OSCILLATORY BEHAVIOR IN ENZYMATIC CONTROL PROCESSES

BRIAN C. GOODWIN

Institute of Animal Genetics, University of Edinburgh, Edinburgh

INTRODUCTION

THE demonstration of negative feedback control processes operating at the molecular level in cells is one of the most significant developments in modern biology. The phenomena of feedback inhibition[1, 2] and feedback repression[3] whereby enzymatic activities are controlled at the level of the enzyme and the gene, respectively, provide a firm experimental basis for the construction of dynamic models which represent the fundamental regulatory activity of cells. The behavior of these and other molecular control circuits thus constitutes the basis of cell physiology, and in effect provides the physiologist with his elementary units of function.

It is of fundamental importance to an understanding of cellular organization whether or not the dynamic activity of molecular control processes involves oscillatory behavior. The traditional view of the cell as a biochemical system is that molecular populations move towards steady-state levels determined by the environment, and that when a steady state is reached the system maintains itself by a constant flow of intermediates. This view regards the cell as a passive system which changes state only in response to environmental stimuli.

However, there is considerable experimental evidence which suggests that cellular processes are intrinsically rhythmic or periodic, evidence which comes largely from studies showing the widespread occurrence and fundamental significance of biological "clocks" and timing devices.[4, 5] More direct observations of periodicities in the dynamics of metabolic processes in cells have been reported by Duysens and Amesz[6] and by Chance, Estabrook and Ghosh.[7] On the theoretical side, the occurrence of negative feedback and time-lags in the operation of molecular control circuits makes it highly probable that many molecular species in cells will undergo continuing undamped oscillations. The purpose of this paper is to illustrate the type of periodic behavior which can arise in model systems incorporating the essential control features of enzymatic regulatory processes, and to discuss the significance of oscillatory motion in relation to the organization of cellular processes in time. In order to handle these ideas mathematically,

certain concepts and quantities of an essentially thermodynamic nature will be introduced, so that it will be possible to pursue an analysis of temporal organization in cells which links the microscopic or molecular features of cellular control processes with the macroscopic or physiological properties of cells. In this I will follow the procedure given in my book dealing with this question.[8]

NON-INTERACTING OSCILLATIONS

The first control system which will be studied is that illustrated schematically in Fig. 1. Here L_i is a genetic locus which produces messenger ribonucleic acid (mRNA) in quantities denoted by X_i. This mRNA then combines with ribosomes to form active protein-synthesizing aggregates (polysomes) designated

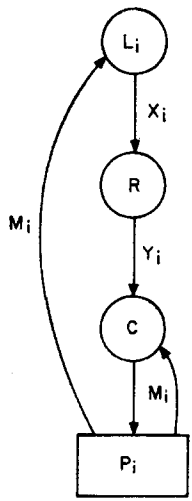

FIG. 1.

by R, producing protein in quantity Y_i. This protein assumed to be an enzyme then directs a metabolic transformation giving rise to a metabolic species M_i which passes through a cellular pool, P_i. A fraction of the metabolite in the pool feeds back to the genetic locus where it serves to repress the activity of the gene, presumably in association with a macromolecule, the aporepressor.[9]

This cycle constitutes a closed-loop negative feedback control circuit whose operation has been called feedback repression.[3] A set of equations describing the dynamics of this system under certain assumptions regarding the

essential control variables involved has been derived by Goodwin.[8] They are:

$$\left.\begin{aligned}\frac{dX_i}{dt} &= \frac{a_i}{A_i + k_i Y_i} - b_i \\ \frac{dY_i}{dt} &= \alpha_i X_i - \beta_i\end{aligned}\right\} \tag{1}$$

where X_i = concentration of mRNA of the ith species,

Y_i = concentration of protein of the ith species,

this protein assumed to be an enzyme. The other quantities are parameters whose significance is discussed in my book. These equations describe what is probably the simplest conceivable control process consistent with certain essential features of genetic control of enzyme synthesis.

The major feature of these equations is the occurrence of Y_i in the denominator of the expression for the rate of mRNA synthesis. This is a consequence of the assumption that the repressor complex acts on the DNA template by a surface adsorption process similar to the action of inhibitors on enzymatic activity. The parameter k_i therefore involves an equilibrium constant for the non-covalent reaction between deoxyribonucleic acid (DNA) and the repressor.

Equations (1) were studied dynamically on a Philbrick Analogue Computer at the Massachusetts Institute of Technology, and the behavior of the variables X_i and Y_i is shown in Fig. 2, for parameter values:

$$a_i = 72,\ A_i = 36,\ k_i = 1,\ b_i = 2,\ \alpha_i = 1,\ \beta_i = 0$$

$$(X_i)_0 = 7,\ (Y_i)_0 = -10 \text{ (initial values of the variables).}$$

The variable with the larger amplitude is Y_i. The scale is in 10 V increments between each heavy horizontal line, zero being given by the line passing through the centre of the oscillation in X_i. Thus X_i varies between +10 and −10 V, for example.

The parameter values are scaled to keep the variables within the limits of machine operation. For actual molecular populations in cells, the amplitude of the oscillation in Y_i (enzyme) would be many times larger than that in X_i (mRNA). In real time, a single complete oscillation would take about 4–8 hr, whereas on the computer it takes about 0.28 sec (the computing interval shown is 1 sec). X_i and Y_i are allowed to take negative values in this model for scaling purposes, although biologically these quantities are always positive or zero, being concentrations. The units of the variables are chosen for convenience to be molecules per cell.

The equations clearly define a non-linear biochemical oscillator. It has been shown[8] that the equations (1) have a first integral of the motion (an invariant integral), so that the dynamic system is conservative in some sense.

A temperature function, θ can thus be defined for the oscillator in the same way that thermodynamic temperature is defined for a molecule in an ideal gas in terms of its Hamiltonian. Thus one can write:

$$p = e^{-(G-\psi)/\theta}$$

as the probability distribution which defines the statistical properties of the oscillator whose invariant integral is G. ψ is in this expression the analogue of the free energy of the oscillator.

The quantity, θ, has been called the talandic temperature of the control circuit (Gk $\tau\alpha\lambda\alpha\nu\tau\omega\sigma\iota\varsigma$ = oscillation), and it is a measure of the amplitude of the oscillation. On the computer this amplitude is determined by the initial values of X_i and Y_i. The steady state of equations (1) is given by the quantities $\bar{X}_i \equiv p_i = \beta_i/a_i$, $\bar{Y}_i \equiv q_i = 1/k_i(a_i/b_i - A_i)$. For the parameter values chosen, these are $p_i = q_i = 0$. Starting the system with X_i and Y_i at these steady-state values, it is observed that there is initially no oscillation at all in the variables. However, when the computer is allowed to "run", noise in the circuitry introduces perturbations and the oscillations build up in amplitude, increasing until the machine reaches its limiting voltages. This behavior reflects the conservative property of this dynamic system, which is only weakly stable: small disturbances produce changes in the trajectories which build up and lead ultimately to instability, the oscillations increasing in amplitude and being bounded only by the limits of machine operation. Strong stability, characteristic of oscillators with limit cycles, will be illustrated below in a slightly modified set of equations.

It is observed in Fig. 2 that the amplitude of oscillation in Y_i is considerably larger than that in X_i. This is consistent with the theoretical calculation of relative amplitudes. Using the procedures of statistical mechanics, approximate expressions for the mean positive amplitudes of the variables X_i and Y_i can be obtained,[8] with the results:

$$\overline{(X_i^+)} \approx \sqrt{\frac{2\theta}{\pi c_i}} \qquad \overline{(Y_i^+)} \approx \frac{\theta Q_i}{b_i k_i}$$

where $c_i = (a_i k_i)/Q_i$, $Q_i = A_i + k_i q_i$. For the parameter values used, these quantities are:

$$\overline{(X_i^+)} \approx \frac{2\times 36\times\theta}{\pi} \approx \frac{24}{5}\sqrt{\theta}, \quad \overline{(Y_i^+)} \approx \frac{36\theta}{2} = 18\theta$$

The talandic temperature, θ, is defined in this system by the relations:

$$\overline{c_i X_i(X_i - p_i)} = \theta = \frac{\overline{b_i k_i^2 Y_i(Y_i - q_i)}}{Q_i(A_i + k_i Y_i)}$$

where the bars signify mean values. A calculation from Fig. 2 gives $\theta \approx 1.33$.

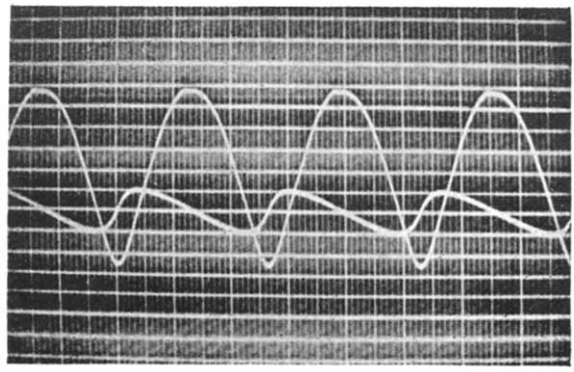

FIG. 2.

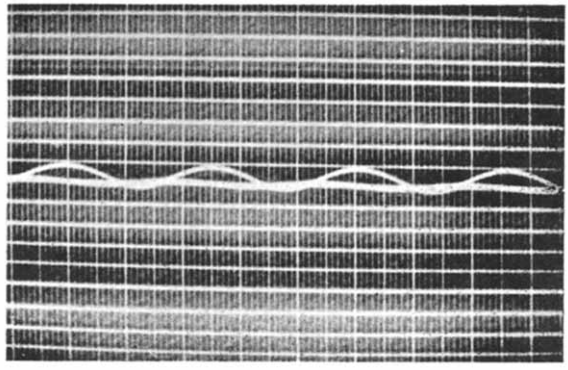

FIG. 3.

[facing p. 428

Thus we get:

$$(\overline{X_t^+}) \approx 5.53, \quad (\overline{Y_t^+}) \approx 24,$$

which values correspond reasonably well with the observed amplitudes.

Another interesting feature of the oscillations is that there is an asymmetry in the variable Y_t about its steady state, excursions above $q_t = 0$ being appreciably greater than those below. This is a consequence of the non-linearity of the oscillation, the non-linearity increasing with θ.

In Fig. 3 is shown the same oscillator as that of Fig. 2, but now the initial conditions are $X_0 = 0.9$, $Y_0 = 0$, values very close to the steady state where, theoretically, there should be no oscillation at all. Clearly the amplitude is greatly decreased, so that θ is much reduced over its value in Fig. 2. Furthermore, the observed oscillation, which is in the variable Y_t, is very nearly sinusoidal (linear).

The reason for using these thermodynamic-like notions of talandic energy, talandic temperature, etc., in the context of an analysis of the dynamic behavior of cellular control processes is the following. Within a single cell hundreds of different proteins, the majority of them enzymes, are being synthesized at any one time. Their synthesis and activity are regulated by negative feedback control processes which interact with one another either directly or via common metabolic pools in such a manner as to achieve coherence and order in the biosynthetic and physiological activities of the cell. In order to study the general dynamic behavior of these hundreds of control circuits operating in a common metabolic space, it is necessary to use some generalized dynamic analysis such as statistical mechanics. From such an analysis there emerge naturally thermodynamic-like quantities, such as the talandic energy of a cell. This quantity is then directly related to the amount of oscillatory activity within a cell, oscillatory activity which I believe to arise from the intrinsic dynamic properties of closed feedback control loops. The fundamental importance of this type of energy in the organization of of cell behavior will be discussed later.

INTERACTION BETWEEN NON-LINEAR OSCILLATORS

It is to be expected from the complexity of intracellular processes that control circuits will interact in some manner. One type of interaction which seems very probable is for the repressor of one genetic locus to have a repressive effect on another locus. This situation can be represented schematically as in Fig. 4, and mathematically by the equations:

$$\left.\begin{aligned} \frac{dX_1}{dt} &= \frac{a_1}{A_1 + k_{11}Y_1 + k_{12}Y_2} - b_1, \quad \frac{dY_1}{dt} = a_1X_1 - \beta_1 \\ \frac{dX_2}{dt} &= \frac{a_2}{A_2 + k_{21}Y_1 + k_{22}Y_2} - b_2, \quad \frac{dY_2}{dt} = a_2X_2 - \beta_2 \end{aligned}\right\} \tag{2}$$

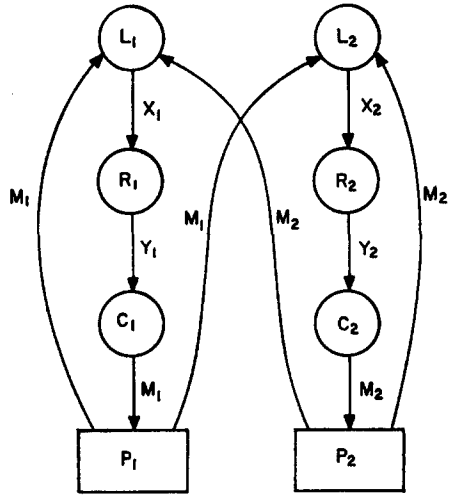

FIG. 4.

where mutual interactions between the oscillators are allowed if both k_{12} and k_{21} have non-zero values. Figure 5 shows the Y-variables of a pair of non-interacting control circuits when the parameters are:

$$a_1 = 360;\ A_1 = 36;\ b_1 = 10;\ k_{11} = 1;\ k_{12} = 0;\ \alpha_1 = 0.5;$$

$$\beta_1 = 0;\ a_2 = 360;\ A_2 = 43;\ b_2 = 10;\ k_{21} = 0;\ k_{22} = 1;$$

$$\alpha_2 = 0.6;\ \beta_2 = 0.$$

A theoretical calculation of the relative frequencies of these oscillators gives:

$$\frac{w_2}{w_1} = \frac{b_2\sqrt{c_2}}{b_1\sqrt{c_1}} = \sqrt{\frac{0.6}{0.5}} \approx \frac{11}{10}$$

Thus the free-running or non-interacting oscillators have different frequencies, O_1 (oscillator 1, variables X_1 and Y_1) completing 10 cycles in about the same time as it takes O_2 to complete 11. Setting now $k_{12} = 0.3$ and keeping $k_{21} = 0$, so that the faster oscillator drives the slower one, the result is as shown in Fig. 6. The slower oscillator is entrained to w_2, the frequency of O_2, and now the behavior of the oscillators is completely coherent. The emergence of an ordered relationship between the oscillators from the disorganized behavior shown in Fig. 5 shows how interactions between control circuits in cells can lead to organization of biochemical processes in the time domain, thus producing coherent behavior. For example, the levels of the enzymes involved in the synthesis of adenine and guanine could be controlled by circuits which interact in this manner, so that there is always a close correlation between the

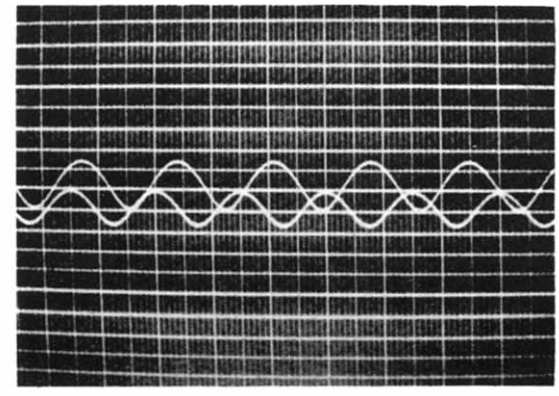

FIG. 5.

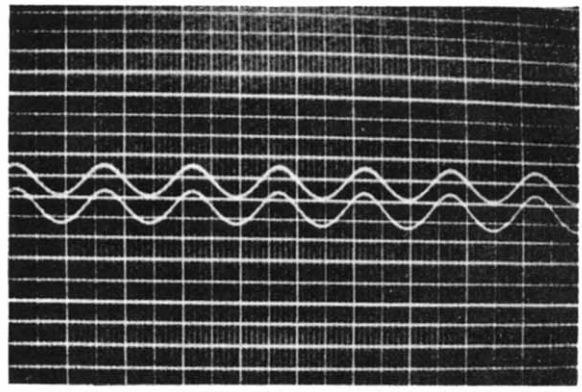

FIG. 6.

[*facing p. 430*]

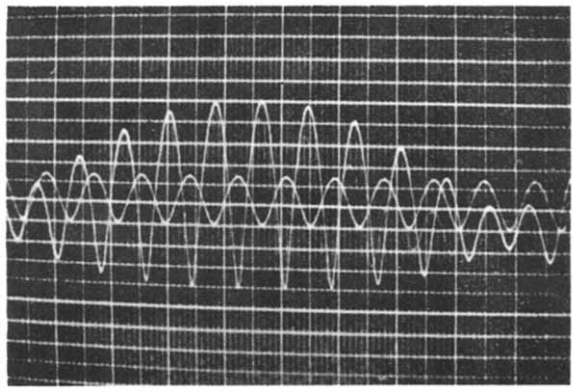

FIG. 7.

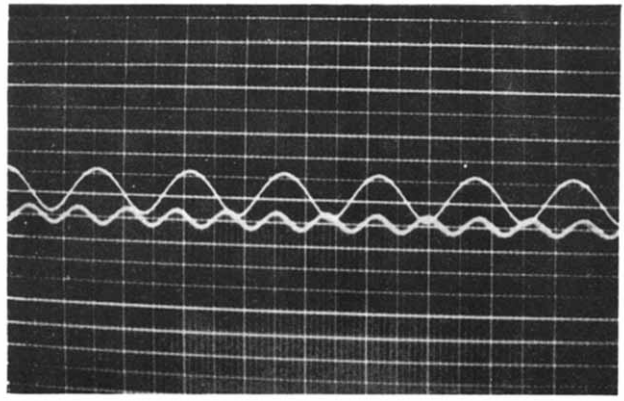

FIG. 8.

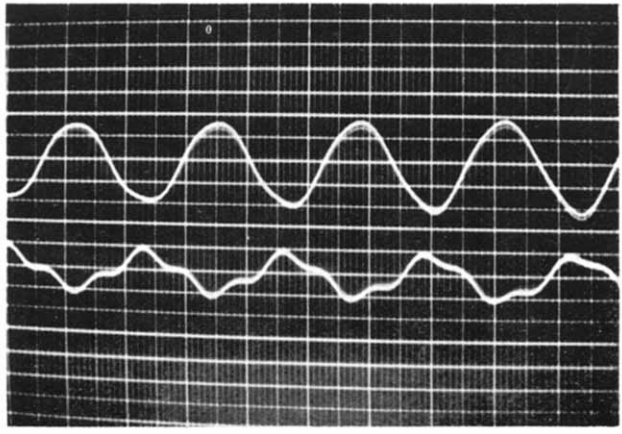

FIG. 9.

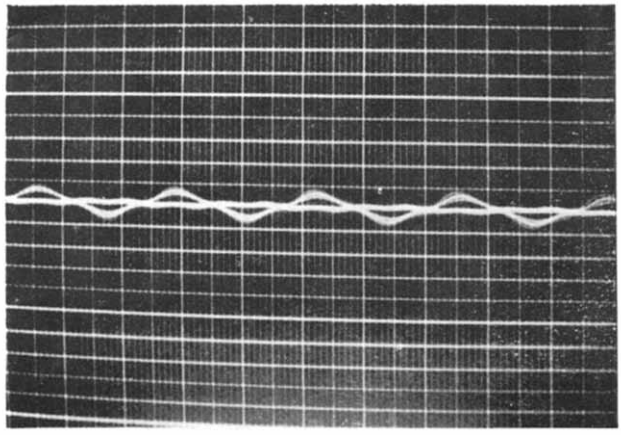

FIG. 10.

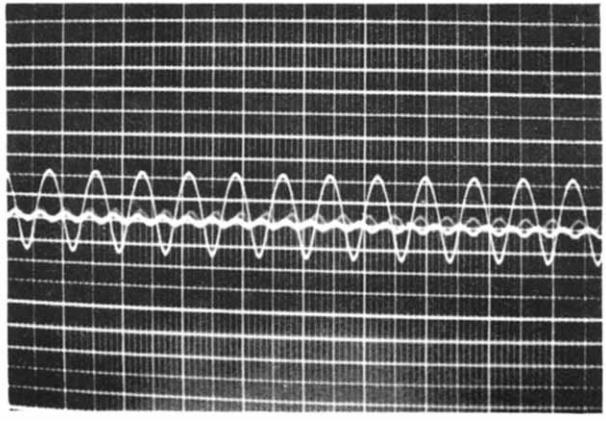

FIG. 11.

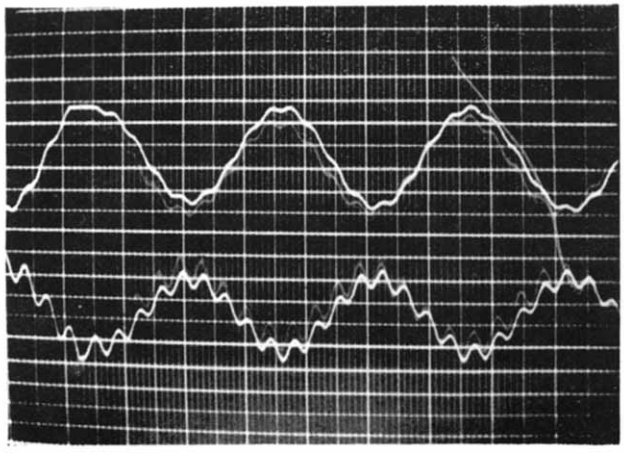

FIG. 12.

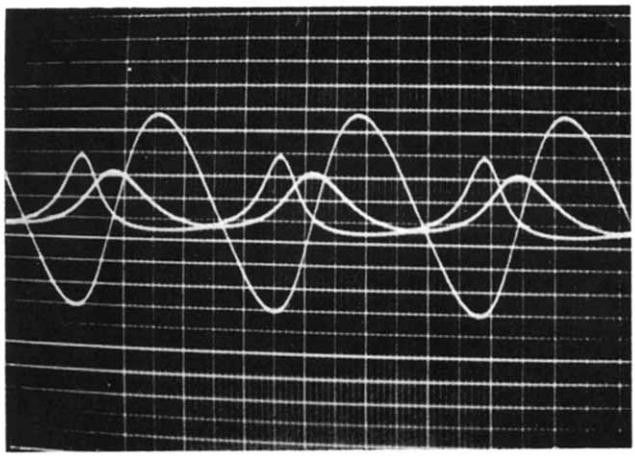

FIG. 13.

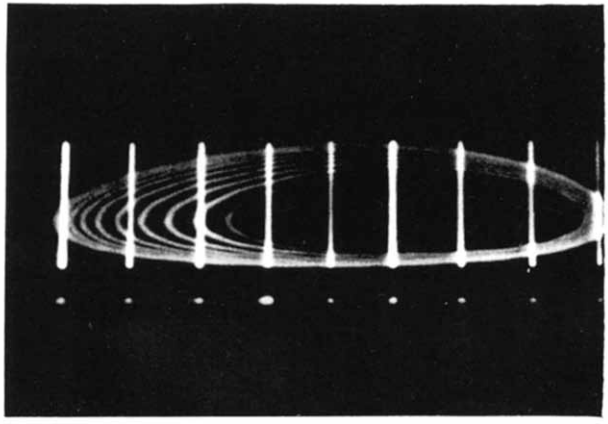

FIG. 14.

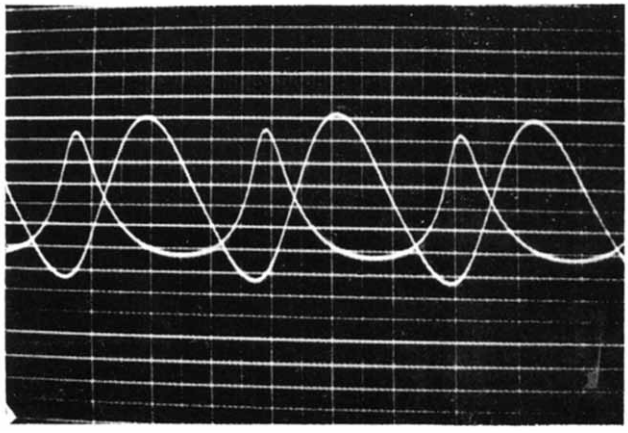

FIG. 15.

[*facing p. 431*]

rates of production of these two metabolites. It is clear that in the economy of a cell there is a distinct advantage in having coherent synthetic rates in related metabolic processes. The suggestion presented here is that the cell employs non-linear interaction to achieve this organization in time of its intrinsically rhythmic biochemical activities.

If the interaction is reversed so that O_1 interacts with O_2, but not vice-versa ($k_{12} = 0$; $k_{21} = 0.3$), one observes a different phenomenon, as shown in Fig. 7 (where the computing time is 2 sec, as compared with 1 sec in Fig. 6). In this case there is no entrainment of frequencies, but a beat occurs in O_2 with a beat frequency equal to about $w_2/11 = w_1/10$, where w_1 and w_2 are the original free-running frequencies. The beat frequency is clearly determined by the ratio $w_2/w_1 = 11/10$.

Figure 8 shows the Y-variables for the pair of oscillators defined by equations (2) when there is no interaction ($k_{12} = k_{21} = 0$) and $a_1 = 0.6$, $a_2 = 2.0$ (other parameters unchanged from above values). The ratio of the frequencies is now, theoretically,

$$\sqrt{\frac{c_2}{c_1}} = \sqrt{\frac{20}{6}} \approx \frac{13}{7}$$

This corresponds roughly to the observed ratio. Introducing mutual coupling between the oscillators, with $k_{12} = 1.2$, $k_{21} = 0.4$, a phenomenon known as subharmonic resonance is observed as shown in Fig. 9. Both variables now have the same frequency, which is considerably smaller than either of the free-running frequencies. The lower curve is Y_2, and in it one can still see the original frequency on which has been superimposed the slower frequency of the subharmonic, of order about 1/3. In Y_1 the order of the subharmonic is about 2/3, the original frequency of Y_1, not being visible.

Two features of this subharmonic resonance are of interest. The first is the increase in amplitude which occurs in both variables Y_1 and Y_2, Y_1 more than doubling over the free-running amplitude while Y_2 is nearly doubled. Secondly, in the subharmonic relation the variables are 180° out of phase. In general it has been found that subharmonics of order smaller than 1/2 in a mutually coupled pair of oscillators of the type described by equations (2) always involve an antiphase relationship in the Y-variables (i.e. the protein populations). Figure 10 shows the behavior of the X-variables (messenger RNA population) in the subharmonic relation. The larger oscillation is X_1, while X_2 shows practically no subharmonic at all, in spite of the well-defined subharmonic in Y_2. This shows that the behavior of mRNA and of protein in interacting control circuits may be quite different, variation in one not being obviously correlated with variation in the corresponding variable.

The importance of this type of phenomenon in the organization of biochemical processes in cells appears to be considerable. We may observe first that the range of frequencies available to a cell for ordering its activities

relative to environmental periodicities is greatly extended over the frequencies of its free-running or primary oscillations. The occurrence of relatively long rhythms, such as circadian (~24 hr) rhythms in unicellular organisms, and monthly rhythms in higher organisms, can thus be explained in terms of non-linear interaction rather than seeking elementary biochemical processes with time constants sufficiently large to generate such long periodicities. In the case of circadian rhythms there is good evidence that subharmonic resonance (or frequency demultiplication, as it is also called) is indeed involved.[10, 11]

Another important aspect of a subharmonic relation of the type shown in Fig. 9 is the phase relation between the variables Y_1 and Y_2. In an organism with circadian organization such as the unicellular dinoflagellate, *Gonyaulax polyedra*, certain processes such as photosynthesis are at a peak of activity during the day, while other processes such as luminescence are maximal at night. These activities are controlled by a variety of factors, among them enzyme and substrate levels in the cells. It seems reasonable to suggest that the timing of maximal photosynthetic and luminescent activity may be determined by mutual interactions between the control circuits regulating the relevant enzyme and giving rise to a subharmonic resonance such as that which has been demonstrated. In this way the organism achieves several desired results at once: a variety of frequencies can be generated, from which the appropriate one may be selected by proper coupling of control processes; an increased amplitude over that of the primary oscillation is obtained; and the biochemical activities can be correctly phased with one another.

For any given pair of coupled circuits, a great variety of subharmonic relations may be observed. Increasing the amplitude of O_1 and doubling the calculation time to 4 sec in the same pair of oscillators, the uncoupled behavior of the Y's is as shown in Fig. 11. When mutual interaction is now introduced by setting $k_{12} = 1.2$, $k_{21} = 0.77$, a subharmonic of relatively low order is obtained as shown in Fig. 12. For O_1 the order of the subharmonic is about 2/9, while for O_2 it is about 1/9.

The stability of this subharmonic relation is strongly dependent upon the amplitudes of the free oscillators prior to coupling. If the amplitudes are small, no subharmonic occurs, and only at larger amplitudes does a stable subharmonic relation arise. It is clear from Figs. 2 and 3 that the amplitude of the oscillations, hence the talandic temperature of the oscillators, is directly related to the "amount" of non-linearity in the dynamics, an oscillator with a small amplitude being almost linear and showing quasi-sinusoidal behavior. Only as the amplitude increases does the essential non-linearity of the oscillator become evident. The non-linear phenomena observed in this study, such as entrainment and subharmonic resonance, depend for their stability upon the talandic temperature of the oscillators, and this becomes increasingly the case as the order of the subharmonic decreases. Thus, the subharmonic of

Fig. 12 requires for stability a significantly larger initial amplitude in one of the oscillators than does the subharmonic of Fig. 9.

As the coupling parameter k_{21} is increased up to 0.8, lower order subharmonics appear in the coupled system which require progressively larger initial amplitudes for stabilization. At $k_{21} = 0.82$, the coupled oscillators become intrinsically unstable, Y_1 increasing to the limit of machine operation while Y_2 decreases. Theoretical studies of this coupled system[8] have shown that there is a critical surface in parameter space which divides a region of stable behavior from one of intrinsically unstable behavior, elliptic surfaces changing to hyperbolic surfaces. The bifurcation values of the parameters are determined by the roots of the equation

$$k_{11}k_{22} - k_{12}k_{21} = 0.$$

For the parameter values used above, $k_{11} = 1 = k_{22}$, $k_{12} = 1.2$, $k_{21} = 0.82$, this parametric function has the value $(1 - 0.984) = 0.016$. The instability observed on the computer is thus clearly due to the close approach to the critical boundary for stable performance.

It is of interest to observe that the order of the subharmonic observed as $k_{21} \to 0.82$ decreases rapidly, so that near the critical value one can get a subharmonic of order 1/36 or less, with a very great amplitude of oscillation. At the bifurcation value, the order of the subharmonic may be said to become zero, while the amplitude becomes infinite.

More complicated dynamic behavior arises when three control circuits are coupled together in various ways. Subharmonic resonances can be observed in such systems wherein all three Y-variables have the same frequency, which is smaller than any of the free-running frequencies. Two of the variables are then in phase, while the third is in antiphase with them. The interaction pattern determines these phase relations, strong mutual coupling between two circuits tending to produce an antiphase condition between the oscillators. Once again it is the Y-variables which show greatly increased amplitudes over the free oscillation under a condition of subharmonic resonance, while the X-variables are only slightly affected.

We may say, then, that third order and higher coupling between control circuits allows the cell to organize the periodic synthesis and activity of enzymes according to temporal patterns which have maximum adaptive value in the prevailing environment. Returning to the case of *Gonyaulax*, non-linear interaction of the type described could provide the cell with the dynamic properties necessary for the timing of enzyme syntheses relative to one another in a very well-defined and stable manner. The synthesis of enzymes relevant to photosynthesis can thus be kept in place with one another, for example, while the synthesis of enzymes involved in generating luminescence could occur in phase with each other but 180° out of phase with the photosynthetic group.

Intermediate phase relations can also be achieved by the appropriate coupling of control circuits.

Other non-linear phenomena, such as asynchronous quenching and asynchronous excitation[12] have been observed in the coupled system described by equations (2), with appropriate parameter values. It seems reasonable to assume that the complete range of non-linear behavior is to be expected from these oscillators and their interaction.

CONTROL CIRCUITS WITH LIMIT CYCLES

The major mathematical feature of the oscillations which have been discussed so far is the existence of a first integral of the motion. Such dynamic systems are conservative, and the occurrence of continuing oscillations is due primarily to the absence of damping in the equation of motion defining the operation of the circuit. These form a rather special class of negative feedback control process, and so it was of interest to investigate the possibility of oscillatory behavior in a more general type of system, one in which damping occurs. Oscillations in such circuits would be expected to show limit-cycle characteristics, or non-conservative behavior.

The type of equation which was studied was the following:

$$\left.\begin{aligned} \frac{dX_1}{dt} &= \frac{a_1}{A_1 + k_1 Z_1} - b_1 X_1 \\ \frac{dY_1}{dt} &= a_1 X_1 - \beta_1 Y_1 \\ \frac{dZ_1}{dt} &= \gamma_1 Y_1 - \delta_1 Z_1 \end{aligned}\right\} \tag{3}$$

There are at least three interpretations of these equations, one using the concept of diffusion delay, one a precursor concept, and the third the notion of a metabolic sequence. In the first case we may consider that there is an appreciable delay before mRNA, which is synthesized in the nucleus of higher cells, diffuses into the cytoplasm and becomes active in a polyribosome complex. Then X_1 is nuclear messenger, Y_1 is active cytoplasmic messenger, and Z_1 is the active enzyme which controls the level of the metabolite functioning as repressor at the DNA level. Secondly, we may regard Y_1 as an enzyme precursor which after primary synthesis on mRNA templates, X_1, passes through a pool of inactive molecules before being transformed into mature, active enzyme, Z_1. Finally, may we take X_1 to be an enzyme population whose rate of synthesis is regulated by feedback control at the polysome level via a metabolite Z_1. Y_1 is then an intermediate in the biosynthetic sequence leading to Z_1.

The only dynamic differences in the possible interpretations of the equations (3) lie in the values assigned to the parameters. It should be observed that there is now self-damping by each of the variables. This means that, for example, mRNA and protein are turning over at rates proportional to their concentrations in the cell, an assumption which is more realistic than the condition of constant degradation rates in equations(1).

When this system is studied with parameter values $a_1 = 360$, $k_1 = 1$, $A_1 = 43$, $b_1 = 1$, $\alpha_1 = 1$, $\beta_1 = 0.6$, $\gamma_1 = 1$, $\delta_1 = 0.8$, one observes oscillatory behavior as shown in Fig. 13. The smaller oscillations are X_1 and Y_1, X_1 leading Y_1 in phase. Regarding X_1 as nuclear messenger, Y_1 is then active cytoplasmic messenger which reaches its peak slightly after X_1, there being a diffusion delay in the passage of the macromolecule from nucleus to cytoplasm. The large oscillation is then the enzyme population, Z_1, which follows Y_1 with a further delay. The system has been scaled so that the amount of cytoplasmic messenger, the area under the Y_1-curve, is just about equal to the amount of nuclear messenger, the area under X_1. The amount of enzyme is appreciably larger, of course.

Oscillatory behavior is by no means a necessary dynamic characteristic of equations (3). There is a relatively restricted range of parameter values over which periodicities are observed, the system being damped elsewhere. However, whenever oscillations do occur in such a system, they have the characteristics of limited surfaces. In Fig. 14 is shown the phase portrait of the variable Z_1 against Y_1 (enzyme as a function of cytoplasmic messenger). Z_1 is on the abscissa, Y_1 is the ordinate, and the positive direction of Z_1-axis is from right to left. In this case the system started at a point inside the limiting trajectory, and one can see the widening spirals as the system approaches the limit cycle. Exposure problems prevented a photographic recording of the complete approach to the final limit cycle.

If the same system was started at a point outside the limit cycle, the spirals fell inwards towards it, converging upon the same final closed trajectory.

The reason for the occurrence of oscillations in such control circuits is quite different from that for the circuits discussed in the earlier section of this report. What we are now dealing with is a system which is damped, but which has an exciting factor in the existence of a type of dynamic time-lag in the solution. This is most easily seen if we assume that X_1 is a periodic signal, say $\cos wt$, an assumption which is clearly incorrect but which allows one to see an essential feature of the equations.

Taking $X_1 = \cos wt$, it is readily shown that $Y_1 = Ae^{-\beta_1 t} + a_1\cos(wt - \tau_1)$ where $\tan \tau_1 = w/\beta_1$, and $Z_1 = Be^{\lambda_1 t} + Ce^{\lambda_2 t} + \alpha_1\gamma_1\cos(wt - \tau_2)$, where $\tan \tau_2 = w(\beta_1 + \delta_1)/(\beta_1\delta_1 - w^2)$ and λ_1, λ_2 are the roots (both negative) of the quadratic $X^2 + (\beta_1 + \delta_1)X + \beta_1\delta_1 = 0$. For t relatively large, the negative exponential terms in these solutions are small, and the dominating terms are the cosine functions. These have the same period as $\cos wt$ but they are shifted

in phase by τ_1 and τ_2 for Y_1 and Z_1, respectively, and there is an amplitude modification. Thus for large t, a major dynamic effect of these equations is a phase shift in the initial signal. In effect this approximates to a time-lag in the transmission of the signal X_1, so that the oscillator shown in Fig. 13 may be regarded as a time-lag oscillator. It is this lag in signal transmission which acts as an exciting force for the oscillations. Figure 15 shows the same basic equations scaled roughly to represent feedback inhibition. The variable showing the sharp peaks and long troughs is then the amount of enzyme, X_1, while the other variable shown is the end-product, Z_1, which acts as the control molecule for enzyme synthesis. The scaling used reduces Z_1 to about 1/100th of its correct value. In real time the period of such an oscillation has an estimated range of about 30 min to 2 hr. Again the oscillation is a limit cycle.

CONCLUSION

It is clear from these studies that oscillatory behavior may be expected to constitute a very important dynamic feature of the operation of cellular control processes. The oscillations which have been studied represent only a small class of the periodic solutions which can occur in negative feedback control circuits, but they serve to illustrate how spontaneous rhythmic activity may be expected to arise in the dynamic organization of cells. This intrinsic rhythmic activity represents a type of biological energy which cells and organisms can use for organizing in time the staggering complexity of biochemical processes which make up living systems, thus achieving coherence and order in these activities. The interactions of non-linear oscillators, illustrated in this paper, provide a dynamic basis for this self-organizing property of oscillating cellular control circuits.

The work reported here and that of other investigators, such as Spangler and Snell[13] and Higgins[14] on the occurrence of periodicities in metabolic control processes, show that oscillatory behavior can arise at different levels of cellular organization. These different levels of control in cells correspond to different frequencies of oscillation, metabolic periodicities such as those observed by Chance, Estabrook and Ghosh[7] having a frequency of about 1.7 per min and almost certainly not involving macromolecular synthesis; while the slower rhythms underlying circadian time structure apparently involve control processes at the level of mRNA synthesis.[15] In higher organisms the range of frequencies is extended by the development of hormonal control circuits which can generate monthly, seasonal, and even annual rhythms; while within the nervous system, neural organization appears to involve the occurrence of rapid rhythms generated by reverberatory arcs of neurones with frequencies of several cycles per sec.[16, 17]

In view of these observations, one is strongly tempted to suggest that the basis of much animal and plant behavior is to be found in the intrinsic

rhythmic or period properties of control circuits, at all levels of organization. Oscillatory activity in these circuits is then indeed a fundamental type of biological energy, and our analysis of behavior should proceed with concepts and quantities which give full weight to the central importance of periodicities in the primary regulatory processes of organisms. It is to this end that a thermodynamic-like theory of molecular control mechanisms has been developed,[8] and some of the physiological implications of this procedure have been discussed by Goodwin.[18] The ultimate goal of these studies is a theoretical physiology of behavior, which will allow one to use the knowledge of elementary control processes such as those governing enzymatic synthesis and activity as the basis for a comprehensive, predictive theory of biological organization.

SUMMARY

The demonstration in recent years of negative feedback control processes operating at the molecular level in cells leads naturally to a study of their dynamic properties. Since such control mechanisms are known to have an intrinsic tendency to oscillate, and since rhythmic processes constitute a prominent dynamic feature of plant and animal physiology, it is suggested that spontaneous oscillatory behavior in an organism's control processes constitutes the dynamic basis of rhythmic behavior patterns. A computer (analogue) analysis is presented of differential equations representing control of enzyme synthesis by feedback repression, and non-linear oscillations are shown to occur. Phenomena such as synchronous locking and subharmonic resonance are shown to arise from the interaction of these oscillators, and the physiological significance of such non-linear behavior is discussed. A thermodynamic-like analysis of the properties of many interacting oscillators is introduced.

ACKNOWLEDGMENTS

This research was carried out under Office of Naval Research Contract Nonr(G)-00012-64 and Project NR 018-308.

REFERENCES

1. H. E. Umbarger, Evidence for a negative feedback mechanism in the biosynthesis of isoleucine, *Science* **123**, 848–849 (1956).
2. B. Magasanik, The metabolic regulation of purine interconversion and of histidine biosynthesis, pp. 485–490, in *The Chemical Basis of Development* (W. B. McElroy and H. B. Glass, eds.), Van Nostrand, Princeton, N.J. (1958).
3. L. Gorini and W. K. Maas, Feedback control of the formation of biosynthetic enzymes, p.p. 469–478 in *The Chemical Basis of Development* (W. B. McElroy and H. B. Glass, eds.), Van Nostrand, Princeton, N.J. (1958).
4. J. L. Cloudsley-Thompson, *Rhythmic Activity in Animal Physiology and Behaviour*, Academic Press, New York and London (1961).

5. *Cold Spring Harbor Symposium Quant. Biol.* **25**, 1961.
6. L. N. M. DUYSENS and J. AMESZ, Fluorescence spectrophotometry of reduced phosphopyridine nucleotide in intact cells in the near ultraviolet and visible, *Biochim. Biophys. Acta* **24**, 19–26 (1957).
7. B. CHANCE, R. W. ESTABROOK and A. GHOSH, Damped sinusoidal oscillations of cytoplasmic reduced pyridine nucleotide in yeast cells, *Proc. Nat. Acad. Sci. (Wash.)* **51**, 1244–1251 (1964).
8. B. C. GOODWIN, *Temporal Organization in Cells*, Academic Press, London and New York (1963).
9. A. B. PARDEE, F. JACOB and J. MONOD, The genetic control and cytoplasmic expression of "inducibility" in the synthesis of β-galactosidase in *E. coli*, *J. Mol. Biol.* **1**, 165–178 (1959).
10. C. S. PITTENDRIGH and V. G. BRUCE, An oscillator model for biological clocks, pp. 75–109 in *Rhythmic and Synthetic Processes in Growth* (D. RUDNICK, ed.), Princeton University Press (1957).
11. C. L. COLE and P. L. ADKINSON, Daily rhythm in the susceptibility of an insect to a toxic agent, *Science* **144**, 1148–1149 (1964).
12. N. MINORSKY, *Nonlinear Oscillations*, Van Nostrand, Princeton, N.J. (1962).
13. R. A. SPANGLER and F. M. SNELL, Sustained oscillations in a catalytic chemical system, *Nature* **191**, 457–461 (1961).
14. J. HIGGINS, A chemical mechanism for oscillation of glycolytic intermediates in yeast cells, *Proc. Natl. Acad. Sci. U.S.* 989–994 (1964).
15. M. W. KARAKASHIAN and J. W. HASTINGS, The inhibition of a biological clock by actinomycin D. *Proc. Natl. Acad. Sci. U.S.* **48**, 2130–2136 (1962).
16. J. W. S. PRINGLE, On the parallel between learning and evolution, *Behaviour* **3**, 174 (1951).
17. E. R. CAIANIELLO, Outline of a theory of thought-processes and thinking machines, *J. Theoret. Biol.* **1**, 204–235 (1961).
18. B. C. GOODWIN, A statistical mechanics of temporal organization in cells, *Symp. Soc. Exper. Biol.* **18**, 301–326 (1964).