BIOCHEMICAL OSCILLATIONS IN "CONTROLLED" SYSTEMS

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It seems possible that the question of how to arrange reactions so that concentrations oscillate will have multiple answers (cf. Spangler and Snell, 1961; Higgins, 1964; Walter, 1966; Strickland and Ackermann, 1966). Particularly appealing is Goodwin's (1963) idea that oscillations will arise naturally in "controlled" systems, and his demonstration thereof for a simplified case wherein control is exerted through enzyme synthesis. Before learning of Goodwin's work we showed, as follows, that concentration oscillations can occur in systems of catalyzed reactions in which control is directly exerted through negative feedback inhibition of the Yates-Pardee (1956) type.

Consider consecutive reactions (each assumed to include an irreversible step) in which formation of substance[1] S_i from S_{i-1} is catalyzed by enzyme E_{i-1}, and conversion to S_{i+1} is catalyzed by enzyme E_i. Assume that S_0 is maintained constant (by vastness of reservoir or by replenishment) and E_0 is inhibited cooperatively (sequential steps ignored) by p molecules of S_n. Assume further that S_i is negligible compared to the ith Michaelis constant, $K_i^{(M)}$, (i.e. the enzymes are "unsaturated") and that $\dot{S}_i$ is slow compared to the rates at which the S_i equilibrate with their enzymes. These assumptions imply first-order rate constants, $k_i = V_i^{(\max)}/K_i^{(M)}$ for $i \geq 1$, and $k_0/(1 + \alpha S_n^p)$ for the 0th reaction, α being the affinity constant of S_n and E_0, and $(1 + \alpha S_n^p)^{-1}$ being the fraction of active E_0. k_n may be considered to be the velocity constant for the exit of S_n from the system. In turn, α, p, and the k_i are parameters in the differential equations governing the system

$$\dot{S}_1 + k_1 S_1 = k_0 S_0/(1 + \alpha S_n^p). \tag{1}$$

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[1] Here and elsewhere the chemical symbol and the molar concentration of a substance are expressed by the same capital letter.

$$\dot{S}_i + k_i S_i = k_{i-1} S_{i-1}, \qquad i = 2, 3, \cdots . \tag{2}$$

Alternatively, these equations are expressible as a single nth order equation,

$$\sum_{i=0}^{i=n} f_i^n S_n^{(n-i)} = \left(\prod_{i=0}^{i=n-1} k_i \right) S_0/(1 + \alpha S_n^p), \tag{3}$$

where f_i^n is the sum of all i-combinations of the n numbers, $k_1, k_2, \cdots k_n$, and $S_n^{(n-i)}$ is the $(n - i)$th derivative of S_n. Equation (3) for S_n shows that among the constants, $k_1, k_2, \cdots k_{n-1}$, each constant occupies an algebraic position equivalent to every other, and *mutatis mutandis* for $S_{i<n}$. Therefore, among the ks before k_i, variation in any k has the same effect on S_i as variation in any other equal k.

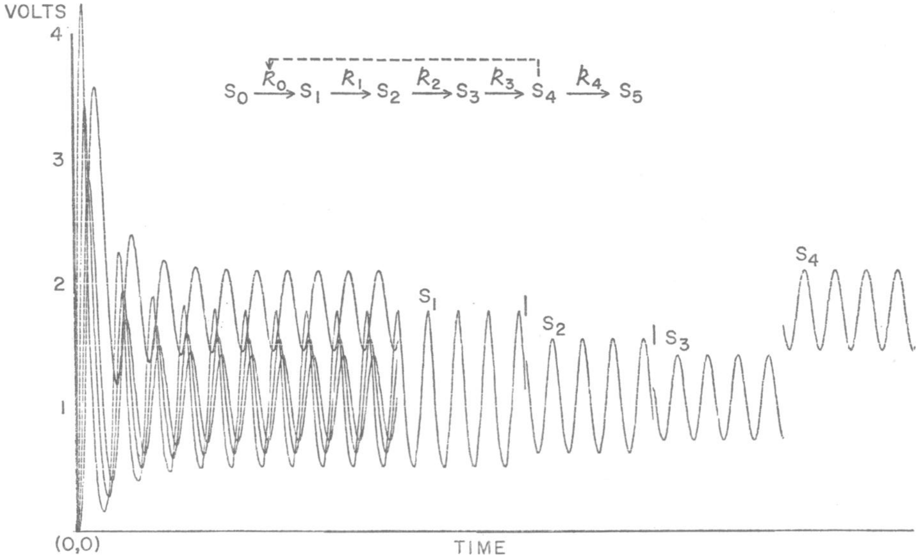

Figure 1 Transient and sustained oscillations. $p = 4$, $\alpha = 0.08$, $k_0 S_0 = 5.1$, $k_1 = k_2 = k_3 = 1.000$, $k_4 = 0.600$. At the left are shown initial oscillations of all four quantities; at the right, oscillations of individual quantities separately.

We have not solved equations (1), (2), or (3) analytically, but have simulated them on an EAI TR-48 analogue computer (verifying the behavior by hand calculation), and have studied certain properties of their solutions. Simulation shows (Fig. 1) that for certain ranges of values of the parameters the system passes through an initial transient phase, then "settles down" to produce sustained oscillations in he S_i for an indefinitely long time; i.e.

$$S_i(t) \rightarrow \langle S_i \rangle + A_i F_i[2\pi(\nu t - \Phi_i)], \tag{4}$$

where $\langle S_i \rangle$ is the time average of S_i, A_i is the (constant) amplitude, and F_i is a

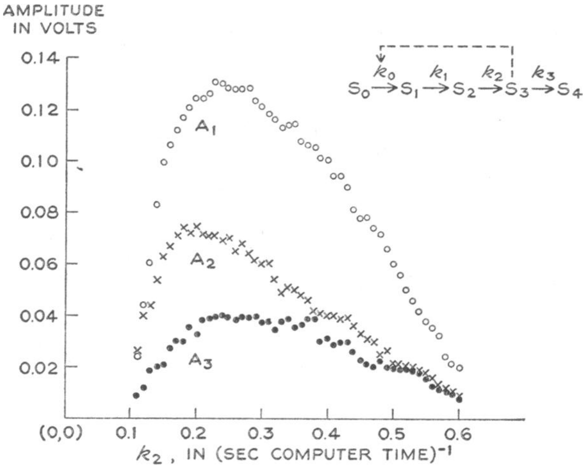

FIGURE 2 Amplitudes A_i, as functions of k_2. $p = 4$, $\alpha = 0.25$, $k_0S_0 = 2.75$, $k_1 = k_3 = 0.250$.

normalized periodic function lagging behind $(1 + \alpha S_n^p)^{-1}$ by a fraction of a period, Φ_i. The following properties of S_i may prove useful in matching experimental data.

During sustained oscillations, integration of equations (1) and (2) over times which are integral multiples of the period shows that the time average of the material flux through any species is the same; i.e.

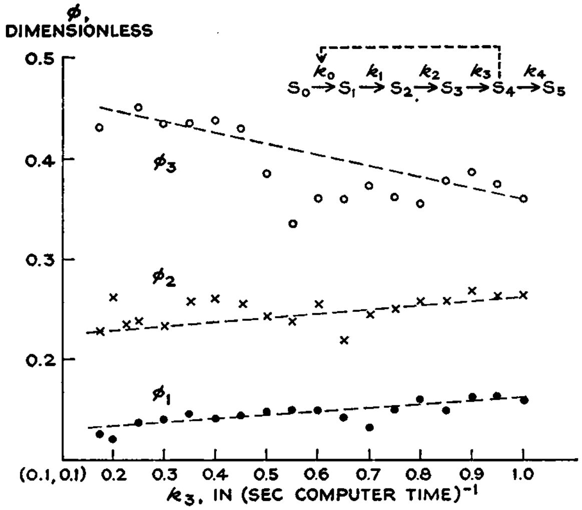

FIGURE 3 Fractional phase differences, Φ_i, as functions of k_3. $p = 4$, $\alpha = 0.18$, $k_0S_0 = 6.2$, $k_1 = k_3 = k_4 = 1.000$. The dotted lines only emphasize sign of the slope, and do not imply linearity.

$$k_0\langle S_0/(1 + \alpha S_n^p)\rangle = k_1\langle S_1\rangle = \cdots = k_n\langle S_n\rangle. \tag{5}$$

In general, the "wave forms" of the S_i are found to be unsymmetrical.

The amplitudes, A_i, pass through maxima when the k_i are varied over certain ranges (Fig. 2).

For fixed values of the k_i, increasing p appears to increase the A_i.

The phase differences, Φ_i, behave as follows: $\Phi_n = \frac{1}{2}$; when k_i is increased, the $\Phi_{j<i}$ either increase or stay constant, and the $\Phi_{j\geq i}$ either stay constant or decrease (Fig. 3).

Because the k_i occupy equivalent algebraic positions, and because ν should vanish when any k vanishes, a simple guess for $\nu(k)$ is

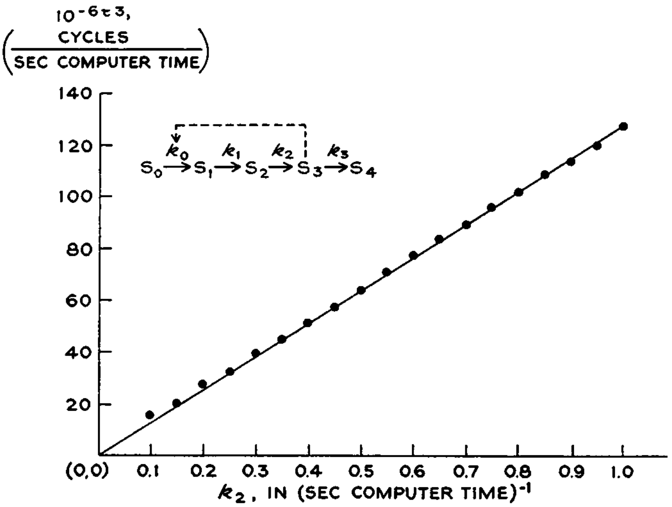

FIGURE 4 $(\text{Frequency})^3$ as a function of k_2. $n = 3, p = 4, \alpha = 0.29, k_0S_0 = 1.76, k_1 = 0.300, k_3 = 0.260$.

$$\nu = N(\alpha, k_0S_0) \prod_{i=1}^{i=n} k_i^{(1/n)}, \tag{6}$$

wherein N is an arbitrary function. This relationship in fact holds very well (Fig. 4).

In summary, the concentrations of intermediates in a "straight chain" metabolic pathway exhibiting negative feedback inhibition will oscillate detectably if certain quantitative characteristics of the intervening enzymes are "right." In this work an attempt has been made to relate general properties such as the frequency, individual species properties such as amplitudes, and relational properties such as phase differences to the characteristics of the enzymes.

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REFERENCES

GOODWIN, B. C. 1963. Temporal Organization in Cells; A Dynamic Theory of Cellular Control Processes. Academic Press, Inc., New York.

HIGGINS, J. 1964. *Proc. Natl. Acad. Sci. U.S.* **51**:989.

SPANGLER, R. A., and F. M. SNELL. 1961. *Nature.* **191**:457.

STRICKLAND, E. H., and E. ACKERMANN. 1966. *Nature.* **209**:405.

WALTER, C. F. 1966. *Nature.* **209**:404.

YATES, R. A., and A. B. PARDEE. 1956. *J. Biol. Chem.* **221**:757.