscillation behavior

k_3	Pattern of X_4
↓	↓ Divergent
0.700	↓ Damped
0.746	↓ Sustained
2.83	↓ Sustained
3.50	↓ Damped
↓	↓

Table 8. Relation between k_3 value and the initial concentration of X_4 .

k_3	Initial X_4			Change in average X_4 at stationary state
	0.0	0.5	1.0	
0.700	Sus. \longrightarrow Sus.	\longrightarrow Dam.	\longrightarrow Dam.	Downward
0.746	slls. \longrightarrow Sus.	\longrightarrow Dam.	\longrightarrow Dam.	Downward
2.83	Sus. \longrightarrow sus.	\longrightarrow Sus.		No
3.50	Dam. \longrightarrow Dam.	\longrightarrow Dam.		No

of feedback system is able to realize the capability of autocontrolling the enzymatic chain reactions. Some limited types of feedback system may act as a controlling device in organisms. The dispensable conditions imposed on the feedback system as controlling device have not yet been established. In other words, the behaviors of biochemical feedback system have not been surveyed enough to establish their qualification for the autocontrolling device.

In the present study, a model of biochemical feedback system was assumed and it was analyzed by analog computer simulation to follow especially the oscillatory behavior and capability to treat the external perturbation. The obtained results may be summarized as follows: (1) For realizing the sustained oscillation, the strictly limited parameter-set was needed. The sustained oscillations of the metabolic intermediate *in vivo* have been widely observed. The results of the present computation, therefore, suggest that the *in vivo* feedback system itself would be regulated further by systems of higher order. It is interesting that oscillating properties of feedback system were considered to be, in the electronic or mechanical dynamics, undesired one, while the biochemical systems generate intentionally the oscillation of metabolic intermediates. (2) It is obvious that the feedback system assumed here exhibits the several ways by which the external perturbation are treated. As described already, under the suitable parameter-set, the average value of X_4 at the stationary state was held at a constant value regardless of the magnitude of the perturbation which was introduced in the form of X_4 to the system, and the perturbation was stored on the intermediate other than X_4 at the stationary state. Thus, in the feedback system, the perturbation can be treated at positions other than the original position where the perturbation was introduced. This will be specially meaningful when one argues the effect of the external perturbation on the metabolic pathway *in vivo*. (3) Another peculiar results are shown in Tables 3 and 4. In the case of (A), the increase in the magnitude of the external perturbation caused the decrease in the average value of X_4 at the stationary state, whereas in the case of (B), the reverse was observed. Thus, the effects of perturbation on the average stationary value of intermediate are changing delicately according to the variation of parameter-set. This may infer that one's simple and qualitative inference on the effect of the perturbation on the biochemical feedback system should be avoided.

In the present model for a chemical feedback system, two important assumptions were made: First, at the summing point, the operation represented by

equation (2) was assumed, and second, the X_4 was assumed to be lost only by the constant Y_2 . On the basis of molecular mechanism, the above operation at the summing point would be realized by some type of allosteric enzymes, and the second assumption would be accomplished by a followed feedback system which can control the flow or consumption of X_4 at Y_2 value.

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