MATHEMATICAL ANALYSIS OF METABOLIC NETWORKS*

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1. Introduction

Although its important reaction sequences and cycles have been known for more than 25 years, we are still far from a satisfactory theory of intermediary metabolism. The empirical material is overwhelming and increases every year, but the development of a general conception is still in its infancy, although the conceptual progress of recent years has been considerable (for reviews, see [1–3]).

In our opinion, however, the emphasis is too much on static concepts which view the intermediary network as if it were a stock of spare parts, energy and material reserves to be used and filled up by appropriate enzymic reactions. The principal bias is towards optimization and control of this supply whereas the dynamic aspects are obviously neglected.

There are preferences which testify to this tendency, for example the predominance of the feedback concept, or, in more general terms, of restrictive and repressive control at the metabolic and epigenetic level as a device to meet material demands and to prevent, or to cope with, sudden affluence. Activation and induction are likewise understood to have the same purpose. Indeed, the very concept of 'control' as an index of what restricts a given state to its own level points to the same inclination. A more miscellaneous symptom is the short-lived fashion of oscillations and the tendency to discount them as a curiosity rather than to take them as a key to the hidden dynamic organization of pathways.

The prevalence of the static concept is most clearly reflected in the widespread opinion that sigmoidal kinetics is a switch device providing a rigorous answer to small changes in effector levels. Simple reflection shows the fallacy of this straightforward interpretation. Admittedly, to increase $\bar{v}$ from, say, 0.03 to 0.97 of V_{max} a 1000-fold effector change is required for a Michaelis-type enzyme. On the other hand, even the most cooperative mechanisms so far observed ($n > 3$ in a Hill plot) still require factors of about 10 to achieve the same goal, and the efficiency is much lower at the more common ranges of cooperativity. A trigger mechanism with such a backlash is a poor gadget in technology as well as in metabolism, even more so as drastic changes are very rarely observed in vivo. Obviously the S-shape alone cannot account for a trigger; in fact, it is even unnecessary to explain it [4].

It should be emphasized that the term 'static' is not meant in a perjorative sense. The progress along such lines is too obvious to be discounted (e.g. the energy charge concept, feed-back control of key-

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enzymes, nucleotide coupling of pathways, multisite control etc. [1–3,5]). This review letter attempts to sketch an outline of the complementary dynamic view. It is intended as a survey of basic ideas rather than of accumulated results.

2. Mathematical analysis of models

2.1. *Choice of model*

The mathematical model is, in a sense which has been elaborated in a different context [6], a link which connects empiricism and theory. It represents or predicts empirical facts, but not in empirical language. This explains why the choice of the model can be made to a certain extent by convenience of theoretical reflection.

This review will be restricted, again because of convenience, to one particular formalism: the kinetic model of metabolic networks. Its general form is a system of ordinary non-linear differential equations derived from the law of mass-action according to the topological rules of a metabolic map, including enzyme mechanisms. The variables are concentrations of substances, the parameters phenomenological rate constants.

2.2. *Basic model*

The basic model is of the following special form

$$dx_i/dt = \sum_p c_{ip} v_p - \sum_q c_{iq} v_q \quad , \qquad (1)$$

i.e. the concentrations of the substances x_i change with a velocity according to the sum of input fluxes minus the sum of output fluxes of the respective substance. The c's are stoichiometric coefficients ($\geqslant 0$), whereas the velocities v are non-linear functions of the x's, involving rate constants.

2.3. *Validity of the model*

With specified start conditions and known rate constants, the time course of all metabolites and enzymic intermediary complexes is determined. Indeed, the famous simulation studies of D. Garfinkel, B. Hess, E.M. Chance and others (reviewed in [7]) started exactly from this position. Apart from numerous special conclusions, they furnished the conceptually important general result that the time behaviour of complex metabolic systems can be essentially explained by the basic model (1); correct predictions and refutation of particular hypotheses are possible. No mystic 'vital force' has to be introduced to explain the observed phenomena – only thermodynamic and kinetic principles are required.

2.4. *Reduction of the model to canonical form*

Regardless of its established formal validity the direct simulation approach meets with practical and conceptual difficulties. The numerical values of the rate constants, for instance, are usually unknown and would require experimental information of enormous accuracy. To a certain degree such values can be guessed at because the result often depends only weakly on the precise value, but this introduces an undesirable element of empirism from the start. A second problem concerns dimensionality. Even small pathways require several hundred equations [8], and the complete intermediary network of a cell would comprise a system of order 10^4 or 10^5. This is beyond the scope if not of the numerical capacity (which depends on computer development) but certainly of the interpretative capacity of the investigator. Reduction is therefore a theoretical and practical necessity.

Closer examination of any real model (1) will reveal that the numerical values of the x's, v's and dx/dt differ by orders of magnitude. Rapid reactions are in the order of μsec^{-1}, whilst the slowest may be of order hr^{-1} or even less. As been aptly discussed by Goodwin [9] and Higgins [10] this introduces a time hierarchy into the system. At a specified time level those variables whose motion is slow will appear as constants ('structural variables' in Higgins's wording), while the rapid reactions freeze to steady-state ('dynamic substructure'). At the metabolic time level (sec^{-1}), for instance, the enzyme activities belong to the structure, while ionic dissociation is part of the dynamic substructure which is represented by stationary equations.

For detailed studies the basic model (1) must therefore be transformed to display the properties at the specified time scale. In principle this amounts to scaling of all parameters and variables, including time, and to finding, by way of transformation, those 'lumped' variables and parameters which are essential at the chosen time scale.

2.5. *Example. One-substrate reaction*

These analytical methods are perhaps best illustrated by the classical mechanism

$$S + E \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} X \xrightarrow{k_{+2}} E + P \quad , \tag{2}$$

which, as is well known, can be described by the following basic model

$$\frac{ds}{dt} = -k_{+1} \cdot e \cdot s + k_{-1} \cdot x$$

$$\frac{dp}{dt} = k_{+2} \cdot x$$

$$\frac{dx}{dt} = k_{+1}\, e \cdot s - (k_{-1} + k_{+2}) \cdot x$$

$$\frac{de}{dt} = -k_{+1}\, e \cdot s + (k_{-1} + k_{+2}) \cdot x \quad . \tag{3}$$

Of the 4 variables only 2 are really *independent,* because of the linear integrals

$$s + p + x = s_0 \text{ (total metabolite concentration)}$$

$$e + x = e_0 \text{ (total enzyme concentration).} \tag{4}$$

Therefore the system is of order 2:

$$\frac{ds}{dt} = -k_{+1} \cdot s \cdot (e_0 - x) + k_{-1} \cdot x \; ; \; s(t=0) = s_0$$

$$\frac{dx}{dt} = k_{+1} \cdot s \cdot (e_0 - x) - (k_{-1} + k_{+2}) \cdot x \; ; \; x(t=0) = 0 \quad . \tag{5}$$

2.6. *Scaling of variables to slow movement*

The system (5) cannot be solved explicity. Its analysis requires transformation to canonical form with dimensionless variables and parameters (which are distinguished by Greek letters). Introduce

$$\epsilon = \frac{e_0}{K_m} \; ; \; \xi = \frac{k_{-1}}{k_{-1} + k_{+2}} \quad \text{with } K_m = \frac{k_{-1} + k_{+2}}{k_{+1}} \quad , \tag{6}$$

and consider the dimensionless variables

$$\sigma = \frac{s}{K_m} \quad , \quad \kappa = \frac{x}{e_0} \quad , \tag{7}$$

and dimensionless time

$$\tau = k_{+1}\, e_0 \cdot t \quad . \tag{8}$$

With two normalized variables σ, κ and two *essential parameters* ϵ, ξ and with quantities of numerical order unity at the right-hand side, the canonical equations are

$$\frac{d\sigma}{d\tau} = -\sigma(1-\kappa) + \xi\kappa \; ; \quad \sigma(0) = \frac{s_0}{K_m}$$

$$\epsilon \frac{d\kappa}{d\tau} = \sigma(1-\kappa) - \kappa \; ; \quad \kappa(0) = 0. \tag{9}$$

If the concentration of enzyme e_0 is, as is often so in practice, very small, i.e. $\epsilon \ll 1$, then κ in comparison to σ is a *rapidly* moving variable. Such a system with separated time scales can, if certain preconditions are fulfilled [11–13], be simplified by the limit transition $\epsilon \to 0$ (steady-state or quasi-stationarity assumption). In our case this gives a 'degenerate' model (with only 1 variable):

$$-\frac{d\sigma}{d\tau'} = \tilde{\kappa}$$

$$\tilde{\kappa} = \frac{\sigma}{1+\sigma} \; (\tilde{\kappa} = \text{quasi-stationary value of } \kappa)$$

$$\tau' = (1-\xi)\tau \quad , \tag{10}$$

which is the famous Michaelis equation. It can be solved in an implicit form (integrated Michaelis equation).

A misunderstanding which is ubiquitous in textbooks should be annotated, namely that the steady-state value of κ be constant. In fact, only ϵ approaches zero, while (from (10) by differentiation)

$$\frac{d\tilde{\kappa}}{d\tau'} = \frac{1}{(1+\sigma)^2} \frac{d\sigma}{d\tau'} = -\frac{\sigma}{(1+\sigma)^3} \neq 0. \tag{11}$$

This shows that $\tilde{\kappa}$ changes with time without affecting the validity of quasi-stationarity.

2.7. *Scaling to fast process*

For consideration of rapid kinetics it is required to introduce a 'fast' time scale $\theta = \tau/\epsilon$. The canonical form now reads

$$\frac{d\sigma}{d\theta} = \epsilon(-\sigma(1-\kappa) + \xi\kappa)$$

$$\frac{d\kappa}{d\theta} = \sigma(1-\kappa) - \kappa \tag{12}$$

which is equivalent to (9), but displays the slow movement of σ at this level: $d\sigma/d\theta \sim \epsilon \ll 1$, while $d\kappa/d\theta \sim 1$. After transition $\epsilon \to 0$, σ becomes a constant parameter:

$$\frac{d\kappa}{d\theta} = \sigma - (1+\sigma)\,\kappa \qquad (13)$$

This equation is again integrable; it yields the so-called Gutfreund exponential equation.

Such simple reduction is no longer possible, when ϵ and $\xi \sim 1$. Then, as eq. (9) shows, κ and σ move with about equal velocity. Nevertheless, a slow component can again be isolated with a surprising result. Introduction of the time scale τ' (i.e. $d\tau' = d\tau\,(1-\xi)$) and elimination of the first term in (9a) gives:

$$-\frac{d\sigma}{d\tau'} = \epsilon\,\frac{d\kappa}{d\tau'} + \kappa$$

$$(1-\xi)\,\epsilon\,\frac{d\kappa}{d\tau'} = \sigma(1-\kappa) - \kappa \qquad (14)$$

Now the transition $\xi \to 1$ gives $\tilde{\kappa}$ as in (10) and hence, by differentiation

$$\frac{d\sigma}{d\tau'} = \frac{\sigma(1+\sigma)}{\epsilon + (1+\sigma)^2} \qquad (15)$$

which at first glance is utterly surprising, because it is formally identical to the Monod–Wyman–Changeux equation for a two-protomer allosteric enzyme [14]. Its validity depends on $\epsilon \sim 1$ and $\xi \sim 1$ (i.e. $k_{-1} \gg k_{+2}$), and it reduces to the Michaelis hyperbola at excess substrate ($\epsilon \to 0$), a behaviour distinct from the Monod model.

2.8. *Essential variables*

Often a clear separation of time scales as in (10) cannot be achieved with the original biochemical variables. Consider, for example, the following system of reactions involving adenine nucleotides:

$$\mathrm{ADP + ADP} \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} \mathrm{ATP + AMP} \quad \text{(adenylate kinase)}$$

$$\mathrm{ADP + P_i} \xrightarrow{k_2} \mathrm{ATP} \quad \text{(ATP synthesis)}$$

$$\mathrm{ATP} \xrightarrow{k_3} \mathrm{ADP + P_i} \quad \text{(ATPase)} \qquad (16)$$

which in a more convenient notation can be represented as

$$\frac{da_1}{dt} = k_{+1}\,a_2^2 - k_{-1}\,a_1 a_3$$

$$\frac{da_2}{dt} = -2(k_{+1}\,a_2^2 - k_{-1}\,a_1 a_3) - k_2 a_2\,p + k_3\,a_3$$

$$\frac{da_3}{dt} = k_{+1}\,a_2^2 - k_{-1}\,a_1 a_3 + k_2\,a_2\,p - k_3\,a_3$$

$$\frac{dp}{dt} = -\,k_2\,a_2\,p + k_3\,a_3\ . \qquad (17)$$

Again, two linear integrals exist:

$a_1 + a_2 + a_3 = a_0$ (conservation of adenylate)

$a_2 + 2a_3 + p = p_0$ (conservation of phosphate), (18)

which reduce the system to order 2. Introduction of

$$\alpha_i = \frac{a_i}{a_0}\ ;\ \rho = \frac{p}{a_0}\ ;\ \tau = k_2\,a_0\,t\ ;$$

$$\beta = \frac{k_3}{k_2 a_0}\ ;\ \epsilon = \frac{k_2}{k_{-1}}\ ;\ K = \frac{k_{+1}}{k_{-1}} \qquad (19)$$

$$\nu_1 = K\,\alpha_2^2 - \alpha_1\alpha_3\ ;\ \nu_2 = \alpha_2\,\rho\ ;\ \nu_3 = \beta\alpha_3 \qquad (20)$$

leads to

$$\frac{d\alpha_1}{d\tau} = \frac{1}{\epsilon}\nu_1\ ;\ \frac{d\alpha_2}{d\tau} = -\frac{2}{\epsilon}\nu_1 - \nu_2 + \nu_3\ ;$$

$$\frac{d\alpha_3}{d\tau} = \frac{1}{\epsilon}\nu_1 + \nu_2 - \nu_3\ ;\ \frac{d\rho}{d\tau} = -\nu_2 + \nu_3\ . \qquad (21)$$

If adenylate kinase activity is high as compared to ATP synthesis ($\epsilon \ll 1$), then the first motion is clearly fast, while the last one is slow. The others contain both types of motion, and separation is impossible. But there exist pools (lumped variables) which move with time scale τ, for instance the *energy charge*, defined by

$$\varphi = \alpha_3 + 0.5\,\alpha_2 \qquad (22)$$

leading to

$$\epsilon\,\frac{d\alpha_1}{\alpha\tau} = \nu_1\ ;\ 2\,\frac{d\varphi}{d\tau} = \nu_2 - \nu_3 \qquad (23)$$

The asymptotic transition $\epsilon \to 0$ gives now:

$$\frac{d\varphi}{d\tau} = \tfrac{1}{2}(\nu_2 - \nu_3) \quad , \tag{24}$$

where the coefficients are determined by (20) and by the equation $\nu_1(\alpha_1, \alpha_2, \alpha_3) = 0$.

The pool variable φ, which was introduced on intuitive consideration by Atkinson [15], makes the construction of a canonic form with separated time scales possible.

2.9. *Properties of the canonical form*

To summarize, the canonical form is characterized by the following properties: i) homogenous time scale; ii) dimensionless variables and parameters of numerical order unity; iii) connection to different time regimes by scale parameters (ϵ etc). The parameters as well as the variables are often combination of the original phenomenological constants and biochemical variables, respectively. The approximate character of the limit transitions does not introduce nor eliminate qualitative features of the time level in question. The quantitative approximation is very good, even more so as numerical subtleties usually surpass the available experimental accuracy.

3. Dynamic properties of metabolic networks

3.1. *Open system*

In a closed system of biochemical reactions nothing except an approach to the stable equilibrium will happen [16]. It has been noted often that the open character of biological systems accounts for most of their typical phenomena, for instance multistability and temporal or spatial organization [17–23]. Metabolic systems, in particular, are suitable models in which to study the theory of such phenomena, because their quantitative properties are excellently known, and their experimental study in open conditions is comparatively easy.

3.2. *Example. Skeleton of glycolysis*

To facilitate the explanation of certain phenomena, a very simple model of glycolysis is introduced for further discussion: (eq. (25) below) with canonical form

$$\frac{d\sigma_1}{\alpha\tau} = \nu_1 - \nu \; ; \; \frac{d\sigma_2}{d\tau} = \alpha_2(\nu - \nu_2) \; ; \; (\alpha_2 = \text{scale factor}) \tag{26}$$

This model can be derived from a detailed basic model of glycolysis [24]; it retains its most important dynamic properties. A detailed model would have at least two precursors, and PKF would be a two-substrate–two-product reaction with multiple back-activation or forward-inhibition [25,26]. Also the consumption of ADP is a more involved process. But inclusion of all these details could only modify, not change, the characteristic qualitative behaviour to be explained now by graphical illustration. To this purpose the three velocities may be specified as follows. The production of σ_1 (skeleton of hexokinase) is assumed to be a precursor-saturated process with inhibition by σ_1. This can be approximated in the critical region by

$$\log \nu_1 \approx \log \nu_m - \beta_1 \log \sigma_1 \text{ (competitive inhibition).} \tag{27}$$

Furthermore, the consumption of σ_2 is to be described by an enzymic model with very high affinity for σ_2. Approximately,

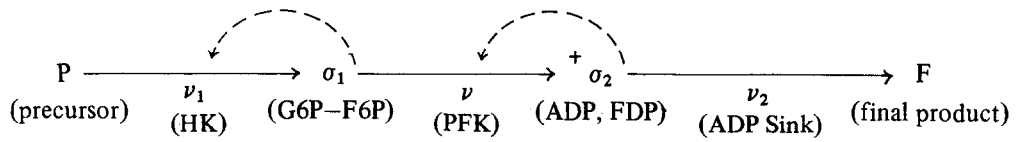
$$\nu_2 = \beta_2 \sigma_2 \; . \tag{28}$$

Finally, PFK activation is to be represented by cooperative kinetics involving σ_2 as effector. To avoid cumbersome formalism, only the graph is displayed in fig. 1. The model so specified is now considered in some detail.

3.3. *Stationary point and input characteristics*

A stationary point of σ_2 is any $(\bar{\nu}, \sigma_2)$-pair for which at a given σ_1-value $\nu = \nu_2$ so that $d\sigma_2/d\tau = 0$. The point is defined by intersection of the ν-curve with the ν_2-curve (fig. 2, left-hand part).

At a given σ_1-value the stationary value of σ_2 is not necessarily unique. This multiplicity of stationary points (fig. 2) is a consequence of the sigmoidal kinet-



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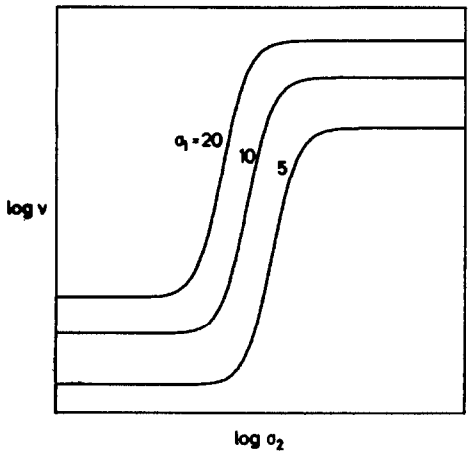


Fig. 1. Activation kinetics of idealized PFK. At different levels of the substrate (σ_1 = F6P) cooperative activation by σ_2 (e.g. ADP) is operative. Only the sigmoidal shape is essential for the qualitative considerations to follow, the particular curves were selected for convenience of demonstration.

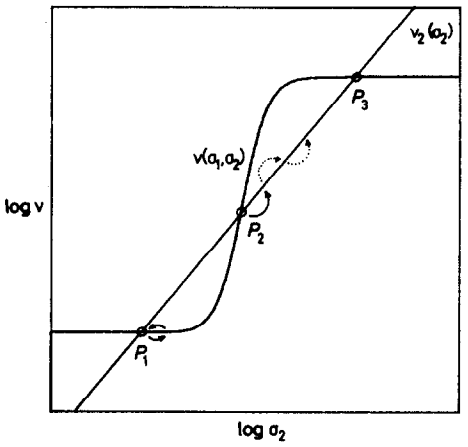


Fig. 3. Stability and instability of stationary points. This is a section from fig. 2, left-hand. Points P_1 and P_3 are *stable* stationary points. Consider, for instance, P_1, where a slight perturbation which increases σ_2 makes $\nu_2 > \nu$, therefore (eq. 26) $d\sigma_2/d\tau < 0$, so the system would return to the stationary point as indicated by the arrows. Accordingly for a decrease perturbation. By contrast, P_2 is *unstable*, because here an increase pertubation leaves $d\sigma_2/d\tau > 0$, so σ_2 would further increase until P_3 is reached. The system does *not* return to the original stationary point.

ics and could not occur if two straight lines or simple hyperbolae were the intersecting curves.

If the stationary velocity $\bar{\nu}$ is plotted against the corresponding σ_1-value, the so-called *input characteristics* result (fig. 2, right-hand side) which can be defined as the stationary flux through the ν—ν_2-system when the input value σ_1 is kept constant. It will be of use for the construction of the stationary state of the open model and its properties.

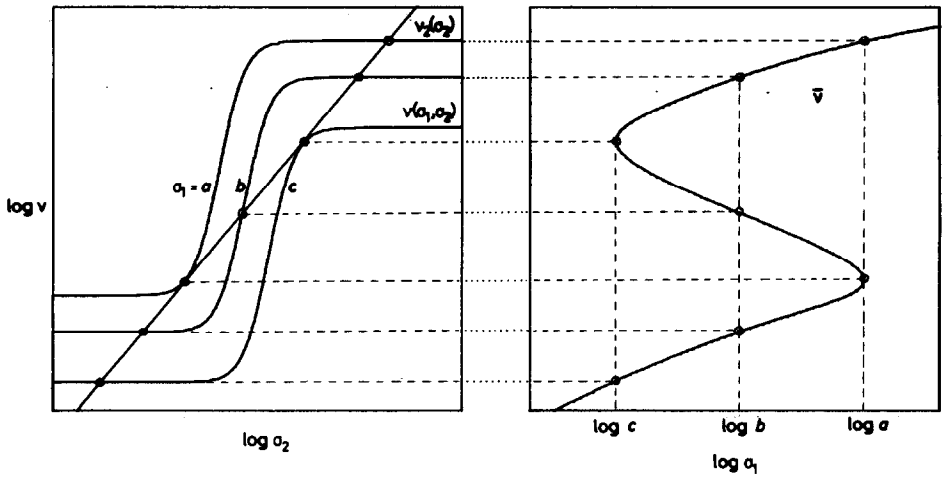


Fig. 2. Stationary points and input characteristics. Left-hand: Stationary points of σ_2. The sigmoidal curves of PFK-activity (ν) versus activator σ_2 have been projected on the kinetics of removal of σ_2 (ν_2, e.g. oxidative phosphorylation of ADP). Intersection points ($\nu = \nu_2$) indicate stationarity of σ_2 as function of σ_1 (by selection of the sigmoidal curve). Note that up to three stationary points for one σ_1-value are possible. Right-hand: The stationary $\bar{\nu}$-values of the left picture are replotted as function of σ_1. The resulting curve is called 'input characteristics' of PFK, and expresses the stationary flux through the enzyme at specified input value of σ_1.

3.4. *Stability of stationary points*

Consider once more the case with three stationary points of σ_2 (fig. 3). As made evident in the legend to this figure by investigation of small perturbations of the stationary point, P_2 is an *unstable* point which will *not* be reached again after the perturbation. A stable point like P_1 or P_3 behaves contrarily.

3.5. *Stationary state*

A stationary state is defined by simultaneous stationarity of all variables, in our case σ_1 and σ_2. It can be constructed by superposition of ν_1 on the input characteristics (fig. 4). The intersection points satisfy $\nu_1 = \nu = \nu_2$. Again, multiple stationary states are possible. Two out of three are stable in the example considered.

3.6. *Critical state. Hysteresis*

The stationary state Q_1 in fig. 4 is not critical, because a slight change of the ν_1-curve would produce only a slight displacement of Q_1. By contrast, in fig. 5 critical states Q_1 and Q_2 are produced by the fact that the ν_1-curve *touches* the input characteristics. A very slight further change would suddenly leave the curve with only one intersection point at a different branch. A very drastic change of the stationary velocity would follow. This, in contrast to the argument as referred to initially, is a really sensitive trigger mechanism to explain switch-on and switch-off of pathways or cycles.

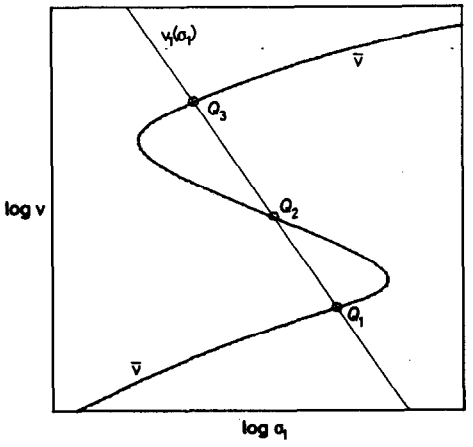


Fig. 4. Stationary state of glycolytic model. Intersection of ν_1 ('hexokinase') and input characteristics $\bar{\nu}$ (σ_1) give the stationary points of the whole system, where $\nu_1 = \nu = \nu_2$. Note that Q_2, owing to the instability of P_2 in fig. 3, is unstable, while Q_1 and Q_3 are stable stationary points.

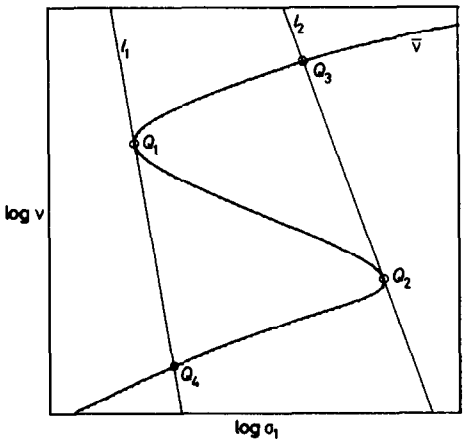


Fig. 5. Critical state of the model. Variation of ν_1 between the limits denoted as l_2 and l_1 leaves the stationary point between Q_1 and Q_3 if it was originally on this branch. But with l_1 a critical state is reached. Even the slightest increase of the inhibition would leave only one intersection left of Q_4 on the lower branch of the input characteristics. If $\alpha_2 >> 1$ (see eq. 26) a drastic jump of an active state (Q_1) to an inactive one (near Q_4) would occur. Note that if now ν_1 moves back, the system would *not* jump back from Q_4 to Q_1 but rather walk along the lower branch towards Q_2, where the critical state of the inhibited regime is reached. From here a sudden jump to Q_3 is possible. The fact that a system does not move back along the same path to the old state if one parameter does so, is called *hysteresis*, and is of conceptual importance.

A further phenomenon, *hysteresis* (not to be confounded with hysteresis of a single enzyme [27]), is detected on closer examination of this example. On slow migration of one parameter the system may approach one critical state, then switch over to a different state as described, but would not return to the old state along the *same* branch, if the parameter returns. The explanation is given in fig. 5.

It should be noted that these effects could likewise be produced by variation of β_2 rather than of β_1 or ν_m. The latter choice was made for the sake of a more comprehensible argument. A variation of β_2 is of more relevance, because it could explain the Pasteur effect.

3.7. *Limit cycle and auto-osciillations*

In fig. 6 'hexokinase' is chosen so that only the unstable branch of the input characteristics is inter-

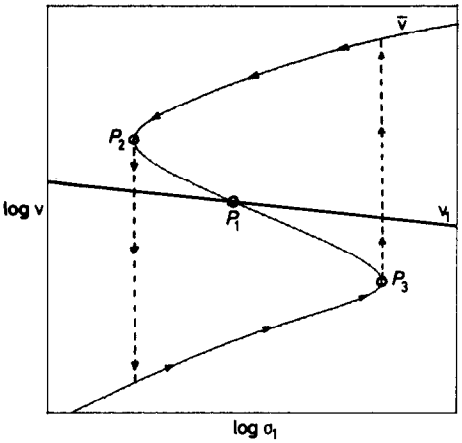


Fig. 6. Auto-oscillations of jump character. Refer to the previous figures. Here the intersection point between ν_1 and ν is an unstable point so that in accordance with the argument in figs. 3 and 4 a perturbation to, say, the left side of the stationary state ($\nu > \nu_1$) would trigger a rapid fall of σ_1 ($d\sigma_1/d\tau < 0$, see eq. 26). If $\alpha_2 >> 1$ the system would move along $\bar{\nu}$ towards P_2. There further decrease of σ_1 along $\bar{\nu}$ is not possible on the upper branch. The system jumps therefore to the lower branch of $\bar{\nu}$ (of course this is no exact derivation!), so now $d\sigma_1/d\tau > 0$ which results in migration towards P_3. There a relaxation to the upper brach takes place. It is clearly seen that σ_1 shows spontaneous oscillations (limit cycle behaviour). σ_2 (Not shown) behaves accordingly.

sected. As explained in the legend, this unstable stationary point is surrounded by a stable *limit cycle* of periodic oscillations which have a jump character (relaxation) when the turnover of σ_2 becomes very rapid.

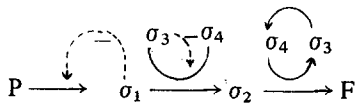
It should be mentioned that auto-oscillations of very long and stable periods can be predicted by this type of model, when a fast depositary mechanism (such as glycogen formation would be) is operative [28]. This could explain circadian and longer rhythms at the metabolic level, a result which at first glance is unexpected because of the different time scales.

3.8. *Spatial organization.*

So far a 'well-stirred' system was considered which restricts dynamic phenomena to the temporal category. In a non-stirred system spatial phenomena can also be predicted from the same type of non-linearity [23, 29]. This is of importance for biological phenemena such as growth and differentiation, but has not been investigated thoroughly so far.

3.9 *Equivalent models [28]*

An important inductive step towards a general theory is the study of equivalent mechanisms which have the same qualitative structure but look quite different on a metabolic map because of their topological structure. The properties of model (25), for instance, could also be predicted from a model like



(29)

which is closer to the real glycolytic system of some cells.

Equivalent mechanisms can be sorted out from the metabolic map. This introduces a systematic order into the network of intermediary reactions, thereby reducing the number of conceptually important models considerably. The necessary inductive work along this line is still in its initial phase.

4. Theory of metabolic networks

In the field of science, no theory can be developed only from axioms. It has to proceed by inductive generalization starting from the empirical facts. An intermediate stage is description of phenomena which can be envisaged as concentrated, purified facts. Their successful explanation by a model connects empirism and theory. The latter is derived from general principles (which are found by a combination of inductive and deductive reasoning) with the aim of predicting phenomena and facts.

At the level of cellular metabolism, such a theory is not at hand at the present time, because our phenomenological knowledge is not sufficient. So far only single enzymes and rather small pathways have been extensively characterized by kinetic methods. The description of more integrated networks is an obvious task for the near future.

The guiding methodical principle of this theory will be, in our opinion, structural simplification. For example, the details of macromolecular structure are not required for a general theory of cellular metabolism, and already a single enzyme model contains their essence only, in distilled form. Their interaction produces new properties which at the elementary level would appear incomprehensible. The enzyme

mechanism, then, is transferred in an abstract form to the next level, organization of enzymes within pathways, then the essential properties of pathways into a complex network such as the cytosol metabolic system etc.

This hierarchial principle and the intermediate position of the mathematical model between empirism and theory explain why interaction with experimental activities is necessary for theoretical work at any level of time or organization. The methodics of such interaction, for instance the systematic investigation of the predictive capacity of models, or a strict concept of its consistency with experimental data as well as its qualification for theoretical deduction, all this forms an integral part of the theory. A more detailed consideration of these methodical aspects of connection between theory and reality will be given elsewhere [6,30–32].

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